*Proc. Natl. Acad. Sci. USA*
Vol. 86, pp. 2331–2335, April 1989
Immunology

# Tumor necrosis factor α induces proteins that bind specifically to κB-like enhancer elements and regulate interleukin 2 receptor α-chain gene expression in primary human T lymphocytes

**(DNA–protein interaction/human immunodeficiency virus enhancer/Tax protein)**

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*Communicated by Robert J. Lefkowitz, December 22, 1988 (received for review November 21, 1988)*

ABSTRACT **We have investigated the biochemical basis for the activation of interleukin 2 receptor α-subunit (IL-2Rα) gene expression in primary human T lymphocytes by a cytokine (tumor necrosis factor α), a T-cell mitogen (phorbol 12-myristate 13-acetate), and the transactivator protein (Tax) from the type I human T-cell leukemia virus. Using *in vivo* transfection techniques specifically designed for these primary T cells in conjunction with *in vitro* gel retardation and DNA footprinting assays, we found that activation of the IL-2Rα promoter by each of these agents involves the induction of nuclear proteins that specifically interact with a κB-like enhancer element (i.e., an element resembling the immunoglobulin κ-chain enhancer sequence recognized by transcription factor NF-κB). DNA–protein crosslinking studies revealed that primary T cells express at least three different inducible DNA-binding proteins (50–55, 70–75, and 80–90 kDa) that specifically interact with this IL-2Rα κB element.**

The proliferation of human T lymphocytes involves the coordinated activation of genes encoding the T-cell growth factor interleukin 2 (IL-2) and its membrane receptor (IL-2R) (1, 2). The functional high-affinity IL-2R complex consists of at least two distinct IL-2-binding protein subunits, IL-2Rα (Tac, p55, CD25) (3) and IL-2Rβ (p70–75) (4–7). Both of these receptor subunits appear to be required for effective growth-signal transduction in the presence of physiological concentrations of IL-2 (8, 9). Since resting T cells constitutively express IL-2Rβ (7), the induction of IL-2Rα gene expression is important for the regulation of high-affinity IL-2R display and T-cell proliferation. Cell surface expression of IL-2Rα can be induced by a variety of stimuli, including antigens, the transactivator protein (Tax) of type I human T-cell leukemia virus (HTLV-I) (10), and nonspecific mitogens such as phytohemagglutinin (PHA) and phorbol 12-myristate 13-acetate (PMA) (11). In addition, tumor necrosis factor α (TNF-α), a macrophage- and T-cell-derived cytokine that displays a wide spectrum of biological activities (12), has been shown to induce IL-2Rα gene expression in both normal and leukemic T cells (13, 14).

Previous studies of the regulation of IL-2Rα gene activation have used long-term cultured leukemic T-cell lines as approximate experimental models for activation events occurring in primary T cells (15–21). Studies of one such T-cell line, Jurkat, suggested that the interaction of inducible DNA-binding proteins with a κB-like promoter element is important in the Tax activation of the IL-2Rα gene (19–21); however, disparate requirements for this element have been described with phorbol ester as the inducer (18–21).

In the studies reported here, we analyzed the cis-acting regulatory sequences and trans-acting factors operational in the activation of IL-2Rα gene expression in primary human T cells induced with TNF-α, PMA, or Tax. Functional transfection studies employing a series of mutated forms of the IL-2Rα promoter, coupled with gel retardation, DNA footprinting, and DNA–protein crosslinking assays, revealed that IL-2Rα gene activation by TNF-α, PMA, and Tax involves the induced expression of at least three distinct DNA-binding proteins that interact specifically with the κB-like element present in the IL-2Rα promoter.

## MATERIALS AND METHODS

**Cell Cultures.** Peripheral blood lymphocytes were isolated from the venous blood of healthy adult volunteers by density gradient centrifugation. Mononuclear cells were initially cultured at a concentration of $10^6$ cells per ml in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine (growth medium), supplemented with PHA (Wellcome) at 1 μg/ml and recombinant IL-2 (Cetus) at 100 units/ml. After 4 days of culture, the nonadherent cells were harvested, resuspended at $4 \times 10^7$ per ml in chilled growth medium, and placed on ice. Plasmid DNA (10 μg) was added to 250 μl of the cell suspension and the cultures were electroporated with a Bio-Rad Gene-Pulser equipped with a capacitance extender (960 μF, 250 mV) (22). The cells were then cultured at $10^6$ per ml in growth medium containing IL-2 (30 units/ml) and either PMA (20 ng/ml) or TNF-α (100 units/ml) or neither of these inducers. In some experiments, these primary cells were cotransfected with a *tax* cDNA expression plasmid (spFMTLTR/82-2C) (23). After an additional 24 hr of culture, cell extracts were assayed for chloramphenicol acetyltransferase (CAT) activity as described (24). All results were normalized according to protein recovery and expressed as percent transacetylation. In each experiment, cells were analyzed at day 4 for surface expression of T-cell markers by using a fluorescence-activated cell sorter. The percentage of positive cells for each marker (mean value of five donors ± SD) was as follows: $CD3^+$, 98.0 ± 1.0%; $CD2^+$, 98.0 ± 1.5%; $CD25^+$, 99.0 ± 1.0%; $UPC10^+$ (negative control), 3.5 ± 1.5%. Mononuclear cells were isolated from fresh human thymic tissue and cultured as described above. After 4 days of culture, the cells were 99% $CD25^+$, 99% $CD2^+$, and 94% $CD3^+$.

**Expression Plasmids.** A nested series of 5′ deletion mutants of the IL-2Rα promoter was generated as described (17). The resultant deletion mutants were designated according to the most 5′ nucleotide retained relative to the major 3′ cap site (+1) as assessed by DNA sequencing. Various IL-2Rα

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Abbreviations: CAT, chloramphenicol acetyltransferase; IL-2, interleukin 2; IL-2R, IL-2 receptor; IL-2Rα, IL-2R α subunit; HIV-1, human immunodeficiency virus type 1; HTLV-I, human T-cell leukemia virus type I; PMA, phorbol 12-myristate 13-acetate; PHA, phytohemagglutinin; TK, thymidine kinase; TNF-α, tumor necrosis factor α.

promoter oligonucleotides were also inserted into a CAT expression plasmid containing the −105 to +51 region of the thymidine kinase gene from herpes simplex virus (TK–CAT) (25). Inserts used were IL-2R III (−291 to −245 of the IL-2Rα promoter), IL-2R III M1 (−291 to −245 of the IL-2Rα promoter, where GGG at positions −266 to −264 of the κB element is replaced by CTC), HIV DR [the direct repeat element of the enhancer of type 1 human immunodeficiency virus (HIV-1)], and HIV 3′ (the 3′ motif of HIV DR). Nucleotides corresponding to the IL-2Rα κB element (−267 to −256) were also deleted from the fully functional −317 IL-2Rα promoter plasmid by oligonucleotide-directed *in vitro* mutagenesis (26) to create the −317Δ construct. Mutants M1–M9 were similarly prepared by specific site-directed mutations of two or three nucleotides traversing the promoter region between −273 and −250.

**Gel Retardation and DNA Footprinting.** The procedures for nuclear extract preparation and gel retardation assays have been published (17, 18, 27). In competition experiments, a 200-fold molar excess of unlabeled oligonucleotide was added to the reaction mixture 10 min prior to the addition of radiolabeled probe. Chemical DNA footprinting was performed using 1,10-phenanthroline copper (28). Nuclear extracts were incubated with radiolabeled IL-2R III and the resultant DNA–protein complexes were resolved by gel retention. Bound and free radiolabeled probe were partially cleaved with 1,10-phenanthroline copper as described (28). Gel slices containing bound or free probe were excised and the relevant DNA species were analyzed by electrophoresis in 10% polyacrylamide/urea sequencing gels.

**DNA–Protein Crosslinking.** A 27-base-pair (bp) IL-2Rα κB probe (−275 to −249) substituted with 5-bromo-2′-deoxyuridine (BrdUrd) and radiolabeled nucleotides was prepared (21). This probe was incubated with nuclear extracts under UV (300-nm) light for 20 min in the absence or presence of a 200-fold molar excess of unlabeled competitor oligonucleotide. The reaction mixtures were then heated to 100°C in the presence of 1% SDS and 5% 2-mercaptoethanol, and the resulting complexes were directly analyzed by SDS/8% PAGE. Alternatively, DNA–protein complexes were first purified by gel retardation and the gel slices containing these complexes were excised separately, heated to 100°C with 1% SDS and 5% 2-mercaptoethanol, and then analyzed by SDS/8% PAGE.

## RESULTS

**Induction of the IL-2Rα Promoter in Primary Human T Cells.** Recently described electroporation techniques (22) were used to introduce a nested series of 5′ deletion mutants of the IL-2Rα promoter into preactivated primary human T cells. The transfected cells were then either cultured in medium alone or stimulated with TNF-α (Fig. 1*A*) or PMA (Fig. 1*B*). In other experiments, these primary T cells were cotransfected with *tax* cDNA expression plasmids (Fig. 1*C*). TNF-α induction of the IL-2Rα promoter occurred within a physiological dose range (1–100 units/ml) of this cytokine, with half-maximal effects observed at 3–10 units/ml (data not shown). TNF-α or PMA stimulation of cells transfected with the −471 promoter construct resulted in a 5- to 8-fold induction of CAT activity (Fig. 1 *A* and *B*). Of note, deletion of sequences located between −340 and −317 produced a consistent increase in TNF-α- and PMA-induced CAT activity, suggesting the presence of a functional negative regulatory element in this deleted region. In contrast, the function of this apparent negative regulatory element was less evident in cells induced with the HTLV-I Tax protein (Fig. 1*C*). Deletion of nucleotides 3′ of −281 resulted in a precipitous decline in both TNF-α- and PMA-induced IL-2Rα promoter activation. However, the region between −281 and −266 appeared fully dispensable for Tax-induced promoter activation.

To explore the potential role of the κB element and flanking sequences in the IL-2Rα promoter activation, additional transfections were performed with a series of −317 promoter constructs specifically altered by site-directed mutagenesis between nucleotide −273 and −250 (Fig. 2*A*). Deletion of the entire κB element (−317Δ) or the introduction of various point mutations within this element (M3–M5 and M7) resulted in a pronounced loss of inducibility by PMA, Tax, and TNF-α (Fig. 2 *B* and *C*). The single exception to these findings was obtained with mutant M6. Of interest, this mutation created a perfect 14-bp palindrome encompassing the κB site. In contrast, mutations introduced immediately 5′ of the κB element (M1 or M2) markedly inhibited PMA (Fig. 2*B*) and TNF-α (Fig. 2*C*) inducibility, but Tax activation was unaffected (Fig. 2*C*). These findings, together with the promoter deletion profiles, suggest that PMA and TNF-α, but not Tax, induction of the IL-2Rα promoter in primary T cells involves a second cis element located immediately 5′ of the κB element.

To examine whether the κB element of the IL-2Rα promoter is sufficient to impart inducibility to an unresponsive heterologous promoter in primary T cells, oligonucleotides containing this element (−291 to −245) or the related direct repeat (enhancer) element from HIV-1 were inserted upstream of the TK promoter in the TK–CAT plasmid. Transfection with plasmids containing a single or reiterated IL-2Rα κB element produced a 4-fold and 13-fold increase in CAT activity, respectively, after stimulation with TNF-α (Fig. 2*D*). In contrast, insertion of the same oligonucleotide containing a 3-base mutation in the κB element revealed

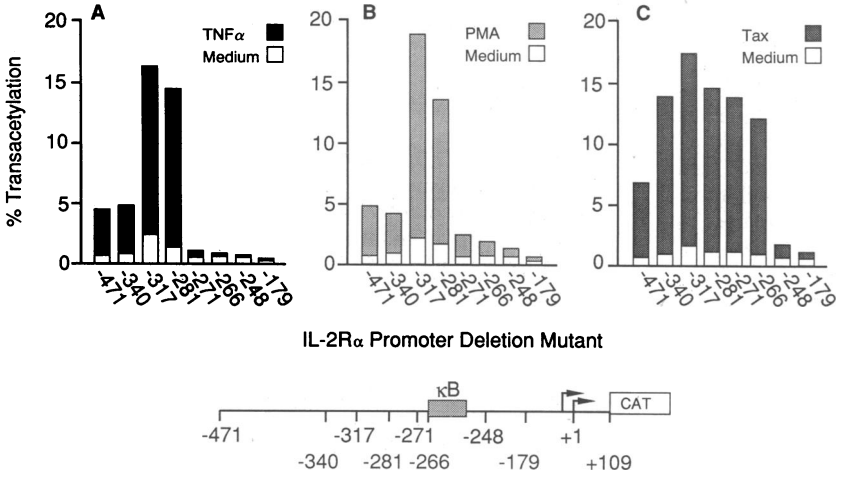


FIG. 1. Induction of IL-2Rα–CAT activity in activated peripheral blood T cells. Cells preactivated with PHA were transfected with various IL-2Rα promoter deletion plasmids (see diagram at bottom) and then stimulated with TNF-α at 100 units/ml (*A*) or PMA at 20 ng/ml (*B*). In other experiments, the cells were cotransfected with *tax* expression plasmids (*C*). Similar results were obtained with cells obtained from four different donors.

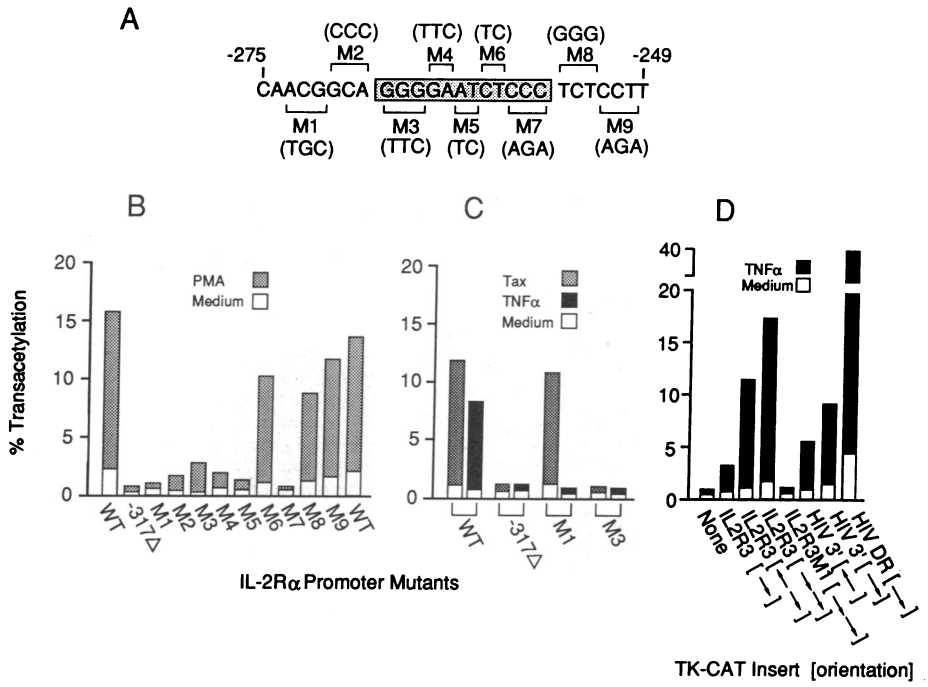


FIG. 2. (*A*) Site-directed mutagenesis of the −317 IL-2Rα promoter. Nucleotides corresponding to the κB element were deleted from the −317 promoter plasmid (−317Δ) by oligonucleotide-directed mutagenesis. Mutants M1–M9 were similarly prepared by specific mutation of two or three nucleotides between −273 and −250. (*B* and *C*) CAT activity from peripheral blood T cells transfected with wild-type (WT) or various mutated forms of the −317 IL-2Rα promoter and stimulated with PMA at 20 ng/ml (*B*), TNF-α at 100 units/ml (*C*) or cotransfected with a *tax* expression plasmid (*C*). Similar results were obtained from four different donors. (*D*) The κB element confers TNF-α inducibility on an unresponsive heterologous promoter. Various oligonucleotides corresponding to IL-2Rα promoter or HIV-1 enhancer sequences were inserted into a TK–CAT plasmid as described in *Materials and Methods* (IL2R3 = IL-2R III). Insert orientation and copy number are indicated by the arrows. Similar results were obtained in three independent experiments.

minimal TNF-α-inducible CAT activity. The 3′ motif of the HIV-1 direct repeat element and the entire HIV-1 direct repeat were similarly induced by TNF-α (6- and 10-fold, respectively). Both of these cellular and viral elements displayed enhancer-like properties, as they functioned in either orientation. Similar results were obtained when PMA or Tax was used as the inducing agent (data not shown).

**Identification of Inducible DNA-Binding Proteins That Specifically Interact with the IL-2Rα κB Element.** Gel retardation assays were employed to investigate the potential binding of inducible nuclear proteins at the κB element of the IL-2Rα promoter. Nuclear proteins extracted from primary human T cells were incubated with radiolabeled oligonucleotides containing the IL-2Rα κB element (IL-2R III). Extracts from peripheral blood T cells activated with PMA for 24 hr (Fig. 3*A*, lanes 1–6) yielded one major retarded complex and a fainter, more slowly migrating complex. The intensity of the upper complex relative to the lower complex varied among donors and may reflect variations due to the nuclear protein extraction procedure (compare lanes 1 and 3 with lanes 4 and 6). Extracts from primary human thymocytes cultured for 5 days in the presence of PMA and PHA yielded a single retarded complex (lanes 7–9). The formation of these complexes was completely inhibited by preincubation of the extracts with a 200-fold molar excess of a 12-bp oligonucleotide corresponding to the IL-2Rα κB element (IL-2R VIII) but not by 12-bp oligonucleotides containing a 3-base mutation (IL-2R VIII M1, GGG → CTC) in this element. Extracts from TNF-α-stimulated peripheral blood T cells similarly produced two κB-specific complexes with the IL-2R III probe (Fig. 3*B*), whereas the levels of these κB-specific proteins in extracts from uninduced T cells (Fig. 3*B*, lane 5) or uninduced thymocytes (data not shown) were lower by a factor of 10–30. A 16-bp oligonucleotide containing the 5′ element of the HIV-1 enhancer also inhibited the binding of nuclear proteins to the IL-2Rα κB element (Fig. 3*B*, lane 4). These findings suggest that these cellular and viral control elements may interact with the same TNF-α-inducible proteins.

Direct evidence that these DNA-binding proteins specifically contact nucleotide residues within the IL-2Rα κB element was obtained by *in situ* footprinting of the retarded DNA–protein complexes. Fig. 3*C* shows that nuclear proteins from peripheral blood T cells induced with PMA (lanes 2 and 3), or TNF-α (lanes 4 and 5), primary human thymocytes induced with PHA and PMA (lane 6), or Jurkat T cells stimulated with PMA (lane 7) each produced a single, strong region of protection within the κB element. Further, the proteins associated with the more slowly migrating B1 complex produced a footprint indistinguishable from that observed with the more rapidly migrating B2 complex. An identical protection pattern was obtained when the complementary DNA strand was analyzed (data not shown).

**DNA–Protein Crosslinking Studies Reveal Multiple κB-Specific Proteins That Interact with the IL-2Rα Promoter.** To further characterize the DNA-binding protein(s) that specifically react with the κB element, crosslinking studies were performed with a photoreactive IL-2Rα κB probe. The resultant covalently bound DNA–protein complexes were directly analyzed in SDS/polyacrylamide gels (Fig. 4*A*) or, alternatively, the B1 and B2 complexes were first resolved by gel retardation prior to SDS/PAGE (Fig. 4*B*). Fig. 4*A* shows that the predominant crosslinked protein species migrated as a doublet at 50–55 kDa, with a less prominent species appearing at 80–90 kDa (lane 1). The detection of each of these proteins was blocked by the prior addition of a 200-fold excess of homologous oligonucleotide (lane 2) but not by the identical oligonucleotide containing a mutation in the κB site (lane 3). Fig. 4*B* shows that the B2 complex (excised from lane 1) contained only the 50- to 55-kDa protein doublet (lane 3), whereas the B1 complex (also excised from lane 1) contained proteins migrating with apparent molecular masses of 70–75 kDa and 80–90 kDa in addition to the 50- to 55-kDa doublet (lane 4). Similar crosslinking results were obtained with nuclear extracts isolated from TNF-α-induced primary T cells (data not shown).

## DISCUSSION

Previous investigations of the biochemical basis for activation of the IL-2Rα gene have been largely restricted to neoplastic cell lines activated with nonphysiological stimulants as an experimental model for events occurring during normal T-cell activation. In this report, we have studied the induction of the IL-2Rα promoter in primary human T lymphocytes following activation by TNF-α as well as by phorbol ester and the Tax protein of HTLV-I. Transfection studies with a nested series of IL-2Rα promoter deletion

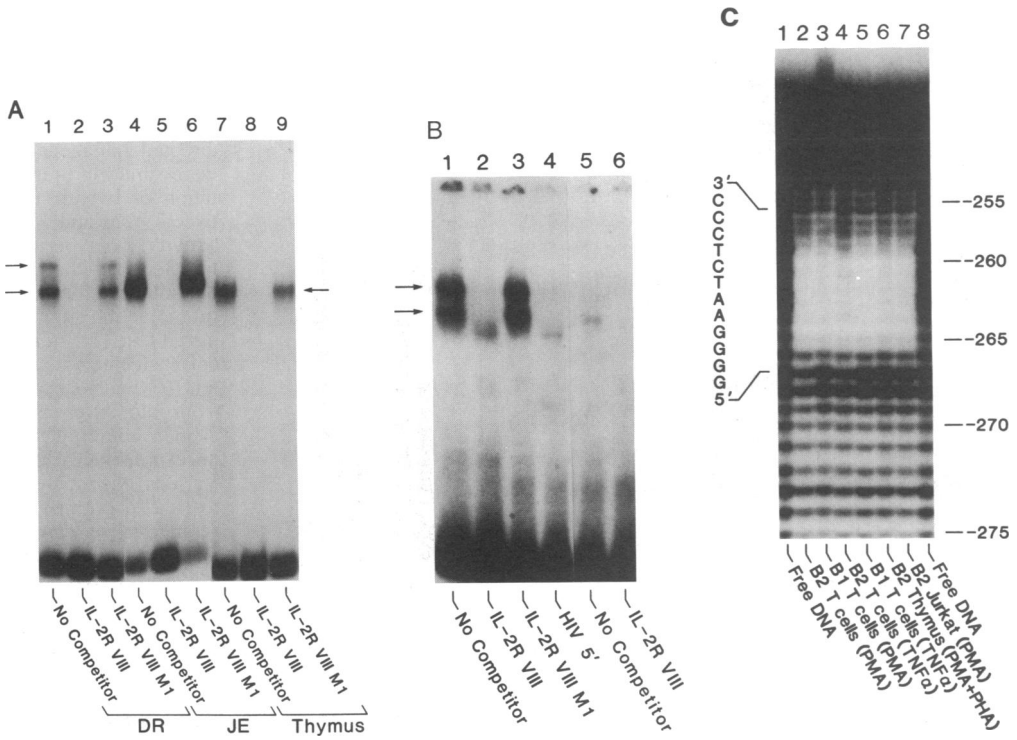


FIG. 3. (*A*) Gel retardation patterns obtained after incubation of radiolabeled IL-2Rα κB probe (IL-2R III) with nuclear extracts from PMA-induced primary T cells. Peripheral blood T cells from two individuals (DR and JE, lanes 1–6) and from primary human thymocytes (lanes 7–10) were cultured with PHA and IL-2 for 4 days and then activated for 24 hr with PMA prior to extraction of nuclear proteins. Competition studies were performed with a 200-fold molar excess of oligonucleotides containing either the 12-bp IL-2Rα κB element (IL-2R VIII; lanes 2, 5, and 8) or a mutated form of this element (IL-2R VIII M1; lanes 3, 6, and 9). (*B*) TNF-α induction of κB-specific binding proteins (lanes 1–3) in peripheral blood T cells whose interaction with the radiolabeled IL-2R III oligonucleotide is blocked by the 5′ element of the HIV-1 enhancer (HIV 5′) (lane 4). The gel retardation pattern obtained with extracts from control, uninduced cells is shown in lanes 5 and 6. (*C*) 1,10-Phenanthroline copper footprinting analysis of IL-2Rα promoter sequences, revealing that nuclear DNA-binding proteins extracted from primary human T cells interact directly with the κB element. Lanes: 1 and 8, free probe; 2 and 3, extract from PMA-induced peripheral blood T cells; 4 and 5, extract from peripheral blood T cells induced with TNF-α; 6, extract from PMA- and PHA-induced primary human thymocytes; 7, extracts from PMA-induced Jurkat T cells. B1 and B2 refer to the slowly migrating (upper) and the more rapidly migrating (lower) complex (arrows in *A* and *B*), respectively.

mutants revealed a 5′ boundary for the TNF-α- and PMA-responsive promoter region at nucleotide −281. In contrast, sequences between −281 and −266 were not required for IL-2Rα promoter activation mediated by Tax. Specific site-directed mutation or deletion of bases within a 12-bp κB-like element located between −267 and −256 abolished TNF-α, PMA, and Tax induction of the IL-2Rα promoter in peripheral blood T cells. Of note, this control element shares a high degree of sequence homology with the NF-κB binding sites present in the regulatory regions of a number of unrelated cellular genes, including those encoding immunoglobulin κ light chain, $\beta_2$-microglobulin, IL-2, and class I major histocompatibility antigens, as well as binding sites present in the enhancers of simian virus 40 and HIV-1 (29–31).

Gel retardation assays were performed with nuclear extracts obtained from induced and uninduced peripheral blood T cells. These studies identified two inducible protein–DNA complexes. DNA footprinting assays precisely mapped the contact points of these protein(s) to nucleotides contained within the κB site, thereby confirming the involvement of this element in the binding of nuclear proteins required for induction of IL-2Rα gene expression. Similar gel retardation and footprint patterns were obtained with nuclear extracts from PMA-induced human macrophage cell lines and mouse EL-4 thymoma cells (J.W.L., unpublished results). These findings suggest that expression of the κB-specific DNA-binding proteins is neither T-cell- nor species-specific. Interestingly, the complexes formed by the interaction of TNF-α-induced proteins with the IL-2Rα κB element was inhibited by a 16-bp oligonucleotide containing the 5′ κB motif of the HIV-1 direct repeat. Furthermore, preliminary transfection results indicate that TNF-α activates the HIV-1 long terminal repeat in primary human T cells (data not shown). Taken together, these results suggest that these TNF-α-inducible proteins bind to the HIV-1 enhancer and that activation of HIV-1-infected T cells by this cytokine may lead to the induction of virus replication *in vivo*.

To examine whether the κB element independently confers TNF-α inducibility, an 18-bp oligonucleotide spanning this region (−272 to −253) was inserted upstream of the TK-CAT promoter. The IL-2Rα, as well as the HIV-1, κB element proved sufficient to confer TNF-α inducibility on this unresponsive heterologous promoter in primary human T cells. Further, the IL-2Rα element displayed enhancer-like properties, as it functioned independent of orientation and the magnitude of induction was increased when the binding site was reiterated. These findings are consistent with our previous studies with PMA- and Tax-induced Jurkat leukemic T cells (17, 18).

DNA–protein crosslinking showed that at least three major PMA- and TNF-α-induced proteins interact with this site, including species of 80–90, 70–75, and 50–55 kDa. Similarly sized proteins were detected in extracts obtained from TNF-α- and PMA-induced Jurkat leukemic T cells (21, 32), human macrophages, and mouse T cells (J.W.L., unpublished results). It is possible that two of these proteins correspond to the 86-kDa HIVEN86A polypeptide (33) and the recently purified 51-kDa NF-κB protein (34). Our recent studies indicate that the induction of both the 80- to 90-kDa and the 50- to 55-kDa proteins is controlled at a posttrans-

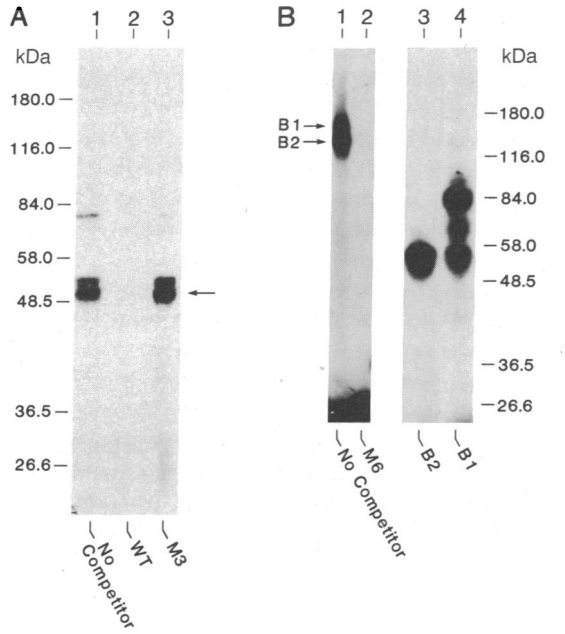


FIG. 4. UV-induced crosslinking of DNA-binding proteins to the κB element of the IL-2Rα promoter. (*A*) A 27-bp IL-2Rα κB photoreactive probe (−275 to −249) was incubated with nuclear extract from PMA-induced peripheral blood T cells in the absence of competitor DNA (lane 1) or in the presence of a 200-fold molar excess of homologous (wild type, WT; lane 2) or M3 mutant (lane 3) oligonucleotide. The reaction mixtures were UV-irradiated and then heated to 100°C in the presence of 1% SDS and 5% 2-mercaptoethanol and the resulting complexes were directly resolved by SDS/8% PAGE. (*B*) The same crosslinking probe was incubated with nuclear extract as described in *A*, in the absence (lane 1) or presence (lane 2) of a 200-fold excess of mutant M6 oligonucleotide, which avidly binds the κB-specific proteins and does not impair functional responsiveness (see Fig. 2). Prior to SDS/PAGE, DNA–protein complexes were resolved by gel retardation. The complexes corresponding to B1 and B2 were excised separately, heated to 100°C in the presence of 1% SDS and 5% 2-mercaptoethanol, and then electrophoresed in SDS/8% polyacrylamide gels (lanes 3 and 4).

lational level, as their induction persists in the presence of cycloheximide at concentrations that inhibit >97% of *de novo* cellular protein synthesis (35). It is unknown whether these proteins are products of different genes or are produced by alternative posttranscriptional or posttranslational modifications of a single gene product.

Transfection studies with IL-2Rα promoter constructs having different deletions or site-specific mutations have identified a second regulatory sequence located immediately 5′ of the κB site. This promoter region is required for both TNF-α and PMA induction but is dispensable for Tax-induced activation. Despite extensive study, no protein interactions were detected at this upstream site by gel retardation, DNA footprinting, or methylation interference assays. However, a solution exonuclease III protection assay has detected the binding of constitutively produced proteins to a site located ≈10 bp upstream from the κB element (D.W.B., unpublished results).

In summary, our results demonstrate that IL-2Rα gene activation in primary human T cells is regulated by the expression of κB-specific DNA-binding proteins that are induced by both physiological (TNF-α) and pathological (HTLV-I Tax) activation signals. The capacity of TNF-α and Tax to induce these κB-binding proteins raises the possibility that these agents may also alter the expression of a variety of cellular genes containing κB elements, including those encoding immunoglobulin κ light chain, IL-2, $\beta_2$-microglobulin, and class I major histocompatibility antigens. In addition, the induced expression of these κB-specific proteins by TNF-α may augment replication of HIV-1 in T cells latently or persistently infected with this virus.


1. Greene, W. C., Leonard, W. J. & Depper, J. M. (1986) *Prog. Hematol.* **14**, 283–301.
2. Taniguchi, T., Matsui, H., Fujita, T., Hatakeyama, M., Kashima, N., Fuse, A., Hamuro, J., Nishi-Takaoka, C. & Yamada, G. (1986) *Immunol. Rev.* **92**, 121–133.
3. Leonard, W. J., Depper, J. M., Crabtree, G. R., Rudikof, S., Pumphrey, J., Robb, R., Kronke, M., Svetlik, P. B., Peffer, N. J., Waldmann, T. A. & Greene, W. C. (1984) *Nature (London)* **311**, 626–631.
4. Tsudo, M., Kozak, R. W., Goldman, C. K. & Waldmann, T. A. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 9694–9698.
5. Teshigawara, K., Wang, H. M., Kato, K. & Smith, K. A. (1987) *J. Exp. Med.* **165**, 223–238.
6. Robb, R. J., Rusk, C. M., Yodoi, J. & Greene, W. C. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 2002–2006.
7. Dukovich, M., Wano, Y., Le thi Bich Thuy, Katz, P., Cullen, B. R., Kehrl, J. H. & Greene, W. C. (1987) *Nature (London)* **327**, 518–522.
8. Lowenthal, J. W. & Greene, W. C. (1987) *J. Exp. Med.* **166**, 1156–1161.
9. Wang, H. M. & Smith, K. A. (1987) *J. Exp. Med.* **116**, 1055–1069.
10. Wano, Y., Feinberg, M., Hosking, J. B., Bogerd, H. & Greene, W. C. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 9733–9737.
11. Depper, J. M., Leonard, W. J., Kronke, M., Noguchi, P. D., Cunningham, R. E., Waldmann, T. A. & Greene, W. C. (1984) *J. Immunol.* **133**, 3054–3061.
12. Beutler, B. & Cerami, A. (1988) *Adv. Immunol.* **42**, 213–231.
13. Scheurich, P., Thoma, B., Ucer, U. & Pfizenmaier, K. (1987) *J. Immunol.* **138**, 1786–1790.
14. Lee, J. C., Truneh, A., Smith, M. F. & Tsang, K. Y. (1987) *J. Immunol.* **139**, 1935–1938.
15. Maruyama, M., Shibuya, H., Harada, H., Hatakeyama, M., Seiki, M., Fujita, T., Inoue, J., Yoshida, M. & Taniguchi, T. (1987) *Cell* **48**, 343–350.
16. Cross, S. L., Feinberg, M. B., Wolf, J. B., Holbrook, N. J., Wong-Staal, F. & Leonard, W. J. (1987) *Cell* **49**, 47–56.
17. Lowenthal, J. W., Böhnlein, E., Ballard, D. W. & Greene, W. C. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4468–4472.
18. Böhnlein, E., Lowenthal, J. W., Siekevitz, M., Ballard, D. W., Franza, B. R. & Greene, W. C. (1988) *Cell* **53**, 827–836.
19. Leung, K. & Nabel, G. J. (1988) *Nature (London)* **333**, 776–778.
20. Ruben, S., Poteat, H., Tan, T.-H., Kawakami, K., Roeder, R., Haseltine, W. & Rosen, C. A. (1988) *Science* **241**, 89–92.
21. Ballard, D. W., Böhnlein, E., Lowenthal, J. W., Wano, Y., Franza, B. R. & Greene, W. C. (1988) *Science* **241**, 1652–1655.
22. Cann, A. J., Koyangi, Y. & Chen, I. S. Y. (1988) *Oncogene* **3**, 123–128.
23. Siekevitz, M., Feinberg, M. B., Holbrook, N., Wong-Staal, F. & Greene, W. C. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5389–5393.
24. Neumann, J. R., Morency, C. A. & Russian, K. O. (1987) *BioTechniques* **5**, 444–447.
25. Miksicek, R., Heber, A., Schmid, W., Danesch, U., Possackert, G., Beato, M. & Shutz, G. (1986) *Cell* **46**, 283–290.
26. Nakamaye, K. & Eckstein, F. (1986) *Nucleic Acids Res.* **14**, 9679–9698.
27. Böhnlein, E. & Gruss, P. (1986) *Mol. Cell. Biol.* **6**, 1401–1411.
28. Kuwabara, M. D. & Sigman, D. S. (1987) *Biochemistry* **26**, 7234–7238.
29. Sen, R. & Baltimore, D. (1986) *Cell* **46**, 705–716.
30. Nabel, G. & Baltimore, D. (1987) *Nature (London)* **326**, 711– 713.
31. Baldwin, A. S. & Sharp, P. A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 723–727.
32. Lowenthal, J. W., Ballard, D. W., Bogerd, H., Böhnlein, E. & Greene, W. C. (1989) *J. Immunol.*, in press.
33. Franza, R. B., Josephs, S. F., Gilman, M. Z., Ryan, W. & Clarkson, B. (1987) *Nature (London)* **330**, 391–395.
34. Kawakami, K., Scheiderest, C. & Roeder, R. G. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4700–4704.
35. Böhnlein, E., Ballard, D. W., Bogerd, H., Peffer, N. J., Lowenthal, J. W. & Greene, W. C. (1989) *J. Biol. Chem.*, in press.