Human Tumor Necrosis Factor α Gene Regulation in Phorbol Ester Stimulated T and B Cell Lines

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Summary

The minimal region of the human tumor necrosis factor α (TNF-α) gene promoter necessary for its transcriptional induction by phorbol esters (PMA) in human T and B lymphocyte cell lines has been localized between -52 and +89 nucleotides (nt) relative to the gene's transcriptional start site. Comparison of these sequences to those required to mediate virus or lipopolysaccharide (LPS) induction of the gene reveal significant differences, and thus, the sequence requirements for PMA induction are distinct from those that mediate induction by virus or LPS. Although three sites in the TNF-α promoter (κ1, κ2, and κ3) specifically bind the transcription factor NF-κB in lymphoid nuclear extracts, TNF-α mRNA induction by PMA does not correlate with NF-κB binding activities displayed by different T and B cell lines. Moreover, κ1–κ3 can each be deleted from the TNF-α promoter with little effect on the gene's inducibility by PMA. Therefore, TNF-α mRNA induction by PMA, like its induction by virus and LPS, is not primarily mediated by NF-κB, but rather is mediated through other sequences and protein factors. Surprisingly, multimers of κ1–κ3 can confer PMA inducibility on a heterologous promoter in a B (Raji), but not a T (HUT78) cell line. However they are not functional on a truncated TNF-α promoter, indicating that promoter context and cell type specificity influence the PMA inducible function of these NF-κB binding sites.

Human TNF-α is a cytotoxic protein that displays a wide range of biological activities that include the hemorrhagic necrosis of certain tumors, inhibition of viral infection and a role in the mediation of septic shock (see references 1 and 2 for review). Historically thought to be a monocyte derived factor, TNF-α is also produced by circulating human peripheral B lymphocytes (3) and tonsillar B lymphocytes (4). In addition, certain human B cell lines produce TNF-α when stimulated by virus (3) and PMA (3, 4). T cell lines have also been shown to produce TNF-α when stimulated by virus and PMA as well as by other T cell activating agents such as anti-CD3 antibody and calcium ionophore (5, 6).

PMA treatment of T and B lymphocytes stimulates protein kinase C activity and therefore provides a model system of lymphocyte activation (7). Induction of TNF-α in B cells and T cells by PMA and other inducers is also of interest in light of the role TNF-α itself plays in the activation and differentiation of lymphocytes and in associated processes. For example, TNF-α promotes T cell growth and differentiation of T cells via induction of IL-2R (8), and stimulates B cell growth and differentiation through its induction of IL-6 (9). In addition, TNF-α increases the expression of HIV in certain latently infected T cell lines (reviewed in reference 10). These effects are presumably mediated through the induction of a variety of transcription factors by TNF-α including T cell derived proteins that bind to NF-κB binding sites (11-13) and the growth factors fos and jun (14). The understanding of TNF-α gene regulation by PMA in B and T lymphocytes may therefore provide insight into these processes of lymphocyte and HIV-1 activation, as well as into mechanisms of inducible gene expression by PMA.

In addition to PMA, virus and LPS are also potent inducers of human TNF-α gene transcription (2, 3). These induction processes are however distinguishable at the level of transcription. For example, virus, LPS, and PMA differ with respect to the cell type in which they can induce TNF-α mRNA expression, the distinct kinetics of TNF-α mRNA accumulation when these inducers act on the same cell type, and their differential interactions with metabolic inhibitors in the same cell type (3). The study of TNF-α gene regulation therefore provides an opportunity to gain insight into the transcriptional mechanisms whereby a single gene is induced by distinct pathways.

The promoter sequences required for virus and LPS induction of human TNF-α gene transcription have recently been identified (15). Although these sequences contain three sites (κ1, κ2, and κ3) that specifically bind the transcription factor NF-κB in vitro, these sites are neither sufficient nor required for virus or LPS induction of the gene in vivo (15).
The transcription factor NF-κB was originally identified as a B cell specific binding activity required for immunoglobulin enhancer function (16). Subsequent studies revealed that NF-κB is present in many cell types, but it is located in the cytoplasm in a complex with an inhibitory protein called IκB. Treatment of these cell types with a variety of inducers including virus, LPS, and PMA leads to the disruption of the NF-κB/IκB complex, and the translocation of active NF-κB to the nucleus where it is thought to be involved in the transcriptional regulation of numerous genes (reviewed in reference 17).

In this study the effect of PMA on the regulation of the cloned human TNF-α gene introduced into cultured human B and T cell lines has been analyzed. As in the case of virus and LPS induction of the human TNF-α gene, κ1-κ3 are not required for the gene’s induction by PMA. Thus, unlike other promoters containing NF-κB binding sites that are inducible by virus, LPS and PMA, the induction of the human TNF-α gene does not appear to be primarily mediated by NF-κB. Moreover, the minimal sequences required for PMA induction of TNF-α (−52 to +89 nucleotides [nt] relative to the mRNA cap site) differ from those minimal sequences required for virus or LPS induction, and thus PMA induction is mediated through distinct regulatory domains. We conclude that TNF-α gene regulation by PMA, virus, and LPS is a very complex process that involves multiple regulatory elements and transcription factors that are active in a cell type specific manner.

Materials and Methods

**Cell Culture, Transfection, and Induction Protocols.** HUT78, P30, HPB-ALL, and Raji cells were grown in RPMI supplemented with 10% FCS, glutamine, penicillin, and streptomycin. Cells were transfected employing lipofectin reagent (Bethesda Research Laboratories, Bethesda, MD) (18), split into two aliquots 18 h after transfection and maintained in RPMI and 10% FCS in the presence or absence of 5 ng/ml of PMA (Sigma Chemical Co., St. Louis, MO) for 48 h. Bacterial chloramphenicol transferase (CAT) assays (19) were performed. These assays were carried out at the time of maximal level of expression post PMA induction (data not shown). Cells used for RNA analysis or nuclear extract preparation were maintained and induced with PMA as above.

**RNA Analysis.** RNA was prepared from HUT78, P30, HPB-ALL, and Raji cells. Uniformly 32P-labeled probes were prepared as described from SP6 γ-actin and T7 TNF-α Bam (3). The γ-actin probe was made to have a specific activity that was five times less than that of the TNF-α probe. The RNA fragments were electrophoresed on a 6% denaturing polyacrylamide gel as described (3).

**Subcellular Fractionation and Mobility Shift Electrophoresis.** Nuclear extracts were prepared and binding assays were carried out as described (20) and as detailed in the figure legends.

**Plasmid Constructions.** The TNF-α promoter CAT constructs (−600, −576, −242, −199, −80, −52, and POCAT), p-128β (κ1)3, p-128β (κ2)4, p-128β (κ3)6, p-61 TNF-α, p-61 TNF-α (κ1)3, p-61 TNF-α (κ2)3, p-61 TNF-α (κ3)6 have been previously described (15), as have been p-128β, 128β(PRI11)4 (21), and p-41β and p-41β(PRI11)4 (22).

**Results**

**PMA Induction of Tumor Necrosis Factor (TNF)-α mRNA in Lymphocyte Cell Lines Is Cell Type Dependent.** B and T lymphocyte cell lines have previously been shown to display different patterns of TNF-α mRNA expression in response to PMA (3, 4, 6). To determine whether TNF-α mRNA expression and NF-κB binding activity could be correlated, four T cell lines and the mature B cell Burkitt’s lymphoma cell line, Raji were compared. The T cell lines were chosen because they represent distinct stages of differentiation as determined by surface expression of lymphocyte antigens (23, 24). The P30 cell line represents the earliest stage in T cell differentiation (T-Blast I) and is TdT+ (terminal transferase activity), CD7+, and MHC class II+ with no detectable γ or δ T cell rearrangements (M. Krangel, personal communication). HPB-ALL (T Blast II) is TdT+, CD4+, CD8+, and αβ TcR+ (24). Jurkat (T Blast III) is TdT+, CD4+, and αβ TcR+ (24). HUT78 represents the most terminally ‘activated’ T cell phenotype (T-Blast V) and is TdT−, CD4+, MHC class II+ and CD3+, but αβ TcR− (24).

These T and B cell lines were mock or PMA–induced and accurately initiated TNF-α mRNA was determined as previously described (3). In HPB-ALL and Jurkat cells no con-
constitutive or inducible TNF-α mRNA was evident (Fig. 1, lanes 3, 4, 7, and 8), although a faint inducible band was detected in both cell types after a long exposure of the autoradiogram (not shown). TNF-α mRNA was constitutively expressed at low levels in P30 cells, but was not inducible by PMA (lanes 5 and 6). By contrast, in HUT78 and Raji cells, TNF-α mRNA levels were highly inducible by PMA (lanes 1, 2, 9, and 10); Raji cells in addition had high levels of constitutive TNF-α mRNA expression. We conclude that constitutive and PMA induced TNF-α mRNA levels vary substantially in the T cell lines tested and may represent stage-specific gene expression.

NF-κB Binding Activity Expression Does not Correlate with Tumor Necrosis Factor (TNF)-α mRNA Expression Patterns. Three sequences within the 5′ regulatory region of TNF-α (κ1, κ2, and κ3; Fig. 2) specifically bind NF-κB in nuclear extracts derived from lymphoid cells (15). Although these sites have previously been shown not to be required for virus and LPS induction of the human TNF-α gene, it was possible that PMA induction of the gene was mediated by NF-κB.

To determine whether NF-κB binding activity could be correlated with TNF-α mRNA expression, nuclear extracts were prepared from mock or PMA induced HUT78, HPB-ALL, P30, and Raji cells. Electrophoretic mobility shift assays were performed using the NF-κB wild type (WT) binding site from the murine κ chain enhancer as a probe (25). The WT sequence bound a protein that was constitutively expressed in HUT78 (Fig. 3, lane 2) and Raji cells (lanes 8 and 10). This binding activity was inducible in HUT78 (lane 3) and HPB-ALL cells (lane 5), but as expected not further inducible in Raji cells (lanes 8 and 9; see reference 16). These binding activities were efficiently competed by WT cold competitor (lane 11), but not by a mutant (MUT) NF-κB site (lane 12) that differs at nucleotides that are crucial for NF-κB binding activity (25). Thus, NF-κB binding activity did not correlate with TNF-α mRNA expression (Fig. 1).

NF-κB binding activity is thought to be present or inducible in most if not all cell types (see reference 17 for review). The absence of NF-κB binding activity in P30 nuclear extracts treated or untreated with PMA under the conditions studied was therefore surprising (Fig. 3, lanes 6 and 7). It will be interesting to test whether other immature T cell lines of the same phenotype as P30 cells also display a lack of PMA-induced NF-κB binding activity. Such cell lines however are not yet available for study.

Taken together with the mRNA expression results discussed above, we conclude that NF-κB binding activity is not necessary for constitutive expression of TNF-α. P30 cells display no such binding activity (Fig. 3, lane 6), but express TNF-α mRNA constitutively (Fig. 1, lane 5). In addition, PMA inducible NF-κB binding activity is not sufficient for significant TNF-α mRNA expression. HPB-ALL cells display PMA inducible NF-κB binding activity (Fig. 3, lane 5), but only barely detectable TNF-α mRNA induction by PMA (Fig. 1, lane 4). However, the T cell type in which TNF-α gene transcription was most highly inducible, HUT78 (Fig. 1, lanes 1 and 2), displayed high levels of constitutive and inducible NF-κB binding activity (Fig. 3, lanes 2 and 3). Similarly, Raji cells constitutively express a high level of TNF-α mRNA that is further inducible by PMA (Fig. 1, lanes 9 and 10). Moreover, Raji cell nuclear extracts display high levels of constitutive NF-κB binding activity (Fig. 3, lanes 8 and 9).

Figure 2. The human TNF-α promoter contains three sites, κ1, κ2, and κ3 that specifically bind the transcription factor NF-κB. A diagram of the human TNF-α promoter and the location of κ1, κ2, and κ3. The rightward arrow indicates the site of initiation of transcription and the numbers indicate the number of nucleotides (nt) upstream (−) or downstream (+) from the site of transcriptional initiation. The nucleotide sequences of the sites are displayed below. The arrows indicate whether the site occurs on the coding (rightward) or noncoding (leftward) strand. Asterisks (*) note divergence from the consensus κB element as compiled by Lenardo and Baltimore (17).

Figure 3. Gel shift experiment with T cell nuclear extracts, using the chemically synthesized NF-κB wild type (WT) element as a probe. Assay mixtures contained either no extract (lane 1) or 5 μg of nuclear extract protein from unstimulated (−) or PMA induced (+) HUT78 (lanes 2 and 3), HPB-ALL (lanes 4 and 5), P30 (lanes 6 and 7), or Raji cells (lanes 8–12). NF-κB WT and mutant (MUT) oligonucleotides were used as probes. Binding assays were carried out as described in reference 20 and the NF-κB WT and HUT oligonucleotides are described in reference 25.
It was therefore possible that although NF-κB was not a primary mediator of TNF-α gene transcription in certain cell types, it could be in other cell types. We were therefore interested in determining whether in HUT78 and Raji cells, κ1-κ3 and NF-κB played a functional role in PMA induced TNF-α gene regulation.κ1, κ2, and κ3 Are Not Required for PMA Induction of Tumor Necrosis Factor (TNF)-α in HUT78 or Raji Cells. To determine the functional contribution of κ1, κ2, and κ3 to the transcriptional expression of TNF-α in HUT78 and Raji cells, 5' deletion TNF-α promoter CAT fusion constructs that progressively deleted κ1-κ3, were transfected into these cell types (Fig. 4 A and B). Deletion of each of the sites κ1-κ3 had little effect on the induction ratio of the promoter by PMA in either cell type (Fig. 4 A and B). Moreover, the −80 TNF-α CAT fusion, in which all three of the NF-κB binding sites have been deleted, is still inducible by PMA in both cell types (Fig. 4 A and B, lanes 9-12). Similarly, this −80 TNF-α construct was inducible in Jurkat cells, which also display NF-κB binding activity (data not shown). We conclude that the NF-κB binding sites from the TNF-α promoter, κ1-κ3, are not required for PMA induction of human TNF-α gene transcription.

Although κ1-κ3 are not required for PMA induction of TNF-α gene transcription, they and other TNF-α promoter sequences have transcriptional activities that vary with cell type. For example, maximal levels of constitutive and induced CAT activity are observed when −199 nt relative to the TNF-α mRNA cap site are present in HUT78 cells (Fig. 4 A, lanes 5-8). Similarly, maximal levels of induced CAT activity are observed when −199 nt of TNF-α 5' sequence are present in Raji cells (Fig. 4 B, lane 8). However, maximal constitutive levels in Raji cells require the sequences between −242 and −199 that contain κ2 (Fig. 4 B, lanes 5-8). Of interest, in HUT78 cells, the largest drop in CAT levels is observed when the sequences between −199 and −118 are deleted (Fig. 4 A, lanes 7-10). Deletion of these sequences has a smaller effect in Raji cells (Fig. 4 B, lanes 7-10). Another slight drop in induced CAT levels occurs in both HUT78 and Raji cells when the sequences containing κ3 are deleted, but the −80 TNF-α CAT fusion, is still highly inducible by PMA in both cell types (Fig. 4 A and B, lanes 9-12). Of note, three other putative NF-κB binding sites are located between −1045 and −608 nt relative to the TNF-α cap site. When a 1045 TNF-α CAT construct was compared to a −608 TNF-α CAT construct in the presence or absence of PMA, little difference in constitutive or induced CAT activities could be discerned (data not shown). Thus, PMA induction of TNF-α gene transcription, like virus and LPS induction, is not primarily mediated through NF-κB.

Multimers of κ1, κ2, κ3 and PRDII Confer Inducibility on the Heterologous β-globin Promoter in Raji but not in HUT78 Cells. To determine whether multimers of κ1, κ2, and κ3 were capable of acting like other PMA inducible NF-κB binding sites in vivo (see reference 17 for review), constructs containing multimers of these sequences upstream from a −128 β-globin promoter fused to the CAT reporter gene were transfected into Raji and HUT78 cells (Fig. 5 A and B). Surprisingly, multimers of κ1, κ2, and κ3 acted as PMA inducible elements when tested in Raji cells but not in HUT78 cells (Fig. 5 A and B, lanes 1-8). Cell type specific activity of these multimers had previously been reported (15). They increased basal transcription of the −128 β-globin promoter in each case in mouse fibroblast L cells but had no effect when tested in mouse monocyte P388D1 cells. Multimers of κ1, κ2, and κ3 were not capable of conferring virus or LPS inducibility on the −128 β-globin promoter in either L or P388D1 cells (15).

PRDII is a critical element involved in the virus induction of the IFN-β gene that has been shown to specifically bind to NF-κB and to mediate virus (26-28) and LPS induction (15). When four copies of PRDII are fused to either the −128 β- or −41 β-globin promoter, basal and PMA induced CAT activity is greatly increased in Raji cells (Fig. 5 A, lanes 1-8). Cell type specific activity of these multimers had previously been reported (15). They increased basal transcription of the −128 β-globin promoter in each case in mouse fibroblast L cells but had no effect when tested in mouse monocyte P388D1 cells. Multimers of κ1, κ2, and κ3 were not capable of conferring virus or LPS inducibility on the −128 β-globin promoter in either L or P388D1 cells (15).

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Transcriptional activities of multiple copies of K1, a2, a3, and PRDII on a heterologous promoter in lymphocyte cell lines. Autoradiogram shows results of CAT assays of extracts prepared from Raji (A) or HUT78 (B) cells transfected with the reporter genes diagrammed in the figure. Cells were transfected and either mock (-) or PMA (+) induced as described above. We note that both the -128 β-globin and -41 β-globin promoters were minimally inducible by PMA.

Multimers of K1, a2, and a3 Do not Increase Transcription when Fused to a Truncated Tumor Necrosis Factor (TNF)-α Promoter. By contrast to the -128 β-globin promoter, the truncated -61 TNF-α promoter is highly inducible by PMA in Raji cells (Fig. 5A and Fig. 6, lanes 1 and 2). In the context of the -61 TNF-α promoter however, multimers of K1-a3 had no effect on either constitutive or induced transcription (Fig. 6, lanes 1-8). This result is consistent with the deletion data presented above; deletion of each of the sites, K1-a3, had little effect on the gene's inducibility by PMA (Fig. 4B). Similarly, multimers of a1-3 fused to the -61 TNF-α promoter also did not augment transcription in HUT78 cells (data not shown). Promoter context has previously been shown to be an important variable in the function of the multimers K1-a3. In P388D1 cells for example, multimers of a2 augmented basal transcription of the -61 TNF-α promoter but not of the -128 β-globin promoter (15). Similarly, the influence of sequence context on the ability of NF-κB binding sites to act as inducible transcriptional activators has also been demonstrated. An NF-κB binding site required for PMA induction of IL-2R-α chain gene does not activate transcription from a heterologous promoter, and sequences immediately adjacent to this site are critical for the gene's induction by PMA (29). We conclude that the in vivo function of the NF-κB binding sites K1-a3 is dependent on the cell type and sequence context in which they are studied.

-52 nt 5’ to the Tumor Necrosis Factor (TNF)-α mRNA Cap Site Are Sufficient for PMA Induction in Raji and HUT78 Cells. To determine whether the minimal sequence requirements for PMA induction of TNF-α are identical to those required for virus or LPS induction of the gene, a -52 TNF-α promoter CAT fusion construct was transfected into HUT78 and Raji cells (Fig. 7A and B). This -52 construct was transfected into HUT78 (Fig. 7A and B, lanes 5 and 6). Deletion of the sequences between -80 and -52 nt caused a decrease in both constitutive and PMA induced CAT levels in both cell types (Fig. 7A and B, lanes 3 and 6). However, the decrease in induction ratio when these sequences were deleted was greater in Raji than in HUT78 cells (Fig. 7A and B, lanes 3-6).

By contrast, the -52 TNF-α construct was not inducible by virus or LPS when studied in L and P388D1 cells. Moreover, the minimal sequence requirements for virus induction of TNF-α gene transcription are cell type specific and differ in L cells where -61 nt are required and in P388D1 cells where -118 nt 5’ to the TNF-α mRNA cap site are required. LPS induction of the gene in P388D1 cells also requires -118 nt 5’ to the TNF-α mRNA cap site. Deletion of the sequences between -61 and -52 nt abolished virus inducibility in L cells and decreased the constitutive expression of the gene in P388D1 cells (15). We conclude that the minimal sequences

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Goldfeld et al. 77
Figure 7. -52 nt 5' to the TNF-α transcription start site are sufficient for PMA induction of the human TNF-α promoter in human lymphocyte cell lines. Autoradiogram shows results of CAT assays of extracts prepared from Raji (A) or HUT78 (B) cells transfected with a -80 TNF-α/CAT and -52 TNF-α/CAT constructs that are diagrammed in Fig. 4 and POCAT, a promoterless CAT construct. Cells were transfected and either mock (-) or PMA (+) induced as described above.

Discussion

Transient transfection of a 5' promoter deletion series of the human TNF-α gene has identified sequences necessary for maximal constitutive and PMA induced expression of the gene in a human B and T lymphocyte cell line. Although there are three sequences in the upstream flanking sequences of the human TNF-α gene that can specifically bind the transcription factor NF-κB in lymphoid nuclear extracts (15), these sequences are not required for PMA induction of the gene. Moreover, PMA inducibility of TNF-α mRNA is not correlated with NF-κB binding activities in various T and B cell lines. Taken together with results that demonstrate that κ1-κ3 are not required for virus and LPS induction, we conclude that TNF-α gene regulation is not primarily mediated by NF-κB. Thus, unlike other genes that are inducible by PMA, virus, or LPS (reviewed in reference 17, 30, and 31) that contain NF-κB binding sites, induction of human TNF-α gene transcription is not accomplished through these sites.

The human TNF-α gene 5' flanking sequences contain six regulatory regions that span the sequences from -600 to +89 nt relative to the mRNA transcription start site (Fig. 8). Region I spans the sequences between -52 and +89 and is sufficient for PMA induction of the gene in both Raji and HUT78 cells (Fig. 7 A and B). Potential regulatory domains in Region I include a purine rich region at -40 nt reminiscent of purine boxes active in Il2 gene transcription (32) and a region at -24 nt that shares some similarity with the multi-response element, a site involved in the phorbol ester, IL-1, TNF-α, and forskolin induction of the Il6 gene promoter (33). Region II includes the sequences between -61 and -52 and are required together with Region I to mediate virus induction of the gene in L cells (15). A putative Sp1 binding site at -54 nt overlaps Regions I and II (Fig. 8). Region III includes the sequences between -80 and -61. Deletion of Regions II and III together results in a decrease in constitutive and PMA induced promoter activity (Fig. 7 A and B). Deletion of these regions also results in a decrease in constitutive activity in P388D1 and L cells and the loss of virus inducibility in L cells (15).

Region IV includes the sequences between -118 and -80 nt and contains the κα3 site. Its deletion results in a slight decrease of PMA induced activity in both Raji and HUT78 cells with little effect on the induction ratio (Fig. 4 A and B). When studied in L cells, its deletion causes an approximately threefold decrease in virus induced mRNA levels, whereas in P388D1 cells there was a slight increase in constitutive activity in P388D1 cells, and LPS or virus induction of the gene could no longer be detected (15). Region V includes the sequences between -199 and -118 nt and its deletion results in an approximately two-fold decrease in both constitutive and PMA induced CAT activity in HUT78 cells (Fig. 4), but has negligible effect in Raji cells (Fig. 5). In both L and P388D1 cells, deletion of Region V results in an approximately three- to five-fold decrease in constitutive and virus and LPS induced activity (15). A second putative Sp1 binding site occurs in Region V at -160 nt (Fig. 8). Neither this site or the Sp1 site at -54 nt are necessary to detect induction of the TNF-α promoter by PMA, they may however contribute to levels of transcription. Region VI includes the sequences between -600 and -199 and contains κ1 and κ2. Sequences included in Region VI contribute to levels of constitutive activity in Raji cells (Fig. 4 B), L and
P388D1 cells (15), and to PMA induced levels in HUT78 (Fig. 4 A) and virus and LPS induced levels in P388D1 cells (15).

We conclude that Regions I-V (−199 to +89 nt relative to the TNF-α mRNA cap site) are sufficient for maximal induction ratio of TNF-α gene transcription resulting from PMA, virus, or LPS treatment in the various cell types tested. However, the minimal sequence requirements for induction of the gene by PMA, virus, and LPS differ. We were unable to compare the sequences necessary for virus and LPS induction of TNF-α to those required for PMA induction in HUT78 and Raji cells because we could not detect induction of the promoter CAT constructs by virus or LPS in these cell types (data not shown).

Previous studies have delineated sequences involved in PMA induction of the human TNF-α gene in other cell types (34, 35). In one of the studies, a 185 nt region spanning sequences from −295 to −110 nt was found to be required for PMA induction of the gene and a −101 TNF-α promoter CAT fusion was found not to be PMA inducible in K562 cells (34). This is in contrast to our findings and to those of another study in which the gene was analyzed in U937 cells (35). In the latter study, PMA induction of TNF-α was localized to a region between −95 and −36 nt upstream of the transcription start site (35). These differences suggest that sequence requirements for PMA induction of TNF-α may be cell type specific. We note that the sequence requirements for virus induction of TNF-α gene transcription are cell type specific (15). This may reflect cell type specific differences in the amounts or types of transcription factors that interact with the promoter in the different cell types.

Surprisingly, multimers of κ1-κ3, and PRDII, when fused to a heterologous promoter, can act as PMA inducible transcriptional activators in a B cell (Raji) but not a T cell line (HUT78) and therefore are active in a cell type specific manner. However κ1-κ3, when fused to a truncated TNF-α promoter, do not augment basal or PMA induced transcription in either cell type. Possibly, proteins that bind to sequences downstream of −61 nt in the TNF-α promoter and that are involved in PMA induction interfere with the binding of NF-κB to κ1-κ3. In this way these sequences and the transcription factors that interact with them could interfere with the ability of κ1-κ3 to act as PMA inducible κB enhancer elements. These observations emphasize the importance of sequence context and cell type in the determination of promoter element function.

It is also important to note that different genes may be activated by common regulatory elements and factors, but these components that are necessary may not be sufficient to activate transcription. For example, multiple copies of the PRDII element from the IFN-β promoter are activated by virus (21), LPS (15), and PMA (Fig. 5 A), presumably achieved at least in part through interactions with NF-κB (15, 26–28). However, the intact IFN-β promoter which only contains one copy of PRDII, responds to virus (21), but not to LPS (15) or PMA (3). The lack of LPS and PMA induction of the intact IFN-β gene is probably due to the requirement of synergistic interactions between PRDII and another promoter element and/or the involvement and release of negative control. Thus, the specificity of transcriptional activation of inducible genes in particular cell types is probably achieved by a unique combination of regulatory sequences and a corresponding unique set of binding activities that are activated by a particular inducer.

TNF-α activates three κB binding proteins in primary T cells (11), and acts via κB sequences to induce the expression of the IL-2R-κ, IL-2, and IL-6 genes (11, 29, 30). TNF-α has also been reported to autoinduce its own transcription in HL-60 cells (36); we were however unable to detect any effect of exogenously added TNF-α protein on TNF-α gene expression in the B and T cell lines studied here (data not shown). TNF-α also stimulates HIV-1 expression from latently infected cell lines either by direct activation of TNF-α responsive sequences within the viral genome (8) or via other mediators such as IL-6 (37), a potent B cell growth factor. Thus, TNF-α may be an important mediator of the polyclonal B cell expansion associated with AIDS and activation of HIV expression associated with the progression of disease. The understanding of TNF-α gene regulation and the role of TNF-α in the activation and differentiation of lymphocytes should therefore enhance our knowledge of both the mechanisms of HIV pathogenicity and of inducible gene expression.
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