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Human tumor necrosis factor α gene regulation by virus and lipopolysaccharide

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ABSTRACT **We have identified a region of the human tumor necrosis factor α (TNF-α) gene promoter that is necessary for maximal constitutive, virus-induced, and lipopolysaccharide (LPS)-induced transcription. This region contains three sites that match an NF-κB binding-site consensus sequence. We show that these three sites specifically bind NF-κB *in vitro*, yet each of these sites can be deleted from the TNF-α promoter with little effect on the induction of the gene by virus or LPS. Moreover, when multimers of these three sites are placed upstream from a truncated TNF-α promoter, or a heterologous promoter, an increase in the basal level of transcription is observed that is influenced by sequence context and cell type. However, these multimers are not sufficient for virus or LPS induction of either promoter. Thus, unlike other virus- and LPS-inducible promoters that contain NF-κB binding sites, these sites from the TNF-α promoter are neither required nor sufficient for virus or LPS induction. Comparison of the sequence requirements of virus induction of the human TNF-α gene in mouse L929 and P388D1 cells reveals significant differences, indicating that the sequence requirements for virus induction of the gene are cell type-specific. However, the sequences required for virus and LPS induction of the gene in a single cell type, P388D1, overlap.**

The human tumor necrosis factor α (TNF-α) gene encodes a protein with complex biological activities that include inhibition of viral infection and possible mediation of lipopolysaccharide (LPS)-induced septic shock (reviewed in ref. 1). TNF-α gene transcription is highly inducible by virus and LPS in certain cell types (reviewed in ref. 2), while in other cell types, virus alone induces the gene (3). Therefore, TNF-α gene regulation provides a model system for the study of inducible and tissue-specific gene regulation. Here we show that the cloned human TNF-α gene is appropriately regulated by virus and LPS when transiently introduced into cultured murine cells, and we identify promoter sequences that are required for constitutive, virus-induced, and LPS-induced transcription of the gene. We find that the sequences required for LPS and virus induction of TNF-α overlap. However, the minimal sequence requirements of TNF-α mRNA induction by virus are cell type-specific.

The 5′ flanking sequence of the TNF-α gene contains three sequences with strong similarity to NF-κB binding sites, which are located in regions of the TNF-α promoter that we show are required for maximal levels of constitutive, virus-induced, and LPS-induced transcription. We demonstrate that these sites, κ1, κ2, and κ3, specifically bind to NF-κB. The active form of the transcription factor NF-κB, which can be activated by virus and LPS, is thought to be involved in the transcriptional activation of numerous genes (reviewed in ref. 4). However, κ1, κ2, and κ3 can each be deleted from the TNF-α promoter with little effect on the induction of the gene by virus or LPS. Therefore, virus and LPS induction of the human TNF-α gene is not primarily mediated by NF-κB. We also show that sequences that lack NF-κB binding sites have strong effects on transcription of the gene. We conclude that TNF-α gene regulation is a complex process that involves multiple regulatory elements and transcription factors that are active in a cell type-specific manner.

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MATERIALS AND METHODS

Cell Culture and Transfection. Procedures for maintenance and transient transfections of L929 fibroblasts (L cells) (5) and P388D1 monocytic cells (6) were as described. Sendai virus and LPS inductions (3) were respectively 8 and 2 hr long, for RNA analysis or nuclear extract preparation, or 12 and 8 hr, for bacterial chloramphenicol acetyltransferase (CAT) assays. CAT constructs were cotransfected with a β-galactosidase (β-gal) expression plasmid and the amount of CAT protein assayed was normalized to β-gal activity (7).

RNA Analysis, Subcellular Fractionation, and Mobility-Shift Electrophoresis. RNA preparation and RNase protection assays (3), nuclear extract preparation (8), and binding assays (9) were as described.

Plasmids. The 5′ TNF-α deletion series (−608, −106, −87, and −61 TNF-αM) was constructed by isolating the TNF-α gene from PLT (a gift from David Goeddel, Genentech) and subcloning it into pSP72 (10); 5′ deletion endpoints were generated by appropriate enzyme digestions. The −600, −576, −242, −199, −118, −80, and −52 TNF-α promoter/CAT gene constructs were prepared by exonuclease III digestion of the −608 TNF-αM construct (11) and subcloning these promoter fragments into the *Hin*cII site of POCAT (12). Plasmids p−128βCAT (−128β) and p−128PII4r [−128β(P)4] have been described (7). p−61TNF-αCAT was made by removing the *Bgl* II–*Cla* I fragment containing the β-globin promoter from −128β and replacing it with a −61TNF-α promoter fragment. p−128β(κ1)3, p−128β(κ2)4, p−128β(κ3)6, p−61TNF-α(κ1)3, p−61TNF-α(κ2)3, and p−61TNF-α(κ3)6 are diagrammed in Fig. 4*B*. The sequences of PRDII×2, κ1, κ2, and κ3 are in the legend to Fig. 2.

RESULTS

DNA Sequences Required for Virus Induction of the TNF-α Gene in L Cells. To determine the minimal sequences necessary for the constitutive and virus-induced expression of the human TNF-α gene, a 5′ deletion series was constructed with deletion endpoints at nucleotides (nt) −608, −106, −87, and −61 and extending approximately to nt +3500 relative to the TNF-α mRNA cap site. The deletion constructs were tested by transient transfection into L cells. RNase protection assays were performed to measure accurately initiated

Abbreviations: TNF-α, tumor necrosis factor α; IFN-β, interferon β; CAT, chloramphenicol acetyltransferase; IL2-rα, interleukin 2 receptor α chain; β-gal, β-galactosidase; LPS, lipopolysaccharide; nt, nucleotide(s).

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TNF-α mRNA before and after virus induction. When nt −608 to −107 were deleted, there was a significant decrease in the virus-induced level of TNF-α mRNA, and the constitutive mRNA level was no longer detectable (Fig. 1*A*, lanes 3 and 4). The virus-induced level of TNF-α mRNA fell again, by a factor of ≈3, when nt −106 to −88 were deleted (lanes 5 and 6). When nt −87 to −62 were deleted, the TNF-α gene was still inducible and there was no further drop in the induced mRNA level (lanes 7 and 8). Therefore, 61 nt 5′ to the TNF-α mRNA cap site are sufficient for virus induction.

Mouse L cells selected for resistance to the killing effects of TNF-α constitutively express small amounts of TNF-α protein (14). However, virus induction of TNF-α mRNA in these cells has not been reported. We found that the endogenous murine TNF-α gene was constitutively transcribed and highly inducible by virus in L cells (Fig. 1*B*, lanes 3 and 4). Furthermore, murine TNF-α and interferon β (IFN-β) mRNA were coinduced by Sendai virus in L cells (lanes 2 and 4). Neither the endogenous nor the transfected TNF-α gene was induced by LPS in L cells (data not shown). Thus, the regulation of the transfected human TNF-α gene in mouse L cells is indistinguishable from that of the endogenous gene.

NF-κB Binding-Site Consensus Sequences κ1, κ2, and κ3 Bind NF-κB. Inspection of the upstream sequence necessary for maximal induction of the human TNF-α gene by virus revealed three putative NF-κB binding-site consensus sequences, κ1 (−587 to −577), κ2 (−210 to −202), and κ3 (−98 to −87). Of note, we have detected numerous sequence differences in the human TNF-α gene promoter (data not shown) from the published sequences (15 and 16). To determine whether κ1, κ2, and κ3 specifically bind NF-κB, we carried out electrophoretic mobility-shift assays (17) with nuclear extracts from the mature-B-cell line Namalwa, which expresses TNF-α mRNA when induced by virus (3). As shown in Fig. 2 *A–C*, κ1, κ2, and κ3 probes all bound a virus-inducible protein (*A*, lane 6; *B*, lane 3; *C*, lane 3), and in each case binding was blocked by unlabeled probe competitor and by the NF-κB wild-type sequence (*A*, lanes 7 and 8; *B*, lanes 4 and 7; *C*, lanes 4 and 9) but not by a mutant NF-κB sequence (lanes 9, 8, and 9 of *A–C*, respectively). Binding of the labeled probes was also blocked by the IFN-β promoter element PRDII (Fig. 2 *A*, lane 13, *B*, lane 6, and *C*, lane 7), which binds NF-κB specifically and is functionally interchangeable with the κB sequence (9, 18, 19). We conclude that κ1, κ2, and κ3 can specifically bind NF-κB. Although κ3 is a good competitor for the binding activities of κ2 and κ1 (Fig. 2 *B* and *C*, lanes 5), κ2 competes poorly with κ1 (Fig. 2*C*, lane 6). κ3 and PRDII (but not κ1, κ2, or wild-type κB) bind another protein that is constitutively expressed, which we designate TNF-BF1 (Fig. 2*A*).

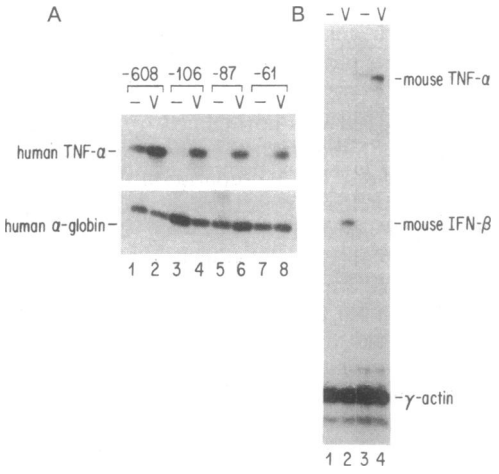

FIG. 1. Virus induction of TNF-α gene expression in mouse L cells. (*A*) Virus induction of transfected human TNF-α gene 5′-deletion constructs. L cells were transiently transfected and either mock-induced (−) or virus-induced (V). Quantitative RNase mapping of TNF-α and α-globin mRNAs was carried out with 20 μg of total cellular RNA. The probe T7TNFMBam (3) was used to measure human TNF-α levels, and the SP6α-globin probe (7) was used to measure the levels of α-globin mRNA produced from a cotransfected human α-globin gene used as an internal control for transfection efficiencies and for RNA recovery. Positions of the TNF-α and α-globin protected fragments are indicated at left. The upper portion is a longer radiographic exposure than the lower portion. (*B*) Virus induction of endogenous mouse TNF-α and IFN-β mRNAs in L cells that were either mock-induced (−) or virus-induced (V). Quantitative RNase mapping of endogenous TNF-α and IFN-β mRNAs was carried out using a ^{32}P-labeled mouse TNF-α (a gift from Bruce Beutler, University of Texas) or a mouse IFN-β (13) RNA probe. An RNA probe complementary to the γ-actin gene was used as an internal control for mRNA levels (3). Prior to *in vitro* transcription with T7 RNA polymerase, the mouse TNF-α probe was linearized with *Nci* I, resulting in a probe that protects a 231-nt fragment, and the mouse IFN-β probe was linearized with *Sca* I to produce a 141-nt protected fragment. Five micrograms of total cellular RNA from each sample was analyzed with either the mouse IFN-β and γ-actin probes (lanes 1 and 2) or the mouse TNF-α and γ-actin probes (lanes 3 and 4). The γ-actin probe was made to have a specific activity one-fifth that of the mouse TNF-α and mouse IFN-β probes. Positions of the mouse TNF-α, IFN-β, and γ-actin protected fragments are indicated at right.

Deletion of κ1–κ3 Has Little Effect on Human TNF-α Gene Induction by Virus. κ1 and κ2 lie between nt −608 and −106, and deletion of this region abolishes constitutive mRNA levels and reduces induced TNF-α mRNA levels (Fig. 1*A*). Therefore, it was possible that although they were not necessary for virus induction, κ1 and/or κ2 played a significant role in constitutive and induced expression of TNF-α mRNA. To test this possibility, 5′ deletion endpoints were constructed at −600 and −576 (deleting κ1), −242 and −199 (deleting κ2), and −118 and −80 (deleting κ3) and fused to the CAT gene (Fig. 3). We also tested a construct with a 5′ endpoint at nt −52 relative to the start site of TNF-α transcription in an attempt to define the minimal sequence necessary for induction. The induction ratios of these constructs were much lower than the induction ratios of entire gene constructs transfected into L cells and analyzed by RNase protection (Fig. 1*A*).

Surprisingly, the deletion of each of these sites had little effect on the viral inducibility of the TNF-α promoter. For example, deletion of κ1 resulted in a slight decrease in constitutive activity (Fig. 3, lanes 1–4) and deletion of κ2 increased the induced level (lanes 5–8), so that the induction ratio of the gene was enhanced by the deletion of κ1 and κ2. Deletion of nt −199 to −118, which do not contain an NF-κB binding site, resulted in a large drop in CAT activity, but the promoter was still inducible (lanes 9 and 10). The only recognizable motif in these sequences is a G+C-rich region at −163 that corresponds to an Sp1 consensus binding site. We conclude that in L cells, maximal constitutive CAT activity requires up to nt −600 and that maximal induced CAT activity requires up to nt −199 relative to the transcription start site. Consistent with the results in Fig. 1*A*, deletion of nt −118 to −81 (including κ3) resulted in a decrease in both induced and constitutive CAT activity (Fig. 3, lanes 11 and 12). When sequences 5′ to −52 were deleted, removing the first 2 nt of another Sp1-like motif, no inducible CAT activity was detected (lanes 15 and 16). Taken together with our observation that −61 nt are sufficient for virus induction, this shows that the minimal sequence required for virus induction of TNF-α in L cells is between −61 and −52. Moreover, κ1–κ3 do not play a significant role in virus induction of the human TNF-α gene promoter in L cells.

Multimers of κ1–κ3 Increase Basal Transcription Levels in L Cells but Do Not Respond to Virus or LPS Induction. To

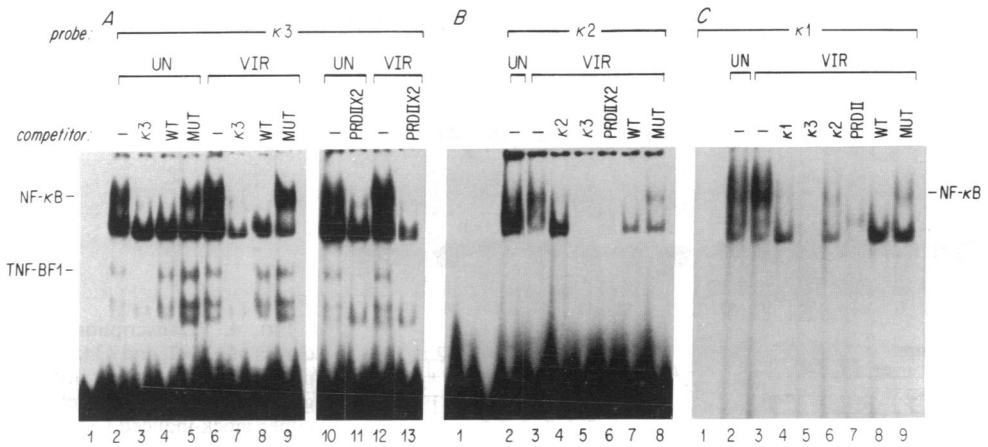

FIG. 2. Gel shift experiments with Namalwa cell nuclear extracts, using chemically synthesized κ1, κ2, and κ3 oligonucleotides as probes. (*A*) Experiments using the κ3 probe. Assay mixtures contained no extract (lane 1) or 5 μg of nuclear extract protein from unstimulated (UN, lanes 2–5, 10, and 11) or virus-induced (VIR, lanes 6–9, 12, and 13) Namalwa cells. Namalwa cells were maintained as described (3). Competitors (100 ng) were the κ3 oligonucleotide (5′-GATCCGAGCTCATGGGTTTCTCCACA-3′) (lanes 2 and 7), the NF-κB wild-type (WT) oligonucleotide (5′-TCGACAGAGGGGACTTTCCGAGAGGC-3′; ref. 6) (lanes 4 and 8), the NF-κB mutant (MUT) oligonucleotide (5′-TCGACAGAATTCACTTTCCGAGAGGC-3′; ref. 6) (lanes 5 and 9), and PRDII×2 (a dimerized PRDII sequence, 5′-GATCTGTGGGAAATTCCGTGGGAAATTCCGGATC-3′) (lanes 11 and 13). (*B*) Experiments using the κ2 probe. Assay mixtures contained no extract (lane 1) or unstimulated (lane 2) or virus-induced (lanes 3–8) Namalwa cell nuclear extract (5 μg). Competitors (100 ng) were the κ2 oligonucleotide (5′-GATCCGGGGTATCCA-3′) (lane 4), κ3 (lane 5), PRDII×2 (lane 6), NF-κB WT (lane 7), and NF-κB MUT (lane 8). (*C*) Experiments using the κ1 probe. Assay mixtures contained no extract (lane 1) or unstimulated (lane 2) or virus-induced (lanes 3–9) Namalwa cell nuclear extract (5 μg). Competitors (100 ng) were the κ1 oligonucleotide (5′-GATCCTGGGACAGCCCA-3′) (lane 4), κ2 (lane 5), κ3 (lane 6), PRDII×2 (lane 7), NF-κB WT (lane 8), and NF-κB MUT (lane 9).

determine the transcriptional activities of isolated copies of κ1, κ2, or κ3 and to test whether in this form they act like other NF-κB binding sites and mediate virus induction, we placed multimers of these sequences upstream of an uninducible −128 β-globin promoter fused to the CAT reporter gene. As a positive control for virus induction, we included a −128 β-globin construct with four copies of PRDII (7). To test whether κ1, κ2, or κ3 could act synergistically with sequences contained in the TNF-α promoter, we placed multimers of these sites upstream of the minimally inducible −61 TNF-α CAT construct (Fig. 4*B*).

Multimers of κ1, κ2, or κ3 did not confer virus inducibility on the heterologous −128 β-globin promoter or truncated TNF-α promoter in L cells (Fig. 4*A*). In both cases all three sites enhanced basal levels of CAT activity and did not augment the induction ratio, and the effect of κ2 was greater than that of κ1 or κ3. Moreover, the transcriptional activity of the multimers fused to the −61 TNF-α promoter was greater than their activity on the −128 β-globin promoter in every case. Therefore, κ1–κ3 are not capable of mediating virus induction in L cells, but they do increase basal levels of transcription in a sequence context-specific manner. The failure of NF-κB binding sites to act as inducible enhancers is not unprecedented. Multiple copies of a site from the interleukin 2 receptor α-chain (IL2-rα) gene failed to mediate phorbol ester induction of a heterologous promoter (20). In addition, sequences immediately adjacent to this site are critical for induction of the gene (21). However, in contrast to the TNF-α gene, this NF-κB binding-site sequence is required for induction of the IL2-rα gene in its normal promoter context.

DNA Sequences Required for LPS Induction of TNF-α. To determine the DNA sequence requirements for LPS induction of the TNF-α gene, we transfected the deletion constructs described above into a murine monocyte cell line (Fig. 5*A*) that produces TNF-α mRNA in response to LPS as well as virus stimulation (Fig. 5*B*). This cell line, P388D1, produces a high level of murine TNF-α mRNA constitutively and can be further induced by virus and to a lesser degree by LPS (Fig. 5*B*, lanes 1–3). As in L cells, murine IFN-β mRNA was coinduced with TNF-α mRNA by virus (Fig. 5*B*, lanes 2 and 4) but not by LPS (lanes 3 and 6) in P388D1 cells.

RNase protection analysis revealed maximal constitutive and LPS-induced expression of accurately initiated human TNF-α mRNA when −608 nt relative to the mRNA cap site were present (Fig. 5*A*, lanes 1 and 2). When nt −608 to −107 were deleted, neither constitutive nor LPS-induced transcripts were detected (lanes 3 and 4). These sequences are also required to detect virus induction of human TNF-α mRNA in P388D1 cells (lanes 13 and 14). Therefore, the

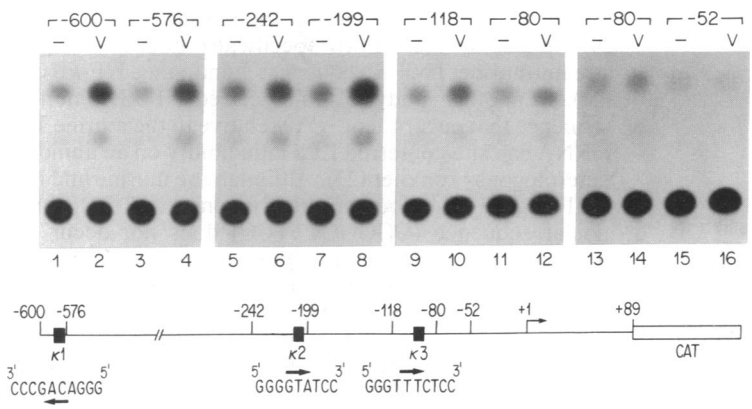

FIG. 3. Virus induction of human TNF-α promoter/CAT gene fusions in L cells. Autoradiogram shows results of CAT assays of extracts prepared from L cells transfected with 5′-deletion constructs of the human TNF-α/CAT fusion reporter gene (diagram at bottom). Arrows indicate the orientation of the binding sites relative to the direction of transcription. Cells were transfected and mock-induced (−) or induced with virus (V) as for Fig. 1*A*, and β-gal and CAT assays were performed as described in *Materials and Methods*. Lanes 1–12 and lanes 13–16 were from two independent transfections.

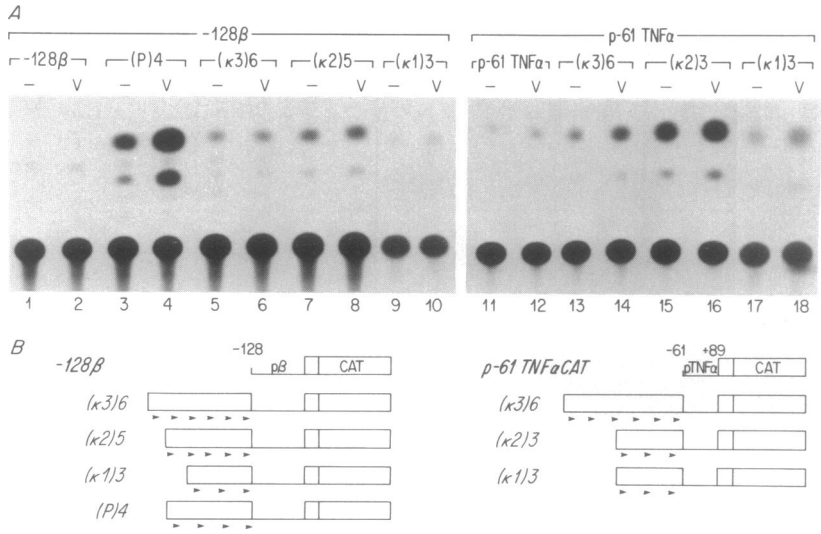

FIG. 4. Transcriptional activities of multiple copies of κ1, κ2, and κ3 (*A*) CAT assays of extracts prepared from L cells transfected with the reporter genes diagramed in *B*. Cells were transfected and either mock-induced (−) or virus-induced (V). (*B*) Reporter genes containing multiple copies of κ1, κ2, κ3, or PRDII×2. Sequences of these oligomers are detailed in Fig. 2 legend. The κ3 oligomer contains nt −106 to −87 relative to the TNF-α cap site.

minimal sequence requirements for detection of virus-induced TNF-α transcripts differ in L and P388D1 cells and thus are cell type-specific. We also conclude that the sequence requirements for LPS and virus induction in a single cell type, P388D1, overlap.

κ1 and κ2 Are Not Required for Virus or LPS Induction of TNF-α. To test whether κ1, κ2, or κ3 plays a role in LPS or virus induction of TNF-α in P388D1 cells, we transfected the 5′-deletion TNF-α/CAT constructs into these cells. The CAT accumulation assay permitted detection of transcriptional activities not detectable by RNase mapping. Deletion of κ1 or κ2 did not abolish virus or LPS induction or greatly affect CAT activity (Fig. 6, lanes 1–12). However, similar to our findings in L cells, deletion of nt −199 to −119 resulted in a significant drop in CAT activity and the promoter was still inducible (lanes 13–15). Therefore, these sequences contain an upstream promoter element that is active in both cell types. Unexpectedly, deletion of nt −118 to −80, which deletes κ3, resulted in a relative increase of constitutive CAT activity (lanes 13 and 16), and induction by virus or LPS was no longer detectable. We conclude that the sequences required for maximal transcription of the TNF-α gene in P388D1 cells are within −242 nt of the gene's transcription start site and that −118 nt are required to detect minimal induction of the gene. As in L cells, κ1–κ3 do not play a significant role in virus and LPS induction of the human TNF-α gene in P388D1 cells nor are sequences 3′ to +89 nt necessary for the expression of the gene. Furthermore, multimers of κ1–κ3 are not capable of conferring virus or LPS inducibility in P388D1 cells (Fig. 7). Although all three elements, κ1–κ3, have transcriptional activity when fused to test promoters in L cells (Fig. 4), only κ2 has transcriptional activity in P388D1 cells and only on the parental TNF-α promoter (Fig. 7, lanes 22–24). Even though the endogenous murine IFN-β gene is not inducible by LPS in P388D1 cells (Fig. 5*C*, lane 6), multimers of the PRDII sequence confer LPS as well as virus inducibility on the −128 β-globin promoter (Fig. 7, lanes 4–6). We conclude that the transcriptional activity of multimers of κ1, κ2, and κ3 is both cell type-specific and dependent on sequence context and that they are not capable of mediating LPS or virus induction in P388D1 cells.

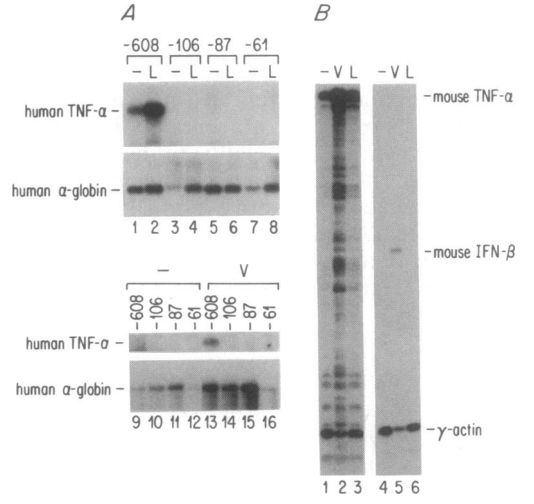

FIG. 5. LPS and virus induction of TNF-α gene expression in mouse P388D1 cells. (*A*) LPS and virus induction of transfected human TNF-α gene 5′-deletion constructs. P388D1 cells were transiently transfected and were either mock-induced or (−) LPS- (L) or virus- (V) induced. RNA was prepared and analyzed as for Fig. 1*A*. (*B*) Virus and LPS induction of endogenous mouse TNF-α and IFN-β mRNAs in P388D1 cells. Quantitative RNase mapping of RNA isolated from P388D1 cell was done as for Fig. 1*B*.

DISCUSSION

NF-κB binding activity is inducible by both virus and LPS, and NF-κB binding sites are involved in the regulation of numerous genes (4, 22–24). We were therefore surprised to find that although the three sites from the TNF-α gene, κ1–κ3, specifically bind NF-κB and increase basal levels of transcription from test promoters, they do not mediate virus or LPS induction of the gene. Of note, these sites are also not required for induction of TNF-α by phorbol 12-myristate 13-acetate (unpublished data).

Recent studies, however, have proposed a significant role for NF-κB in the regulation of the murine TNF-α gene (23, 24). In one study, NF-κB specifically bound multiple upstream murine TNF-α sequences containing NF-κB motifs (24). In another study, a multimerized NF-κB binding site sequence that occurs at nt −510 relative to the murine TNF-α mRNA cap site conferred LPS inducibility on an uninducible heterologous promoter (23). Although the murine and human TNF-α gene 5′ regions are highly conserved (23), this particular sequence (GGGGCTTTCC) does not occur in the human sequence. Therefore, other conserved sequences may be involved in LPS inducibility of the two genes, or the relative contributions of different regulatory elements could differ in the mouse and human TNF-α genes.

We have demonstrated that the sequences required for virus induction of TNF-α are cell type-specific. Cell type-

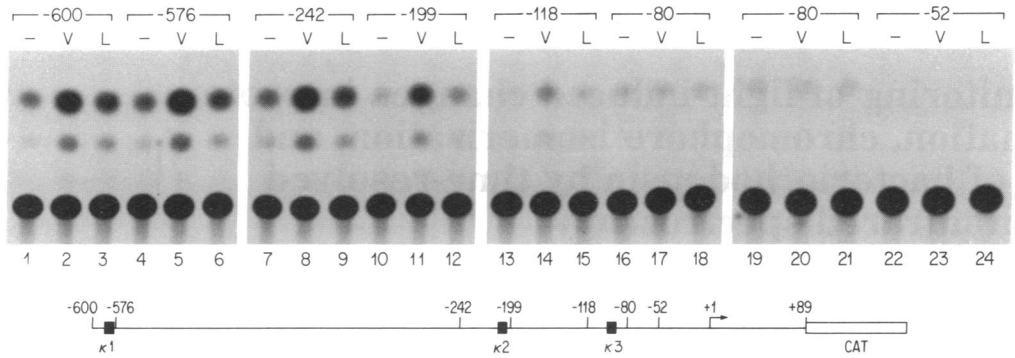

FIG. 6. Virus and LPS induction of human TNF-α promoter/CAT gene fusions in P388D1 cells. Cells were transfected with 5'-deletion constructs (diagram at bottom) and mock-induced (−) or induced with virus (V) or LPS (L). Extracts were analyzed for β-gal and CAT activity. Lanes 1–18 and 19–24 were from two independent transfections.

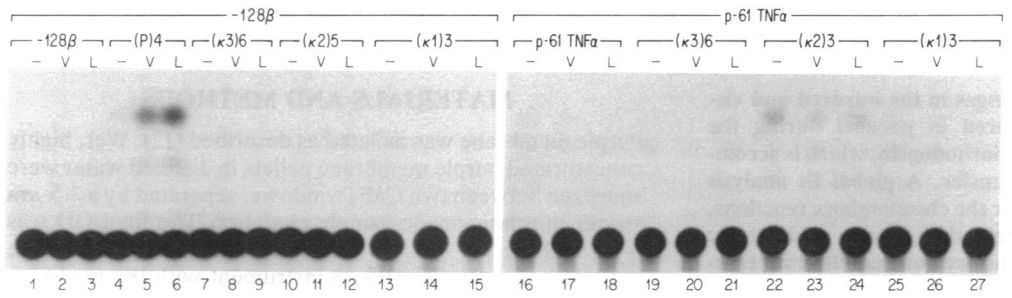

FIG. 7. Transcriptional activities of multiple copies of κ1, κ2, and κ3. P388D1 cells transfected with the reporter genes diagramed in Fig. 4*B* were either mock-induced (−) or virus- (V) or LPS- (L) induced, and β-gal and CAT assays were performed.

specific differences in the sequence requirements for virus induction of the human IFN-β gene have also been reported (reviewed in refs. 25 and 26). These differences are likely to reflect cell type-specific differences in the types or amounts of transcription factors that interact with the TNF-α or IFN-β promoters. We previously observed that the TNF-α and IFN-β genes are coinduced by virus (3). Detailed analysis of the human IFN-β gene promoter has revealed that a crucial element involved in virus induction, PRDII, binds to NF-κB and acts as a virus-inducible element (9, 18, 19). Here we demonstrate that PRDII is also LPS-inducible. We were therefore surprised that κ1, κ2, or κ3 is not functionally interchangeable with PRDII. Virus induction of the IFN-β gene also requires other promoter elements (25, 26). The region between nt −61 and −52, which we demonstrated is required for virus induction of the TNF-α gene in L cells, contains on its negative strand the sequence GAAAGA, which is reminiscent of the GAAANN sequence that occurs in multiple copies in PRDI and in the IFN-α promoter (5, 27). Other similarities to PRDI or PRDIII do not appear in the TNF-α promoter. We conclude that coordinate viral induction of TNF-α and IFN-β mRNA is probably accomplished through a unique constellation of transcription factors and regulatory domains in each case.

In conclusion, although our results argue against a major role for NF-κB in the virus and LPS induction of the human TNF-α gene, they have failed to identify discrete regulatory elements that do mediate these processes. Our data indicate that the cell type- and inducer-specific transcriptional regulation of the human TNF-α gene involves multiple promoter regions and factors that act in a cell type-specific manner. Moreover, these studies emphasize the difficulty of predicting the *in vivo* transcriptional regulatory properties of promoter elements from their *in vitro* binding activities.

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1. De Maeyer, E. & De Maeyer-Guignard, J. (1988) *Interferons and Other Regulatory Cytokines* (Wiley, New York).
2. Goeddel, D. V., Aggarwal, B. B., Gray, P. W., Leung, D. W., Nedwin, G. E., Palladino, M. A., Patton, J. S., Pennica, D., Shepard, H. M., Sugarman, B. J. & Wong, G. (1986) *Cold Spring Harbor Symp. Quant. Biol.* **60**, 597–609.
3. Goldfeld, A. E. & Maniatis, T. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 1490–1494.
4. Lenardo, M. J. & Baltimore, D. (1989) *Cell* **58**, 227–229.
5. Kuhl, D., de la Fuente, J., Chaturvedi, M., Parimoo, S., Ryals, J., Meyer, F. & Weissman, C. (1987) *Cell* **50**, 1057–1069.
6. Pierce, J. W., Lenardo, M. J. & Baltimore, D. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 1482–1486.
7. Fan, C.-M. & Maniatis, T. (1989) *EMBO J.* **8**, 101–110.
8. Dignam, J. D., Lebovitz, R. M. & Roeder, R. G. (1983) *Nucleic Acids Res.* **11**, 475–489.
9. Lenardo, M. J., Fan, C.-M., Maniatis, T. & Baltimore, D. (1989) *Cell* **57**, 287–294.
10. Krieg, P. & Melton, D. (1987) *Methods Enzymol.* **155**, 397–415.
11. Sambrook, J., Fritsch, E. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
12. Prost, E. & Moore, D. (1986) *Gene* **45**, 107–111.
13. Higashi, Y., Sokawa, Y., Watanabe, Y., Kawade, Y., Ohno, S., Takaoga, E. & Taniguchi, T. (1983) *J. Biol. Chem.* **258**, 9522–9529.
14. Rubin, B. Y., Anderson, S. L., Sullivan, S. A., Williamson, B. D., Carswell, E. A. & Old, L. (1986) *J. Exp. Med.* **164**, 1350–1355.
15. Nedwin, G. E., Naylor, S. L., Sakaguchi, A. Y., Smith, D., Jarrett-Nedwin, J., Pennica, D., Goeddel, D. V. & Gray, P. W. (1985) *Nucleic Acids Res.* **13**, 6361–6373.
16. Nedosparov, S. A., Shakhov, A. N., Turetskaya, V. A., Mett, M. M., Azizov, G. P., Georgiev, V. G., Korobko, V. N., Dobrynin, S. A., Fillipov, N. S., Bystrov, E. F., Boldyreva, E. F., Chuvpilo, S. A., Chumakov, A. M., Shingarova, L. N. & Ovchinnikov, Y. A. (1986) *Cold Spring Harbor Symp. Quant. Biol.* **511**, 611–624.
17. Fried, M. & Crothers, D. M. (1981) *Nucleic Acids Res.* **9**, 6505–6525.
18. Visnavathan, K. V. & Goodbourn, S. (1989) *EMBO J.* **8**, 1129–1138.
19. Hiscott, J., Alper, D., Cohen, L., Leblanc, J. F., Sportza, L., Wong, A. & Xanthoudakis, S. (1989) *J. Virol.* **63**, 2557–2566.
20. Cross, S. L., Halden, N. F., Lenardo, M. J. & Leonard, W. J. (1989) *Science* **244**, 466–469.
21. Lowenthal, J. W., Ballard, D. W., Bohnlein, E. & Greene, W. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 2331–2335.
22. Liebermann, T. & Baltimore, D. (1990) *Mol. Cell. Biol.* **5**, 2327–2334.
23. Shakov, A. N., Collart, M. A., Vassali, P., Nedosparov, S. A. & Jongeneel, C. V. (1990) *J. Exp. Med.* **171**, 35–37.
24. Collart, M. A., Bauerle, P. & Vassali, P. (1990) *Mol. Cell. Biol.* **4**, 1498–1506.
25. Maniatis, T. (1988) *Harvey Lect.* **82**, 71–104.
26. Taniguchi, T. (1988) *Annu. Rev. Immunol.* **6**, 439–464.
27. MacDonald, N. J., Kuhl, D., Maguire, D., Naf, D., Gallant, P., Goswamy, A., Hug, H., Bueler, H., Chaturvedi, M., de la Fuente, J., Ruffner, H., Meyer, F. & Weissman, C. (1990) *Cell* **60**, 767–779.