# Reports

## Induction of κ Transcription by Interferon-γ Without Activation of NF-κB

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**The induction of immunoglobulin κ light chain expression in 70Z/3 pre-B cells treated with bacterial lipopolysaccharide (LPS) requires the activation of the B cell–specific factor NF-κB, which binds to the κ enhancer motif, GGGACTTTCC. This sequence alone can function as a tissue-specific enhancer for LPS-induced gene expression. A potent inhibitor of B lymphopoiesis [transforming growth factor–β (TGF-β)] was used to explore the mechanisms in the activation of κ transcription by LPS and by interferon-γ (IFN-γ). TGF-β inhibited LPS-induced κ transcription but not the activation and in vitro binding of NF-κB. This indicates that NF-κB activation, while necessary, is not sufficient for LPS-induced κ transcription. TGF-β had no effect on IFN-γ–induced κ transcription, and NF-κB was not activated by IFN-γ. These results reveal that LPS and IFN-γ activate transcription through different mechanisms.**

THE 70Z/3 CELL LINE REPRESENTS A useful system for studying the events in the development of pre-B cells into early B cells. These cells express κ light chains and produce surface immunoglobulin M in response to a variety of mitogens and lymphokines. The induction of κ light chain expression in 70Z/3 pre-B cells treated with lipopolysaccharide (LPS) is associated with the activation of the B cell–specific DNA binding factor, NF-κB, which interacts with the κB motif in the κ enhancer (*1*). Alterations in this sequence abolish κ enhancer function, which indicates that the κB motif is essential for enhancer activity (*2*). This κB sequence alone can function as an enhancer for B cell–specific transcription and LPS induction (*3*).

Activation of NF-κB binding is induced by LPS in the presence of protein synthesis inhibitors and by phorbol esters [such as phorbol myristate acetate (PMA)], which suggests that this factor is activated through posttranslational mechanisms, possibly involving protein kinase C (*4*). Recently, it has been reported that active NF-κB can be generated by treatment of cytoplasmic extracts from uninduced 70Z/3 cells with dissociating agents that presumably release inhibitors blocking NF-κB DNA binding activity (*5*). Phorbol esters also release the inhibitors of binding and promote NF-κB translocation to the nucleus (*5*). It is unclear if other processes beyond the activation and nuclear translocation of NF-κB binding activity are required for the induction of κ transcription.

In analyzing the mechanisms controlling the activation of κ transcription, we recently discovered that TGF-β selectively inhibited the induction of κ expression by LPS, interleukin 1, or NZB serum factor, but not induction by interferon-γ (IFN-γ) (*6*). To distinguish whether TGF-β affected κ transcription or acted at another point in the production of κ chains, we analyzed the effect of TGF-β on the induction of κ transcription in run-on transcription assays with nuclei prepared from LPS-induced 70Z/3 cells. TGF-β inhibited the LPS-induced increase in κ transcription by 65% (Fig. 1). This approximates the reduction in κ mRNA previously observed (*6*), suggesting that TGF-β directly affects κ gene transcription. TGF-β had no effect on IFN-γ–induced κ transcription in 70Z/3 pre-B cells (Fig. 1). This suggests that IFN-γ and LPS (as well as the other inducers whose activity is inhibited by TGF-β) activate κ transcription through different molecular mechanisms.

Because of the reported requirement for NF-κB activity in LPS-induced κ transcription (*3, 4*), we examined the activity of NF-κB nuclear extracts from nuclei of 70Z/3 cells induced with LPS in the presence or absence of TGF-β. NF-κB binding to a restriction fragment of the κ enhancer (containing the complete κB motif) was analyzed by gel retardation assays (*1*). TGF-β had no detectable effect on NF-κB activation and binding was seen even after a 24-hour treatment (Fig. 2), whereas κ mRNA induction by LPS was substantially inhibited (*6*).

Indistinguishable electrophoretic mobilities were seen for NF-κB DNA-factor complexes obtained from either TGF-β–treated or untreated LPS-induced 70Z/3 cells. The DNA-factor complexes from both cell extracts were abolished by competitor NF-κB DNA (Fig. 2). The DNA factor complexes from TGF-β–treated or untreated cells also gave identical NF-κB binding sites when analyzed by gel footprints (*7*) with the chemical nucleases 1,10-phenanthroline-copper (OP-Cu) (*8*) and methidiumpropyl-EDTA-Fe(II) (MPE) (*9*). These findings indicate that TGF-β does not affect the activation or binding specificity of NF-κB.

Since κ induction by IFN-γ is not inhibited by TGF-β (*6*) (Fig. 1), it seemed likely that NF-κB might not be induced by this lymphokine. The induction of κ transcription by IFN-γ exhibits similar kinetics to those reported for LPS, resulting in a 10- to 20-fold increase after 24 hours (Fig. 3). The activation of NF-κB by IFN-γ was assayed in gel retardation assays as described in Fig. 2. During the 24-hour induction, in which κ transcription was stimulated 16-fold by IFN-γ (Fig. 3), no demonstrable NF-κB activation was observed (Fig. 4). This con-

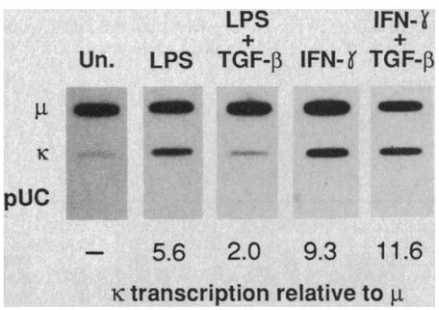


**Fig. 1.** Effect of TGF-β on LPS or IFN-γ induction of κ transcription. The μ, κ, or pUC gene probes were immobilized on nitrocellulose and hybridized with labeled RNA from nuclear transcription assays. Preparation of nuclei and transcription reactions were carried out as previously described (*21*). DNA (3 μg, an amount experimentally determined to be in excess of the RNA hybridization input) was hybridized to labeled RNA ($5 \times 10^6$ cpm/ml) for 48 hours at 65°C. Hybridization intensities were determined by densitometric scanning with an LKB Ultrascan. Levels of κ were determined by normalization of κ intensities to values of μ. (Neither LPS nor IFN-γ have any effect on μ mRNA levels.) The 70Z/3 cells were treated for 24 hours as follows: lane 1, uninduced; lane 2, LPS at 10 μg/ml; lane 3, LPS at 10 μg/ml and TGF-β at 2 U/ml; lane 4, IFN-γ at 50 U/ml; and lane 5, IFN-γ at 50 U/ml and TGF-β at 2 U/ml.

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firms that IFN-γ induction of κ transcription does not involve NF-κB activation or binding to the κ enhancer. The failure to detect NF-κB binding in IFN-γ–induced nuclear extracts was not due to generalized protein degradation, since the binding of another κ enhancer binding factor, NF-μE3, was unchanged in either treated or untreated extracts (*10*).

These results indicate that different transcription control signals and nuclear factors mediate the induction of κ transcription by LPS and IFN-γ. Two other lines of evidence support this conclusion. (i) Mutant cells lines of 70Z/3, selected for loss of responsiveness to LPS, retain inducibility of κ expression by IFN-γ (*11*). (ii) The 70Z/3 pre-B cells, stably transformed with pSV2-*neo*, show increased levels of *neo* mRNA after induction by LPS, but not by IFN-γ (*12*). The SV40 enhancer controlling the expression of the *neo* gene contains a NF-κB site identical to that in the κ enhancer (*1*).

These findings raise the possibility that IFN-γ induction may be mediated by sequences located outside the κ enhancer. One candidate is the octamer motif in the κ promoter, which binds factors found in all cells and an LPS-induced factor restricted to B cells (*13, 14*). However, the B cell octamer-binding factor is not increased by IFN-γ treatment (*15*). The region 5′ of the octamer in the rearranged 70Z/3 κ gene (*16*) contains several stretches with 7/9 nucleotide similarity to conserved nucleotide sequences in the 5′ region of major histocompatibility complex class II genes reportedly involved in IFN-γ induction (*17, 18*). It remains to be determined if these stretches are involved in IFN-γ induction of κ transcription in 70Z/3 pre-B cells.

The inhibition of κ transcription, but not NF-κB activation by TGF-β was unexpected. Several possibilities can be envisioned for the inhibitory effect of TGF-β. First, TGF-β could affect the factor binding in the κ enhancer at a site distinct from the NF-κB site. The κ enhancer contains three elements homologous to the E motifs in the heavy chain enhancer first reported by Ephrussi *et al.* (*19*). These elements contribute additively to the functional activity of the κ enhancer and bind ubiquitous nuclear factors that are not restricted to B lineage cells (*1, 2*). However, we think it is unlikely that these other enhancer sites are affected by TGF-β. The factor binding to these κ enhancer sites is unchanged by LPS induction (*2*), and we observed no effect of TGF-β on the binding of one of these factors (that is, NF-μE3) (*11*).

Second, TGF-β may block κ transcription by affecting certain effector functions of activated NF-κB that are distinct from its DNA binding domain. In this case, TGF-β might prevent the interaction of NF-κB with other regulatory proteins required for κ transcription.

Finally, changes in κ enhancer chromatin structure [that is, deoxyribonuclease I hypersensitivity] are known to occur with the LPS activation of κ transcription (*20*). It is conceivable that TGF-β may block κ transcription by preventing alterations in enhancer chromatin structure that make the κB motif accessible for activated NF-κB binding. This reopens the possibility that negative regulatory factors bound to the κ enhancer may control κ transcription (*21*).

These findings reveal a high degree of complexity still to be resolved in the regulatory circuits and mechanisms in κ gene activation and transcription. TGF-β is a useful reagent for further deciphering the processes in κ gene activation.

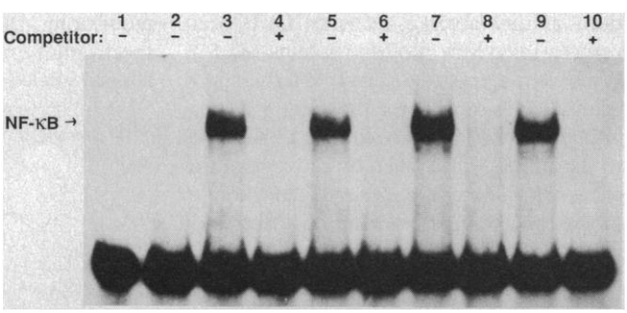


**Fig. 2.** Effect of TGF-β on LPS induction of NF-κB binding. Electrophoretic mobility shift assays were performed with nuclear extracts prepared from 70Z/3 cells (*1*). Binding reactions were done with a 70-bp Dde I–Hae III fragment [designated κ3 in (*1*)] end-labeled with polynucleotide kinase. Nuclear extracts for binding were prepared according to Dignam *et al.* (*22*). Samples are as follows: lane 1, no extract; lane 2, uninduced 70Z/3; lanes 3 and 4, 70Z/3 induced with LPS at 10 μg/ml for 4 hours; lanes 5 and 6, LPS at 10 μg/ml and TGF-β at 2 U/ml for 4 hours; lanes 7 and 8 are the same as lanes 3 and 4, respectively, except that cells were treated for 24 hours; and lanes 9 and 10 are the same as lanes 5 and 6, respectively, but cells were treated for 24 hours. Competitor oligonucleotide NF-κB DNA 5′-GCTGGGGACTTTCC-3′ was added at 100 ng per binding assay to 3′-CCCTGAAAGGCGAC-5′ samples analyzed in lanes 4, 6, 8, and 10.

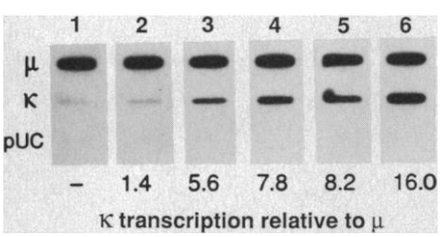


**Fig. 3.** Kinetics of IFN-γ induction of κ transcription in 70Z/3 cells. Nuclear transcription assays were performed as described in Fig. 1. The 70Z/3 cells were induced with IFN-γ at 50 U/ml as indicated: lane 1, uninduced; lane 2, 1 hour; lane 3, 2 hours; lane 4, 4 hours; lane 5, 6 hours; and lane 6, 24 hours. Induced κ transcription is normalized to μ values.

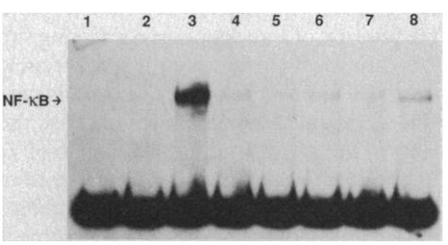


**Fig. 4.** Effect of IFN-γ on the induction of NF-κB binding in 70Z/3 cells. DNA-factor binding reactions and electrophoresis were carried out as described in Fig. 2. Nuclear extract (6 μg) was used in each reaction. Lane 1, no extract; lanes 2 and 4, uninduced; lane 3, LPS at 10 μg/ml for 4 hours; lanes 5 to 8, IFN-γ at 50 U/ml for 1, 2, 4, and 24 hours, respectively.


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