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A Gamma Interferon-Unresponsive Variant of Cell Line 70Z/3, IFN-4, Can Be Partially Rescued by Phorbol Myristate Acetate

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Lipopolysaccharide (LPS) and gamma interferon (IFN-γ) induce kappa transcription in 70Z/3 cells by different mechanisms; LPS treatment induces both NF-κB and OTF-2 transcription factors, but IFN-γ treatment does not. We have dissected these two activation pathways by selecting and analyzing an LPS$^+$ IFN$^-$ variant of 70Z/3.

The murine B-cell lymphoma 70Z/3 has been used as a model system to investigate the control of immunoglobulin kappa (κ) light-chain expression. Treatment of these cells with lipopolysaccharide (LPS) (12) or with gamma interferon (IFN-γ) (23) rapidly induces κ gene transcription and protein expression.

The molecular mechanism by which LPS activates κ transcription has been intensively studied in 70Z/3. Two critical regulatory sequences have been positively identified in this response: the octamer, located 70 base pairs 5′ of the TATA box (13, 16), and a site called $E_\kappa B$, within the enhancer (14, 15). LPS induces κ transcription by activating transcription factors which bind these sites. Treatment of these cells with either LPS or phorbol 12-myristate 13-acetate (PMA) causes very rapid activation and nuclear translocation of transcription factor NF-κB, which binds to the $E_\kappa B$ element (2–4, 7, 17, 18). In contrast to $E_\kappa B$, several different proteins bind to the octamer (19; for a recent review, see reference 11); among these is the B-cell-specific factor, OTF-2, which is transcriptionally induced by LPS (20).

The mechanism of κ transcriptional induction by IFN-γ is less well understood. The isolation of LPS$^+$ IFN$^-$ and LPS$^-$ IFN$^+$ variants of 70Z/3 in our laboratory clearly demonstrated that the two pathways are at least partly separate (22). Briskin et al. (5) showed that the increase in κ mRNA 24 h after IFN-γ treatment of 70Z/3 is transcriptional and that NF-κB is not found in nuclear extracts of IFN-γ-treated cells after 4 or 24 h.

We isolated an LPS$^+$ IFN$^-$ variant of 70Z/3, called IFN-4, by an immunoselection method previously described (9). These cells have wild-type levels of IFN-γ receptors (Karol Bomsztyk, personal communication). To determine their basic phenotype, equivalent numbers of wild-type and IFN-4 cells were treated for 24 h with LPS (10 μg/ml), IFN-γ (50 U/ml), PMA (50 ng/ml), or IFN-γ plus PMA, stained with fluorescein isothiocyanate (FITC)-coupled anti-κ antibodies and the level of membrane immunoglobulin M (IgM) was measured by flow cytometry (Fig. 1A and B). Wild-type cells were 13.6-fold more fluorescent after LPS treatment and 3.1-fold more fluorescent after IFN-γ treatment. In contrast, IFN-4 cells were 3.8-fold more fluorescent after LPS treatment but showed an increase of only 60% after IFN-γ treatment. In both populations, PMA alone had a very small effect, ranging within 30% of the uninduced population (data not shown). The combination of PMA with IFN-γ caused a small increase in surface IgM expression in wild-type cells (about 10%) but had a clear effect on IFN-4 cells (from 0.6- to 1.3-fold more fluorescent). The addition of PMA also increased the LPS response of IFN-4 roughly 2.5-fold but had no effect in wild-type cells (data not shown).

The differences in κ induction were even more striking at the level of κ mRNA. Both populations were stimulated as above, and total RNA was prepared and analyzed by hybridization of Northern (RNA) blots with ^{32}P-labeled probes for κ or actin messages (Fig. 1C). Figure 1D summarizes the densitometric analysis of the autoradiograms. As observed previously (23), LPS was about three times more potent than IFN-γ as an inducer of κ mRNA in wild-type cells. In the mutant, the LPS response was reduced by about fivefold, but the response to LPS was still evident. However, even a long exposure of the blot of IFN-4 RNA showed only a faint κ signal, emphasizing its poor response to IFN-γ stimulation. Darker exposure of this blot and Northern blots comparing a series of dilutions allowed us to estimate that the increase in κ mRNA after IFN-γ treatment of IFN-4 cells was 25- to 30-fold lower than that observed in wild-type cells (data not shown).

This Northern analysis also emphasized the augmentation of IFN-γ action by PMA (Fig. 1C); densitometric analysis of the autoradiograms showed that in wild-type cells, κ mRNA in cultures treated with IFN-γ plus PMA was about 3 times that in IFN-γ cultures, while for IFN-4 cells, the κ message increased about 15 times. In the mutant, neither IFN-γ nor PMA alone increased κ mRNA, but the combination was clearly effective in inducing mRNA and in increasing surface IgM expression.

Next, we determined if the lack of IFN-4 response to IFN-γ or reduced response to LPS was due to a failure of either NF-κB or OTF-2 to appear in the nuclear extracts from these cells. 70Z/3 and IFN-4 cells were treated with various combinations of inducing agents for 24 h, and the nuclear extracts were prepared by the method of Dignam et al. (6), as modified by Mather (10). Each extract was assayed for NF-κB and OTF-2 by gel shift analysis (8, 17), using appropriate DNA fragments (2) or deoxyoligonucleotides (21) (Fig. 2A and B). As a control, the level of NF-κD (also called NF-κE3 [3]), a constitutive nuclear DNA-binding factor specific to another κ enhancer element, $E_\kappa D$ (2), was also determined (Fig. 2C). Each assay contained 5 μg of nuclear extract protein, ~0.5 ng of ^{32}P-labeled DNA fragments, and an appropriate amount of poly(dI-dC) as cold

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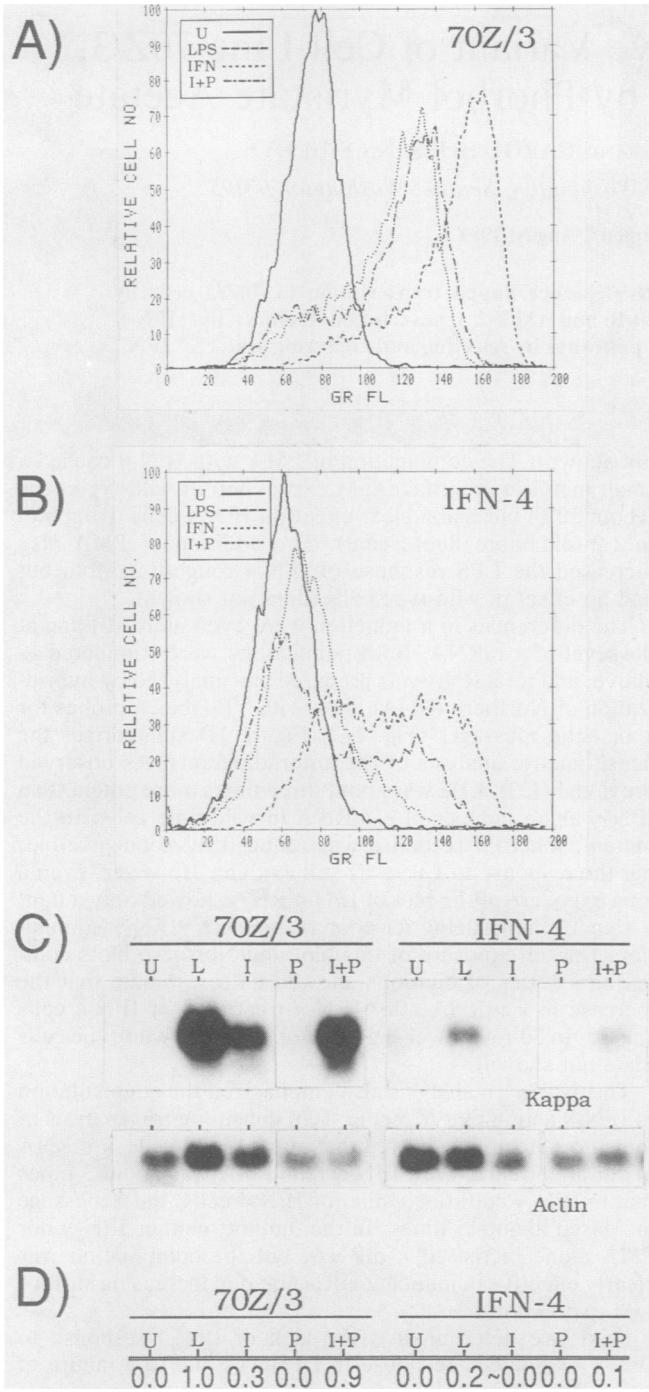

FIG. 1. Analysis of induced membrane IgM and κ message levels in 70Z/3 and IFN-4 cells. Cells were left untreated (U) or were treated with LPS (L), IFN-γ (I), or IFN-γ plus PMA (I+P) for 24 h and assayed for surface IgM and κ message levels. Treated 70Z/3 (A) and IFN-4 (B) cells were stained with FITC-conjugated anti-κ antibodies, and surface IgM levels were determined cytofloremetrically. GR FL, Green fluorescence. (C) Total RNA was prepared from treated cells, and κ and actin messages were analyzed by Northern blots by using appropriate probes. (D) κ message levels were determined by densitometric scanning of appropriate autoradiograms and normalizing to actin message levels. The maximum level obtained by LPS induction of wild-type cells was set at 1.0, and all other levels were reported as a fraction of that level.

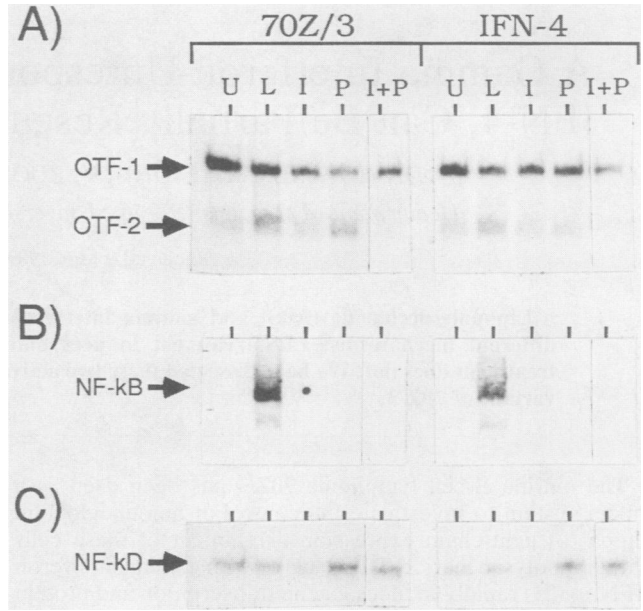

FIG. 2. Gel shift analysis of nuclear factors in 70Z/3 and IFN-4 cells. 70Z/3 and IFN-4 cells were treated with either LPS (L), IFN-γ (I), PMA (P), or IFN-γ plus PMA (I+P) for 24 h, and the nuclear extracts were prepared as described in the text. Each extract was assayed for the presence of the indicated binding factors, NF-κB (B), OTF-2 (A), and NF-κD (C) (as a control) by gel shift analysis, using the appropriate, ^{32}P-labeled probes.

competitor: 0.7 μg for the octamer double-stranded deoxyoligonucleotide (34 base pairs), 9.0 μg for the 65-base-pair $E_\kappa B$-containing fragment, and 3.5 μg for the 85-base-pair $E_\kappa D$-containing fragment.

The first three lanes in each set show the factors derived from untreated wild-type or IFN-4 cells (U) or after LPS (L) or IFN-γ (I) treatment of the cells. As expected, LPS treatment of wild-type cells caused a substantial increase in both OTF-2 and NF-κB, but there was no difference between the uninduced and IFN-γ-treated cells. Further, IFN-4 cells showed the same response; a clear increase in both NF-κB and OTF-2 after LPS treatment, but no change in either factor after IFN-γ treatment.

PMA increases NF-κB only transiently in 70Z/3 (18), so no increase was observed at the time point at 24 h. Both 70Z/3 and IFN-4 cells showed the expected increase in NF-κB at 4 h (data not shown). When extracts from cells treated with combinations of these agents were tested, two further observations were made: IFN-γ or PMA had no effect on the LPS-induced factors, and IFN-γ plus PMA caused no increase in OTF-2 in wild-type or IFN-4 cells. These observations show that neither NF-κB nor OTF-2 is required for IFN-γ action; the mechanism of IFN-γ activation is different from that for LPS.

Whatever the mechanism of IFN-γ action, the addition of some PMA-activated factor increased it by severalfold. The fact that PMA partly rescued the IFN-γ response in IFN-4 cells suggests that their defect is in the IFN-γ activation pathway. PMA causes multiple changes in phosphorylation in cells (1). There are two simple interpretations of the rescue of the mutant. The effect of PMA might be to phosphorylate an intermediate in the IFN-γ pathway; in this view, PMA could bypass the defect in IFN-4 cells by phosphorylating an intermediate downstream of the defect.

Since PMA alone does not induce κ, this model requires that the pathway of IFN-γ induction comprises two limbs, one involving phosphorylation and the other not. An alternative is that the defect in IFN-4 cells is a deficiency of one among several factors required for the IFN-γ response, and the transient induction of NF-κB by PMA partly substitutes for the missing factor. This is not supported by the observation that PMA also augments the LPS response in IFN-4, since the cells fully activate NF-κB after LPS treatment.

We have recently selected a large number of independent IFN$^-$ 70Z/3 cells, and their characterization should allow us to begin to better define the factor(s) involved and the effect of PMA on these induction pathways.

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