Brief Definitive Report

Functional NF-IL6/CCAAT Enhancer-binding Protein is Required for Tumor Necrosis Factor α–inducible Expression of the Granulocyte Colony-stimulating Factor (CSF), but not the Granulocyte/Macrophage CSF or Interleukin 6 Gene in Human Fibroblasts

By Michael Kiehntopf, Friedhelm Herrmann, and Marion A. Brach

From the Department of Medical Oncology and Applied Molecular Biology, Universitätsklinikum Rudolf Virchow der Freien Universität Berlin, Robert Rössle Cancer Center, and Max Delbrück Center for Molecular Medicine, D-13122 Berlin, Germany

Downloaded from http://rupress.org/jem/article-pdf/181/2/793/1106262/793.pdf by guest on 05 October 2022

Summary

Tumor necrosis factor (TNF) α participates in the regulation of the acute-phase, immune, and inflammatory responses. Target genes known to be transcriptionally activated by TNF-α include the granulocyte (G)–colony-stimulating factor (CSF) gene, the granulocyte/macrophage (GM)-CSF gene, as well as the interleukin (IL) 6 gene. Functional nuclear factor (NF)-IL6 recognition sites have been identified in regulatory regions of these genes by transient transfection studies using deleted promoter constructs. In addition, NF-IL6 is known to form heterodimeric complexes with the NF-κB transcription factor, which is also engaged in the transcriptional regulation of these genes. The indispensable importance of NF-IL6 for regulating gene expression of proinflammatory cytokine genes in response to inflammatory stimuli in vivo remains, however, unclear. We here report, by using both antisense (AS) oligodesoxyribonucleotide (ODN) and ribozyme (RZ)-mediated specific elimination of NF-IL6 transcripts in human fibroblasts, that TNF-α–induced synthesis of G-CSF, but not of GM-CSF or IL-6, is abolished in the absence of functional NF-IL6 in vivo. Both AS ODN and RZ targeting of the NF-IL6 transcript eliminate NF-IL6 protein, as shown in Western blot analysis and electrophoretic mobility shift assays. Similarly, fibroblasts exposed to either the AS NF-IL6 ODN or the NF-IL6 RZ, but not to the sense or nonsense ODN or a mutated ribozyme, also failed to respond with functional activation of NF-IL6 as assayed in transient transfection studies using heterologous promoter constructs harboring the NF-IL6 recognition site. In contrast, protein synthesis, DNA-binding activity, and transcriptional activation capacity of the NF-κB transcription factor is not impaired upon exposure to either ODN or RZ. Fibroblasts that had been cultured in the presence of the AS NF-IL6 ODN or the NF-IL6RZ failed to synthesize G-CSF protein in response to TNF-α, while TNF-α–inducible transcription and release of GM-CSF and IL-6 was preserved.

The nuclear factor (NF)-IL6 transcription factor was originally identified as an IL-1–inducible DNA-binding molecule mediating the transcription of the IL-6 gene in fibroblasts (1). Besides coupling IL-1 to IL-6 expression, NF-IL6 was shown to also transmit other stimuli to the nucleus, including those delivered by LPS, TNF-α, and IL-6 (for review see reference 2). More recently, it has been shown that NF-IL6 possesses functional sites in regulatory sequences of the IL-1, IL-8, G-CSF, and GM-CSF genes as well as immunoglobulin genes (2). Molecular cloning of NF-IL6 has revealed its homology to the CCAAT enhancer binding protein (C-EBP), previously viewed as a hepatocyte-specific transcription factor (3). NF-IL6/C-EBP was also shown to be identical to IL-6-induced DNA-binding protein, perceived to be crucial for IL-6–mediated activation of several acute phase response genes in the liver (4). Based on these findings, NF-IL6 is thought to play a key role in the regulation of the acute-phase, immune, and inflammatory responses (2). Similar to NF-IL6, the transcription factor NF-κB is also recognized as a major regulatory molecule in the cellular response to infection and inflammation (for review see reference 5) and is also targeted by TNF-α, IL-1, or LPS (5). Moreover, both transcription factors recognize binding sequences and exert transcriptional regulation control of the same target genes.

793 J. Exp. Med. © The Rockefeller University Press • 0022-1007/95/02/0793/06 $2.00
Volume 181 February 1995 793–798

There is also evidence to suggest that NF-IL6 and NF-κB may cooperate to achieve transcriptional activation of their target genes not only by coordinated binding and transcriptional activation but also by functional interaction. NF-IL6 has been shown to heterodimerize with the NF-κB transcription factor and thereby potentiates its transcriptional activation capacity (6, 7). Other studies have indicated that, despite the presence of functional NF-IL6 binding sites, transcriptional activation of several genes, including GM-CSF (8) and IL-6 (9–11), may rely predominantly on the binding activity and transcriptional activation capacity of NF-κB and various other transcription factors (12–14). However, the role of NF-IL6/NF-κB heterodimers targeting the NF-κB recognition sequence has not yet been addressed. In addition, previous studies have relied on transient transfection assays using deleted promoter–reporter gene constructs. Though these experiments are suitable to identify functional domains within regulatory regions of single genes, they do not point to the nature of the proteins bound and mediating functional activation. To further elucidate the potential redundancy of NF-IL6 and NF-κB in the regulation of cytokine gene expression and to clarifiy the functional role of NF-IL6 in mediating transcriptional activation of cytokine genes, we have examined TNF-α–inducible expression of three different target genes, namely, G-CSF, GM-CSF, and IL-6. This analysis was performed on human fibroblasts that had been deprived of functional NF-IL6 by previous exposure to an antisense (AS) oligodesoxyribonucleotide (ODN) or a synthetic hammerhead ribozyme (RZ) directed to NF-IL6 transcripts.

Materials and Methods

Cell Culture. The human embryonic lung fibroblast cell line FH 109 (15) was maintained in standard culture medium (SCM) RPMI-1640 medium supplemented with 10% low-endotoxin heat-inactivated FCS (Hazelton, Vienna, UT), 100 U/ml penicillin, 10 μg/ml streptomycin, and 2 mM L-glutamine (referred to as standard culture medium [SCM]). FH 109 cells (10^6/ml) were exposed to recombinant human TNF-α (kindly provided by G. Adolf, Bender KG, Vienna, Austria) in concentrations ranging from 1 to 150 U/ml.

Western Blot Analysis. Immunoprecipitation and Western blot analysis were performed as previously described (16). Briefly, cells were lysed in RIPA buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.5% Deoxycholic acid (DOC), 1% Triton X-100, 0.1% SDS) followed by centrifugation at 4°C for 20 min at 20,000 *g* to remove insoluble material. Lysates were separated by SDS-PAGE and transferred to Hybond C^+ membrane (Amersham Buchler GmBH, Braunschweig, Germany). Filters were probed with a specific anti–NF-IL6 antiserum (kindly provided by S. Akira, Osaka University, Osaka, Japan) or a specific mAb directed against the p65/rel A protein (Santa Cruz Biotechnology, Santa Cruz, CA), stripped, and then reprobed with a specific mAb directed against β_2-microglobulin (Dianova, Hamburg, Germany).

AS ODN-mediated Elimination of NF-IL6. AS experiments were performed essentially as previously described (16). An AS ODN corresponding to the ATG sequence of the human NF-IL6 gene (5′-CAGGCGTTGCATGAACGCGG-3′), the corresponding sense (S) ODN (5′-CCGCGTTCATGCAACGCCTG-3′) and an unrelated nonsense (NS) ODN (5′-CCAGAGAGGGCCCGTGTGGA-3′) with the same overall basepair compositon as the AS ODN were synthesized and stabilized by addition of thiosulfate groups, HPLC purified, and subsequently sequenced (Genset, Paris, France). ODN were complexed to cationic liposomes (Boehringer-Mannheim GmBH, Mannheim, Germany) as recommended by the manufacturer and added to cell cultures in SCM every 12 h for up to 36 h. Uptake of labeled ODNs, intracellular ODN stability, and intracellular duplex formation of the AS ODN with its target sequence had been assessed and was identical as previously described in detail (16).

RZ-mediated Elimination of NF-IL6. A hammerhead RZ recognizing the UUC sequence at position −3 to −1 relative to the translation initiation site (designated NF-IL6 RZ) was designed according to previously described criteria (17). The sequence of the NF-IL6 RZ is as follows: 5′-GCGTTGCATCUGAAGAGGCCG-AAAGGCCGAAAACGCGGGT-3′ (the sequence complementary to the NF-IL6 transcript is underlined). In addition, a mutated NF-IL6 RZ (RZm) carrying two base mutations (5′-GCGTTGCAT-CUaAAGAGGCCcAAAGGCCGAAAACGCGGGT-3′; mutated bases are in lower case letters) (NF-IL6 RZmt) was also designed. The mutations introduced are known to interfere with the catalytic potential of hammerhead RZ (17). Both RZ harbor several chemical modifications according to previously published criteria (17). RZ were complexed to cationic liposomes (Boehringer-Mannheim GmBH) as recommended by the manufacturer and added to cell cultures in SCM every 12 h for up to 36 h.

Preparation of Nuclear Proteins and Electrophoretic Mobility Shift Assays. Electrophoretic mobility shift assays were performed essentially as previously described (16). Briefly, nuclear extracts were prepared from FH 109 cells that had received (or not) liposome-complexed ODN or liposome-complexed RZ followed (or not) by exposure to TNF-α according to the Dignam method. Protein concentrations were determined by the Bradford assay. S and AS ODN (NF-IL6 S: 5′-TCGAGGACGTCATTGCACAATCTTAAT-AAG-3′; NF-IL6 AS 5′-TCGAACCTTATTAAGATTGTGCAA-TGAGT-3′; NF-κB sense: 5′-TCGAGAGA GGGGACTTTCCG-AGAGGC-3′; NF-κB antisense 5′-TCTCGGAAAGTCCCCTCT-CTCGAGGC-3′, binding sites are underlined) corresponding to the NF-IL6–binding site of the IL-6 gene and the κB enhancer, respectively, were synthesized, annealed, and endlabeled. The endlabeled ODN (1 ng; ~10,000 cpm) were incubated with 10 μg nuclear proteins in an incubation buffer as previously described (10) for 20 min at room temperature. For competition assays, incubation was performed in the presence of 25-fold molar excess of unlabeled double-stranded oligomers containing the NF-IL6 or NF-κB recognition sequence or a mutated binding sequence. The reaction products were analyzed by electrophoresis in a 5% polyacrylamide gel followed by autoradiography.

Transient Transfection Assays. Promoter constructs used for transient transfection assays have been described elsewhere (10). Transient transfections were performed by use of commercially available liposomes (Boehringer Mannheim GmBH) as detailed by the manufacturer. Cells were cultured in the presence of liposome-complexed DNA for 24 h, split, and cultured in the presence or absence of TNF-α (100 U/ml) for an additional 24 h, followed by analysis of human growth hormone (hGH) activity in cell-free culture supernatants using a hGH-specific enzyme immunoassay (Eurogenetics, Tessenderio, Belgium).

Reverse Transcription PCR and Southern Blot Analysis. RNA preparation and reverse transcription (RT) PCR reactions were performed as previously described (17). Primers used were as follows. G-CSF primers: S, 5′-CAGAGCCTGCTGCTCTTG-3′; AS, 5′-AGC-AGTCAAAGGGGATGAC-3′; GM-CSF primers: S, 5′-CAGAGC-CTGCTGCTCTTG-3′; AS, 5′-AGCAGTCAAAGGGGATGAC-3′;

Downloaded from http://rupress.org/jem/article-pdf/181/2/793/1106262/793.pdf by guest on 05 October 2022

IL-6 primers: S, 5'-ACAAATTCGGTACATCCTCG-3'; AS, 5'-ATG-AGTTGTCATGTC CTGCA-3'; β_2-microglobulin primers: S, 5'-CCAGCAGAGAATGGAAAGTC-3'; AS, 5'-GATGCTGCTTAC-ATGTCT-3'. One fifth of each RT-PCR reaction mixture was run on 1% agarose gels, followed by Southern blotting and hybridization to specific cDNA probes.

Determination of Cytokine Concentrations in Cell-free Culture Supernatants. Concentrations of G-CSF, GM-CSF, or IL-6 were determined in cell-free culture supernatants by commercially availabe ELISA assays (R&D Systems, Minneapolis, MN [IL-6]; Amgen Biochemicals, Thousand Oaks, CA [G-CSF]; Medical Resources Ltd., Darlington, Australia [GM-CSF]).

Results

Upon exposure to TNF-α, FH 109 fibroblasts responded with enhanced synthesis of both the NF-IL6 and the NF-κB transcription factor (Fig. 1). The capacity of TNF-α to promote synthesis of NF-IL6 or NF-κB was dose dependent in the range of 1–100 U/ml and also time dependent (data not shown). Maximum protein accumulation was observed at a concentration of 100 U/ml after 6 h. Therefore, this dose and time point were chosen for further experiments. Cells that had received NF-IL6–specific liposome-complexed AS ODN for 24 h failed, however, to display the NF-IL6 protein after TNF-α exposure, while the level of NF-κB protein remained unchanged. In contrast, exposure to S or NS ODN did not interfere with TNF-α–inducible accumulation of either transcription factor. Likewise, cells that had been exposed to liposome-complexed NF-IL6–specific RZ failed to produce NF-IL6 upon exposure to TNF-α, while cells that had received an RZm continued to produce NF-IL6 protein in response to TNF-α.

In line with these findings, we also failed to detect enhanced NF-IL6–binding activity after TNF-α stimulation of cells that had previously been treated with liposome-complexed AS ODN or liposome-complexed RZ, while liposome-complexed S or NS ODN and liposome-complexed RZm failed to interfere with TNF-α to promote NF-IL6–binding activity. The ability of the NF-κB protein to bind DNA in response to TNF-α stimulation was not impeded by either AS, S, or NS ODN nor by the active or inactive ribozyme (Fig. 2).

The next set of experiments aimed at elucidating the functional activation of both NF-IL6 and NF-κB by TNF-α via transient transfection assays. FH 109 cells that had (or not) been exposed to liposome-complexed ODN or liposome-complexed RZ were transiently transfected with heterologous reporter gene constructs harboring the NF-IL6– or the NF-κB–binding sequence followed by TNF-α stimulation. While functional activation of the NF-κB construct in response to TNF-α was unaffected under all experimental conditions, the functional activation of the NF-IL6 construct was completely abolished in cells that had previously received liposome-complexed AS ODN or liposome-complexed active RZ (Fig. 3).

Among the genes known to be induced by TNF-α in fi-

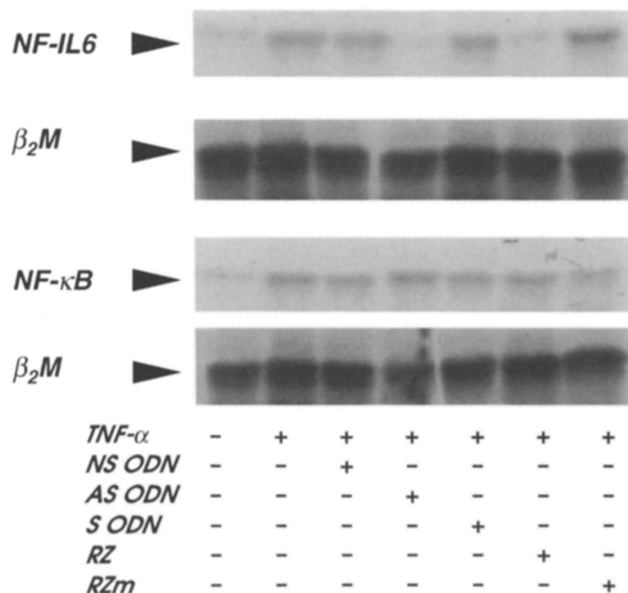

Figure 1. NF-IL6 AS ODN or NF-IL6–directed RZ prevent TNF-α–inducible accumulation of NF-IL6 in fibroblasts. FH 109 cells were cultured in the presence or absence of liposomes (*LS*), liposome-complexed AS, S, or NS ODN or to liposome-complexed NF-IL6 RZ or NF-IL6 RZm for 24 h and then exposed to TNF-α (100 U/ml) for 6 h. Cell lysates were separated and analyzed by immunoblotting as detailed in Materials and Methods for expression of NF-IL6 and p65 NF-κB. Shown is one representative experiment. Two additional experiments gave comparable results.

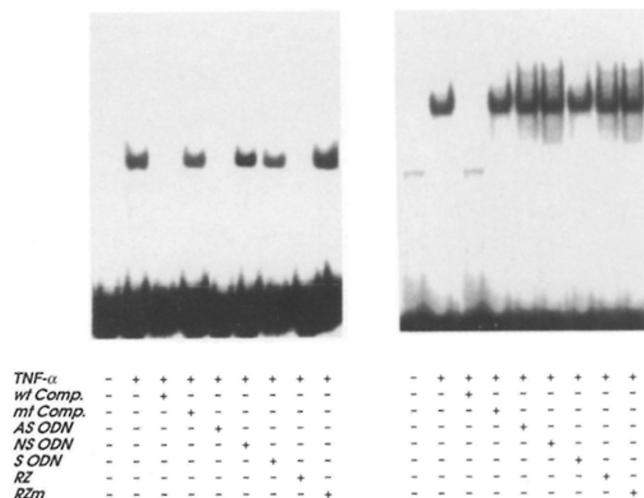

Figure 2. NF-IL6 AS ODN and NF-IL6 RZ prevent enhanced NF-IL6 binding activity after TNF-α stimulation of fibroblasts. FH 109 cells were cultured in the presence or absence of liposomes (*LS*), liposome-complexed AS, S, or NS ODN or liposome-complexed NF-IL6 RZ or NF-IL6 RZm for 24 h and then exposed to TNF-α (100 U/ml) for 1 h. Cell lysates were prepared and analyzed for binding activity by use of labeled double-stranded oligomers containing the NF-IL6 (*left*) or the NF-κB (*right*) recognition sequence. Competition assays were performed in the presence of 25-fold molar excess of double-stranded oligomers harboring the respective binding site (*wt*) or a mutated binding sequence (*mt*). Shown is one representative experiment. One additional experiment gave identical results.

Downloaded from http://rupress.org/jem/article-pdf/181/2/793/1106262/793.pdf by guest on 05 October 2022

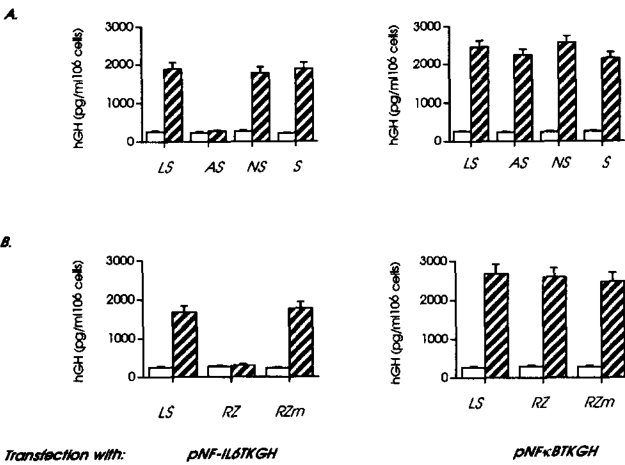

Figure 3. NF-IL6 AS ODN and NF-IL6 RZ prevent functional activation of NF-IL6 by TNF-α in fibroblasts. FH 109 fibroblasts were cultured in the presence or absence of liposomes (*LS*), liposome-complexed AS, S, or NS ODN (*A*) or liposome-complexed NF-IL6 RZ or NF-IL6 RZm (*B*) for 24 h and then transiently transfected with heterologous promoter constructs harboring the NF-IL6 (*left*) or the NF-κB (*right*) binding sequences 5' of the herpes thymidine kinase (HTK) promoter linked to the hGH gene as a reporter. Transfectants were maintained in the presence of liposomes, liposome-complexed ODN, or liposome-complexed RZ for an additional 24 h, split, and either left untreated or exposed to TNF-α (100 U/ml) for an additional 12 h. Thereafter, hGH activity was determined in cell-free culture supernatants with a commercially available EIA assay. Transfectants harboring the control construct (pTKGH) failed to respond to TNF-α with enhanced hGH synthesis under all experimental conditions (not shown). □, SCM; ▨, TNF-α.

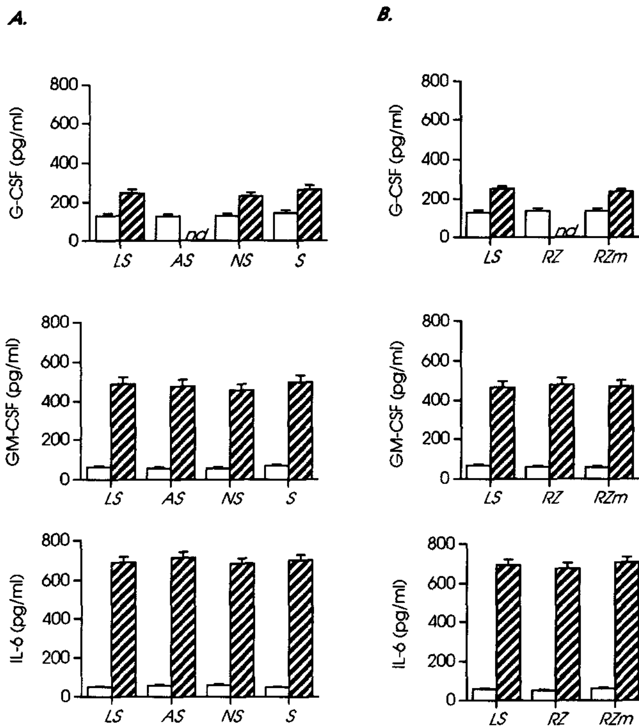

Figure 4. NF-IL6 AS ODN and NF-IL6 RZ interfere with the ability of TNF-α to induce release of G-CSF, but not GM-CSF or IL-6 by fibroblasts. FH 109 cells were cultured in the presence or absence of liposomes (*LS*), liposome-complexed AS, S, or NS ODN (*A*) or liposome-complexed NF-IL6 RZ or NF-IL6 RZm (*B*) for 24 h and then exposed to TNF-α (100 U/ml) for an additional 12 h. Thereafter, cell-free supernatants were assayed for G-CSF, GM-CSF, and IL-6 by use of a commercially available ELISA. Data are shown as mean value ± SD of three independent experiments. *nd*; not detectable. □, SCM; ▨, TNF-α.

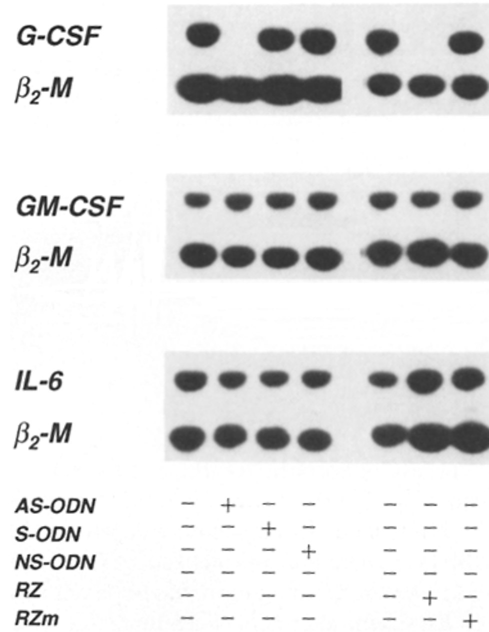

Figure 5. NF-IL6 AS ODN and NF-IL6 RZ interfere with the ability of TNF-α to induce G-CSF, but not GM-CSF or IL-6 mRNA, in fibroblasts. FH 109 cells were cultured in the presence or absence of liposomes (*LS*), liposome-complexed AS, S, or NS ODN, or liposome-complexed NF-IL6 RZ (*RZ*) or NF-IL6 RZm (*RZm*) for 24 h and then exposed to TNF-α (100 U/ml) for an additional 4 h. Thereafter, cellular RNA was prepared and analyzed for the expression of G-CSF, GM-CSF, IL-6, and β_2-microglobulin by RT-PCR and Southern blotting analysis. β_2-microglobulin was coamplified in all reactions to control for comparable amounts of RNA subjected to RT-PCR analysis and allow internal standardization. Shown is one representative experiment; one additional experiment gave identical results.

broblasts are G-CSF, GM-CSF, and IL-6. Pilot experiments, not shown here, had indicated that TNF-α time dependently enhanced the release of G-CSF, GM-CSF, or IL-6 in FH 109 cells, which was maximal at 24 h (15 and Kiehntopf, M., F. Herrmann, and M. A. Brach, unpublished data). This time point was therefore chosen for further experiments. TNF-α–mediated release of G-CSF was completely relieved in cells that had been exposed to liposome-complexed AS ODN or liposome-complexed RZ, while TNF-α–mediated release of GM-CSF or IL-6 was unaffected by this procedure (Fig. 4). Cells that had received liposome-complexed S or NS ODN or liposome-complexed RZm responded to TNF-α with enhanced release of G-CSF, GM-CSF, and IL-6.

These genes harbor in their promoter both NF-IL6– and NF-κB–binding sites. Functional activation of all three genes involving either NF-IL6 or NF-κB has previously been reported

Downloaded from http://rupress.org/jem/article-pdf/181/2/793/1106262/793.pdf by guest on 05 October 2022

(8–14, 18). We have therefore also analyzed the expression of G-CSF, GM-CSF, and IL-6 in response to TNF-α in FH 109 cells that had (or not) received liposome-complexed ODN or NF-IL6–directed liposome-complexed RZ by RT-PCR and Southern blotting (Fig. 5). In previous experiments, TNF-α was shown to time dependently induce mRNA accumulation of G-CSF, GM-CSF, and IL-6 in FH 109 cells, with maximum mRNA accumulation seen after 4 h of exposure (15 and Kiehntopf, M., F. Herrmann, and M. A. Brach, unpublished data). This time point was therefore chosen for further experimentation. FH 109 cells responded to TNF-α with enhanced mRNA accumulation of G-CSF, GM-CSF, or IL-6 when previously exposed to liposome-complexed S or NS ODN or to lipsosome-complexed RZm. TNF-α–mediated accumulation of G-CSF mRNA was completely abolished in cells that had received liposome-complexed AS ODN or liposome-complexed RZ. In contrast, TNF-α–mediated accumulation of both IL-6 or GM-CSF mRNA was unaffected by the presence of the AS ODN or the NF-IL6–directed RZ. These findings indicate that TNF-α–mediated regulation of G-CSF gene expression requires NF-IL6, while TNF-α–mediated regulation of GM-CSF or IL-6 expression also occurs in its absence.

Discussion

We have designed an NF-IL6–specific AS ODN and an NF-IL6–specific RZ. Both the AS ODN and the RZ were incorporated into human FH 109 fibroblasts by liposomal transfer (not shown). The specificity of NF-IL6 elimination was shown at the protein level by Western blot analysis, DNA binding studies, and functional assays using transient transfection assays of heterologous promoter constructs. This model system allowed investigation of the functional role of NF-IL6 in regulating fibroblast gene expression of G-CSF, GM-CSF, and IL-6 in response to TNF-α stimulation. We show that TNF-α–mediated induction of G-CSF requires functional NF-IL6. In contrast, TNF-α–induced activation of GM-CSF and IL-6 gene expression also occurs in the absence of NF-IL6 and thus may demand the presence of the NF-κB molecule or other transcription factors. This view is supported by previous transient transfection assays indicating that deletion of the NF-κB recognition site interfered with inducible transcriptional activation of the IL-6 gene (9–11), while transcriptional activation of the GM-CSF gene required both NF-κB and other transcription factors (8, 12–14). Several lines of evidence have indicated that transcriptional activation of the G-CSF promoter may involve the NF-IL6 transcription factor (14, 18–21). The mouse G-CSF promoter contains a NF-κB recognition sequence (21), which is, however, not recognized by nuclear proteins obtained from mouse macrophages. Although the p65 subunit of the NF-κB transcription factor may target the G-CSF promoter in human cells, it requires the NF-IL6 protein for binding (18). We further support and extend these findings by demonstrating that TNF-α–mediated transcriptional activation of the G-CSF gene in vivo in fibroblasts strictly depends on the NF-IL6 protein and cannot be achieved by NF-κB or other transcription factors including members of the C-EBP family in the absence of NF-IL6.

Regulation of both GM-CSF and IL-6 gene expression in response to various extracellular stimuli has been extensively studied (8–14), and data have been accumulated to suggest that the NF-κB recognition sequence is crucial for transcriptional activation of the IL-6 gene, despite the presence of an NF-IL6–binding sequence. However, the nature of the proteins binding to the NF-κB recognition site has not been precisely characterized. NF-κB can contact its recognition site both as a p50/p65 heterodimer or as complexes consisting of both NF-κB and NF-IL6 (6, 7). Therefore, NF-IL6 may participate in IL-6 gene activation, engaging the NF-κB recognition sequence. While the NF-κB site within the GM-CSF promoter is functional, transcriptional activation of the GM-CSF gene has also been shown to rely on various other transcription factors, including AP-1 (8, 12–14). Our data provide conclusive evidence to suggest that despite the presence of functional NF-IL6–binding sequences in both the GM-CSF and IL-6 promoter, transcriptional activation of both genes in response to TNF-α does not require NF-IL6.

The cytokines under investigation in this study exert unique biological functions: GM-CSF is a known activator of macrophages and APC, IL-6 plays a major role in initiating the acute phase response in the liver, and G-CSF primarily stimulates function and survival of neutrophils and their precursors, but does not target mononuclear phagocytes (22). Based on their distinct in vivo functions, it is reasonable to allow distinct transcription factors to govern the expression of their genes. DNA-binding activity of NF-IL6 is enhanced in vivo after threonine phosphorylation through the microtubule-associated protein (MAP) kinase and appears therefore to be controlled by an activated ras and c-raf-1 protein (23). Taken together with these findings, our data demonstrate that TNF-α–mediated activation of MAP kinase (24) leads to activation of the G-CSF gene by engaging the NF-IL6 transcription factor.

This work was supported by grants from the Deutsche Forschungsgemeinschaft and the Deutsche Krebshilfe to F. Herrmann and M. A. Brach.

Address correspondence to Dr. Friedhelm Herrmann, Universitätsklinikum Rudolf Virchow, Robert Rössle Cancer Center, Lindenberger Weg 80, D-13122 Berlin, Germany.

Received for publication 3 October 1994.

Downloaded from http://rupress.org/jem/article-pdf/181/2/793/1106262/793.pdf by guest on 05 October 2022

References

1. Isshiki, H., S. Akira, O. Tanabe, T. Nakajima, T. Shimamoto, T. Hirano, and T. Kishimoto. 1990. Constitutive and interleukin-1-inducible factors interact with the IL-1-responsive element in the IL-6 gene. *Mol. Cell. Biol.* 10:2757–2764.
2. Akira, S., and T. Kishimoto. 1992. IL-6 and NF-IL6 in acute-phase response and viral infection. *Immunol. Rev.* 127:25–50.
3. Akira, S., H. Isshiki, T. Suigata, O. Tanabe, S. Kinoshita, Y. Nishio, T. Nakajima, T. Hirano, and T. Kishimoto. 1990. A nuclear factor for IL-6 expression (NF-IL6) is a member of a C-EBP family. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:1897–1906.
4. Poli, V., F.P. Mancini, and R. Cortese. 1990. IL-6 DBP, a nuclear protein involved in interleukin-6 signal transduction, defines a new family of leuzine zipper proteins related to C-EBP. *Cell.* 63:643–653.
5. Baeuerle, P.A., and T. Henkel. 1994. Function and activation of NF-kappa B in the immune system. *Annu. Rev. Immunol.* 12:141–179.
6. Le Clair, K.P., M.A. Blanar, and P.A. Sharp. 1992. The p50 subunit of NF-kappa B associates with the NF-IL6 transcription factor. *Proc. Natl. Acad. Sci. USA.* 89:8145–8149.
7. Matsusaka, T., K. Fujikawa, Y. Nishio, N. Mukaida, K. Matsushima, T. Kishimoto, and S. Akira. 1993. Transcription factors NF-IL6 and NF-kappa B synergistically activate transcription of the inflammatory cytokines, interleukin 6 and interleukin 8. *Proc. Natl. Acad. Sci. USA.* 90:10193–10197.
8. Schreck, R., and P.A. Baeuerle. 1990. NF-kappa B as inducible transcriptional activator of the granulocyte-macrophage colony-stimulating factor gene. *Mol. Cell. Biol.* 10:1281–1286.
9. Liebermann, T.A., and D. Baltimore. 1990. Activation of the interleukin-6 gene expression through the NF-kappa B transcription factor. *Mol. Cell. Biol.* 10:2327–2334.
10. Brach, M.A., H.J. Gruss, T. Kaisho, Y. Asano, R. Mertelsmann, T. Hirano, and F. Herrmann. 1993. Ionizing radiation induces interleukin 6 transcription involving activation of the nuclear factor kappa B. *J. Biol. Chem.* 268:8466–8472.
11. Gruss, H.J., M.A. Brach, and F. Herrmann. 1992. Involvement of nuclear factor-kappa B in induction of the interleukin-6 gene by leukemia inhibitory factor. *Blood.* 80:2563–2570.
12. Tsuboi, A., M. Muramatsu, A. Tsutsumi, K. Arai, and N. Arai. 1994. Calcineurin activates transcription from the GM-CSF promoter in synergy with either protein kinase C or NF-kappa B/AP-1 in T cells. *Biochem. Biophys. Res. Commun.* 199:1064–1072.
13. Wang, C.Y., A.G. Bassuk, L.H. Boise, C.B. Thompson, R. Bravo, and J.M. Leiden. 1994. Activation of the granulocyte-macrophage colony-stimulating factor promoter in T cells requires cooperative binding of Elf-1 and AP-1 transcription factors. *Mol. Cell. Biol.* 14:1153–1159.
14. Himes, S.R., L.S. Coles, R. Katsikeros, R.K. Lang, and M.F. Shannon. 1993. HTLV-1 tax activation of the GM-CSF and G-CSF promoters requires the interaction of NF-kappa B with other transcription factor families. *Oncogene.* 8:3189–3197.
15. Mantovani, L., R. Henschler, M.A. Brach, R. Wieser, M. Lübbert, A. Lindemann, R.H. Mertelsmann, and F. Herrmann. 1990. Differential regulation of interleukin-6 expression in human fibroblasts by tumor necrosis factor-alpha and lymphotoxin. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 270:152–156.
16. Brach, M.A., H.J. Gruss, C. Sott, and F. Herrmann. 1993. The mitogenic response to tumor necrosis factor-α requires c-jun/AP-1. *Mol. Cell. Biol.* 13:4284–4290.
17. Kiehntopf, M., M.A. Brach, T. Licht, L. Karawajew, S. Petschauer, C. Kirschning, and F. Herrmann. 1994. Ribozyme mediated cleavage of MDR-1 mRNA, a possible approach to reversal multiple drug resistance phenotype in cancer chemotherapy. *EMBO (Eur. Mol. Biol. Organ.) J.* 13:4645–4652.
18. Dunn, S.M., L.S. Coles, R.K. Lang, S. Gerondakis, M.A. Vadas, and M.F. Shannon. 1994. Requirement for nuclear factor (NF)-kappa B p65 and NF-interleukin-6 binding elements in the tumor necrosis factor response region of the granulocyte colony-stimulating factor promoter. *Blood.* 83:2469–2479.
19. Kuczek, E.S., M.F. Shannon, L.M. Pell, and M.A. Vadas. 1991. A granulocyte-colony-stimulating factor gene promoter element responsive to inflammatory mediators is functionally distinct from an identical sequence in the granulocyte-macrophage colony-stimulating factor gene. *J. Immunol.* 146:2426–2433.
20. Shannon, M.F., L.S. Coles, R.K. Fielke, G.J. Goodall, C.A. Lagnado, and M.A. Vadas. 1992. Three essential promoter elements mediate tumor necrosis factor and interleukin-1 activation of the granulocyte-colony stimulating factor gene. *Growth Factors.* 7:181–193.
21. Nishizawa, M., and S. Nagata. 1990. Regulatory elements responsible for inducible expression of the granulocyte colony-stimulating factor gene in macrophages. *Mol. Cell. Biol.* 10: 2002–2011.
22. Arai, K., F. Lee, A. Miyajima, S. Miyatake, N. Arai, and T. Yokata. 1990. Cytokines: Coordinators of immune and inflammatory responses. *Annu. Rev. Biochem.* 59:783–836.
23. Nakajima, T., S. Kinoshita, T. Sasagawa, K. Sasaki, M. Naruto, T. Kishimoto, and S. Akira. 1993. Phosphorylation at threonine-235 by a ras-dependent mitogen-activated protein kinase cascade is essential for transcription factor NF-IL6. *Proc. Natl. Acad. Sci. USA.* 90:2207–2211.
24. Vietor, I., P. Schwenger, W. Li, J. Schlessinger, and J. Vilcek. 1993. Tumor-necrosis factor-induced activation and increased phosphorylation of mitogen-activated protein (MAP) kinase in human fibroblasts. *J. Biol. Chem.* 268:18994–18999.

Downloaded from http://rupress.org/jem/article-pdf/181/2/793/1106262/793.pdf by guest on 05 October 2022