Characterization of the Promoter for the Human Long Pentraxin PTX3

ROLE OF NF-κB IN TUMOR NECROSIS FACTOR-α AND INTERLEUKIN-1β REGULATION

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The “long pentraxins” are an emerging family of genes that have conserved in their carboxy-terminal halves a pentraxin domain homologous to the prototypical acute phase protein pentraxin (C-reactive protein and serum amyloid P component) and acquired novel amino-terminal domains. In this report, a genomic fragment of 1371 nucleotides from the human “long pentraxin” gene PTX3 is characterized as a promoter on tumor necrosis factor-α (TNFα) and interleukin (IL)-1β exposure in transfected 8387 human fibroblasts by chloramphenicol acetyltransferase and RNase protection assays. In the same cells, the PTX3 promoter does not respond to IL-6 stimulation. Furthermore, IL-1β and TNFα responsiveness is not seen in the Hep 3B hepatoma cell line. The minimal promoter contains one NF-κB element which is shown to be necessary for induction and able to bind p50 homodimers and p65 heterodimers but not c-Rel. Mutants in this site lose the ability to bind NF-κB proteins and to respond to TNFα and IL-1β in functional assays. Sp1- and AP-1 binding sites lying in proximity to the NF-κB site do not seem to play a major role for cytokine responsiveness. Finally, cotransfection experiments with expression vectors validate that the natural promoter contains a functional NF-κB site.

The human gene hPTX3 has been recently cloned from interleukin-1β (IL-1b)3-stimulated endothelial cells (1) and from tumor necrosis factor-α (TNFα)-stimulated fibroblasts (2). PTX3 belongs to the family of pentraxins (so named because they are assembled in pentamers) that include C-reactive protein (CRP) and serum amyloid P component (SAP) from several different species (3) and which are markers of the acute phase. Moreover, while the 3’ half of PTX3 can be aligned with the full-length sequences of CRP and SAP (4) (pentraxin domain), the 5’ half of the protein does not show significant homology with other known proteins. PTX3 is indeed the first isolated member of a new group of proteins, known as “long pentraxins,” which have different 5’-termini upstream from their pentraxin domains (5–9).

While the classical pentraxins CRP and SAP are almost exclusively produced by the liver in response to IL-6 in combination with IL-1 and TNF, PTX3 shows a more promiscuous response in that its expression in vitro can be induced in endothelial cells, hepatocytes, fibroblasts, and monocytes. In all cases, the gene is rapidly and directly induced by exposure to IL-1β, TNFα, and lipopolysaccharide (LPS), but not by IL-6, the mRNA peaking 4–6 h after the stimulation (1, 4, 10). This induction is paralleled by de novo transcription of the gene (10) and is transient in that no more message is detectable after 24 h (1, 10).

The mouse homologue, mPTX3, shows a similar exon/intron organization and 82% identity at the amino acid level with hPTX3 (4). When C57BL mice were injected i.v with LPS to induce an acute phase response, mPTX3 expression was markedly induced in vivo after 4 h in several muscular organs, including the heart and the thigh (4). In situ hybridization studies showed that endothelial cells within the muscular tissues were the major responder cell type. Interestingly, in striking contrast with CRP and SAP, no mRNA for mPTX3 could be detected by Northern analysis in the liver (4).

Similar promiscuous in vivo expression has also been observed for the other “long pentraxins” in organs as diverse as the brain and the testis (6–9).

To begin to understand the molecular mechanisms underlying the regulated expression of the first cloned long pentraxin PTX3, we cloned and characterized the promoter of hPTX3.

EXPERIMENTAL PROCEDURES

Cell Culture—The human fibrosarcoma 8387 (11) and the human hepatoma Hep 3B (12) cell lines were maintained in Dulbecco’s modified Eagle’s medium (Seromed, Biochrom KG, Berlin, Germany), supplemented with 10% fetal calf serum (Hyclone, Logan, UT), 50 μg/ml gentamycin (Life Technologies, Inc., Paisley, Renfewshire, United Kingdom) and 20 mM l-glutamine (Seromed). Plasmid Construction—A 1.37-kilobase EcoRI-ProII genomic fragment from Ap2 phage (1), which spans nucleotides -1317 to +54 relative to the transcription start site, was blunt-end subcloned into the XhoI-digested and blunt-ended pBl CAT 3 vector (13) (giving -1317-CAT). Following PstI-XhoI digestion, the latter plasmid was used as a substrate to generate a set of deletion clones with EcoIII (Stratagene, La Jolla, CA) digestion, mung (Stratagene) blunting, and subsequent religation. Deletion clones covering the whole 5’-flanking region of the hPTX3 gene were sequenced by the Sanger dideoxy method (14). Three of them, -387-CAT, -180-CAT, and -74-CAT, were subsequently used in functional assays.

The plasmid carrying a mutation in the -96 NF-κB site (-180 m NF-κB-CAT) was produced by polymerase chain reaction (PCR) tech-
The plasmid – 180 CAT – was used as a substrate for amplification with four synthetic oligonucleotides (Duotech srl, Milan, Italy): oligonucleotide I (5'-CTTACGCCAGCTGGCGG-3') was designed on a pUC-derived sequence from the pBL CAT 3 vector in sense orientation; oligonucleotide IV (5'-CAGCTGTTATACCATGGCAG-3') was designed on the same orientation. In antisense orientation, oligonucleotide I (5'-TGCTCTAGAATCTGAATTGGTGGGGGAGG-3') was designed in antisense orientation on hPTX3 promoter from nucleotide –115 to nucleotide –97 with an additional 11 nucleotides at the 5' end; 6 of them (underlined) give rise to a XbaI restriction site. Oligonucleotide III (5'-TGCTCTAGACCGTATACCATGGCAGTTCCACCAATATT-3', spanning from nucleotide –131 to nucleotide –101). This mutagenic primer, together with the flanking oligonucleotides I and IV and with the linker linearized wild-type –180 CAT plasmid as a substrate, was used to produce a DNA fragment carrying the desired mutations according to the PCR mutagenesis technique as described (15). Similarly, to obtain the –180 m AP-1-CAT mutant plasmid, we used a mutagenic primer (oligonucleotide VI 5'-CCACCGTATTACCATGTTCCACCATTCCACCAATATT-3' spanning from nucleotide –74 to nucleotide –46) carrying three substitutions (underlined) in the –65 AP-1 site. The double-mutant –180 m NF-B/Cat plasmid was used as a substrate for PCR reaction. The mutagenized fragments obtained were purified by polyacrylamide gel electrophoresis, eluted, and cut with HindIII/XbaI and XbaI/XhoI, respectively. Finally, the digested fragments were inserted into a HindIII/XhoI-digested pBL CAT 3 vector. The resulting construct (–180 m NF-B/CAT) carries a substitution of 8 bases at the NF-B site (AT-TC TTAGA instead of GGAAGCTT).

To obtain the mutant plasmid –180 m Sp1-CAT, we used an oligonucleotide carrying two mutations (underlined) in the –123 Sp1 site (oligonucleotide V 5'-CTTCCCCACCATACTCCCTCCCACCAATATT-3', spanning from nucleotide –131 to nucleotide –101). This mutagenic primer, together with the flanking oligonucleotides I and IV and with the linker linearized wild-type –180 CAT plasmid as a substrate, was used to produce a DNA fragment carrying the desired mutations according to the PCR mutagenesis technique as described (15). Similarly, to obtain the –180 m AP-1-CAT Sp1 mutant plasmid, we used a mutagenic primer (oligonucleotide V 5'-CCACCGTATTACCATGTTCCACCATTCCACCAATATT-3' spanning from nucleotide –74 to nucleotide –46) carrying three substitutions (underlined) in the –65 AP-1 site. The double-mutant –180 m NF-B/Sp1-CAT plasmid was used as a substrate for PCR reaction. The mutagenized fragments obtained were purified by polyacrylamide gel electrophoresis, eluted, and digested with HindIII/XhoI, and cloned into a pBl CAT 3 vector. All the PCR reactions above described were carried out using Pfu DNA polymerase (Stratagene).

Transfection and CAT Assays—8387 human fibrosarcoma (11) and Hep 3B (12) human hepatoma cells were grown to confluence, collected after trypsinization, and cultured at a density of 8 × 10^6 (8387 cells) or 1 × 10^6 (Hep 3B) in 100-mm dishes 24 h before the transfection. Cells were transfected by the calcium phosphate precipitation method (16) with 1 μg of CAT reporter plasmid together with 1 μg of poly(dI-dC) (Pharmacia Biotech, Uppsala, Sweden) in 15 μl of binding reaction buffer (40 mM Tris (pH 7.5), 120 mM NaCl, 0.5 mM EDTA, 40 mM PIPES, pH 6.7). The hybridization was carried out for 18 h at 55 °C. The hybridization buffer was then diluted 10 times in a buffer containing 10 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.1% N-lauroyl sarcosine, 0.5 unit/ml RNase A (Ambion, Austin, TX), and 100 units/ml RNase T1 (Ambion). Incubation was carried out for 45 min at 37 °C. In the same hybridization mixture, 5 × 10^6 cpm of a β-actin riboprobe transcribed from the plasmid pTR3-βACT-human (Ambion) by SP6 RNA polymerase were added.

After inactivation of the enzymes, the hybridization products were extracted in phenol-chloroform, precipitated, and loaded onto an urea/polyacrylamide gel 6% gel.

Electrophoretic Mobility Shift Assay—Nuclear extracts were prepared from 8387 cells that were stimulated with TNFa (500 units/ml) for 3 h or left untreated as described (19). The oligonucleotides utilized (Duotech) correspond to the NF-B site at position –96 and its flanking sequences both in a wild type (5'-AATTACGGGGAACTCCCGTTACC-3') and a mutated form (5'-AATTACGGGTACCCGGTTACC-3') (500 units/ml) and 1 μg of poly(dI-dC) (Pharmacia Biotech, Uppsala, Sweden) in 15 μl of binding reaction buffer (40 mM Tris (pH 7.5), 120 mM KCl, 5% Ficoll, 4 mM EDTA, 1 mM dithiothreitol, and 10% glycerol) for 20 min at room temperature. A 1000-fold molar excess of cold oligonucleotide was used for competition assays. Competition assays were performed using oligonucleotides carrying wild-type or mutant NF-B sites together with an oligonucleotide carrying a functional NF-B site from human immunodeficiency virus type 1 (HIV-1) long terminal repeat (LTR) (5'-GATTCAGAGGGGAACTTCCAGAGGAGC3'-3') which was also used as a standard binding reaction as a positive control. The resulting complexes were separated from the free probe by electrophoresis in a 5% native polyacrylamide gel in 0.5% Tris-buffered EDTA. In supershift analysis we used serum 1141 against an amino-terminal peptide of human p50 (21), serum 1126 raised against a carboxy-terminal peptide of human p65 (21), and serum 1136 raised against a carboxy-terminal peptide of human c-Rel (21); after 20 min of preincubation on ice, a standard binding assay was performed.

RESULTS

Cloning and Sequencing of the 5'-Flanking Region of hPTX3—To analyze the promoter region of the hPTX3 gene, genomic DNA sequences upstream the cDNA-encoding sequence were studied. An EcoRI-PvuII fragment, which spans nucleotides –1317 to +54 (and does not include the ATG) (Fig. 1A), was subcloned for further investigation in the pBl CAT 3 expression vector (13) and identified as –1317-CAT (Fig. 1B). A series of deletion mutants obtained by EcoRI/mung directional deletions was selected as shown in Fig. 1B.

The sequence of the 5'-flanking region of the hPTX3 gene (Fig. 1A) revealed features of an eukaryotic promoter, such as the presence of a number of potential binding sites for transcription factors. We have identified one NF-IL6, two NF-kB, one AP-1, two Pu.1, three PEA 3, one Ets-1, and two Sp1 consensus sequences (Fig. 1A). No obvious TATA or CAAT consensus box was found. The previously identified transcription start site (1), however, corresponds to a pyrimidine-rich 7-nucleotide consensus (22) sequence which has been reported to act in TATA-less promoters and is underlined in Fig. 1A.

While this article was in preparation, the sequence of the mPTX3 became available (23), and the alignment shows an overall 50.1% conservation, with the last 380 nucleotides showing a 66% conservation (Fig. 1A). Furthermore, two of the reported potential binding sites, the NF-kB and the AP-1 sites
at positions −96 and −65, respectively, are maintained at approximately the same positions in both sequences (Fig. 1A).

Functional Analysis of the Promoter—The deletion mutants schematically shown in Fig. 1B were used for expression studies in 8387 human fibrosarcoma cells and in Hep 3B human hepatoma cells. After calcium phosphate-mediated transfection of the cells, cultures were left untreated or were treated with TNFα or IL-1β for 24 h. Data from at least four separate experiments are shown in Fig. 2. The 21317-CAT construct shows a 5.3-fold basal activity with respect to the empty vector, and a comparable level is observed also with the 2387 construct, implying that the 1000 intervening nucleotides do not contribute significantly to this basal activity. In contrast, the 2180 construct has a 2-fold higher basal level, while further deletion up to 274 abolishes almost completely the activity.

TNFα exposure (gray bars) results in a 2.5-fold induction with the −1317, −387, and −180 CAT constructs, but it is completely inactive on the −74 construct. IL-1β exposure (hatched bars) induced a quite similar effect in the conditions tested. On the contrary, IL-6 assayed on 8387 cells transfected with the 21317 construct was completely inactive and did not modify the responsiveness to TNFα (data not shown). In the same experimental setting, an artificial CAT reporter construct containing four tandem NF-κB sites from IL-6 promoter (24) cloned into a pBL CAT 2 vector (13) gave a mean fold induction of 4.5 ± 0.35 times on TNFα induction compared with the untreated cells (data not shown). To validate these results with a different approach, we analyzed the 8387-transfected cells by RNase protection. As shown in Fig. 3, lane 1, the undigested riboprobe corresponds to the predicted size of 383 nucleotides. In the transfected 8387 cells (lanes 4–13), the protected band is 309 bp in all cases, thus

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**Fig. 1. Alignment of the human and murine PTX3 promoters and scheme of the deletion mutants.** A, nucleotide sequence of the hPTX3 promoter (h) aligned with the murine sequence (m). The transcription start sites are indicated as +1 and by arrows. Numbers on the right refer to the position in the 5′ regions. Consensus sequences for binding of transcriptional factors and the pyrimidine-rich region are underlined. The translation initiation codon is indicated. The EcoRI and PvuII sites are shown. B, the different constructs shown were used for transfection.
PTX3 Promoter

Fig. 1—continued

**B**

![Diagram](Imagex.png)

**Fig. 2.** CAT analysis of the transcriptional activity of the hPTX3 promoter. The different constructs were transfected in 8387 cells, subsequently treated or not with TNFα and IL-1β. Extracts were analyzed for CAT levels. Fold induction is referred to CAT levels with respect to empty vector (pBL CAT3)-transfected cells (which produces a mean value of 1.8% acetylation). Mean values and standard deviations from four independent experiments are shown.

NF-κB proteins to the wild-type sequence (lane 4) and, on the other hand, when used as a probe, did not show binding activity in either untreated (lane 6) and TNFα-treated (lane 7) 8387 cells.

Supershifting with antibodies clearly indicated that the two bands correspond to the p50/p65 heterodimer and to the p50/p50 homodimer, respectively (Fig. 4B, lanes 3 and 4). Furthermore, c-Rel is not present in this complex (lane 5) as demonstrated by the lack of supershifting, similar to what is observed with an irrelevant antibody (lane 6).

We further compared under the same experimental conditions the binding activity of 8387 nuclear extracts on an oligonucleotide containing the PTX3 NF-κB site and on an oligonucleotide containing a canonical NF-κB-binding site from HIV-1 LTR, which has been previously reported to give rise to only one retarded complex (20). As shown in Fig. 4C, while the binding on the PTX3 oligonucleotide gave rise to two retarded complexes, corresponding to p50/p50 and p50/p65 homo- and heterodimers, only the upper band was present in the binding to the HIV-1 LTR NF-κB site.

**The NF-κB Site Is Functionally Relevant in the hPTX3 Promoter**—To more directly assess the functional relevance of the NF-κB site, we mutagenized this site in the −180 construct, (−180 m NF-κB-CAT). The mutated sequence is identical to the degenerated oligonucleotide that we had used in the gel retardation experiments (Fig. 4A, lanes 6 and 7). When the −180 m NF-κB-CAT construct was analyzed by CAT analysis and RNase protection, it was evident that despite a detectable level of basal activity, cytokines exposure did not lead to any significant induction of its transcription (Fig. 2, −180 m NF-κB-CAT, and Fig. 3, lanes 10 and 11), thus implying an NF-κB mediated induction of the transcriptional activity of the hPTX3 promoter by TNFαs and IL-1β.

To validate the hypothesis that NF-κB is the key responsive element, we also mutagenized the Sp1 and AP-1 sites which are present in the minimal promoter (as shown in Fig. 1b). The −180 m Sp1-CAT was indeed still responsive (Fig. 2), although at a lower level compared with the wild type −180-CAT construct (1.7 mean fold induction over four separate experiments).

showing the use of the same transcriptional start site in the artificial constructs as in the wild-type gene (1).

While no precise measurement can be made for the baseline values of the different constructs among them due to possible variation in transfection efficiency, for each experimental group the untreated and treated cells can be compared. TNFα indeed increases the transcription of the three responsive constructs by 3–6-fold (as determined by densitometric scanning) (lanes 4–9), whereas construct −74 shows no activity in both unstimulated and stimulated cells (lanes 12 and 13). These data have been reproduced in three separate experiments.

To study the cellular specificity of the TNFα/IL-1β responsiveness, we transfected the same constructs in the human hepatoma cell line Hep 3B; although PTX3 mRNA is inducible in these cells by TNFα and IL-1β exposure (1), we could not observe, either in unstimulated or in TNFα-stimulated cells, a significant CAT activity with respect to the empty vector-transfected cells (data not shown) despite a good transfection efficiency. On the other hand, the artificial CAT reporter construct containing four tandem NF-κB sites (see above) showed, under the same experimental conditions, a full responsiveness to TNFα (−15-fold; data not shown). All these data together imply that further genomic elements or posttranslational modifications may crucially contribute to the hepatic transcription of PTX3 detectable in vitro.

**NF-κB p50 and p65 Can Bind to the hPTX3 Promoter**—The sharp difference in the activity between −180 and −74, the presence of an NF-κB element at position −96 (Fig. 1A), and the known effect of NF-κB in TNFα- and IL-1β-mediated responses prompted us to analyze the involvement of NF-κB in PTX3 regulation by electrophoretic mobility shift assay. An oligonucleotide corresponding to the sequence from position −103 to −81 of hPTX3 was utilized. A low level of binding activity was detectable in untreated 8387 cells as two separate bands (Fig. 4A, lane 1), and they were clearly increased after a 3-h TNFα stimulation (lane 2). The specificity of the binding activity is documented by the complete competition with the cold specific oligonucleotide (lane 3) and with an oligonucleotide containing a canonical NF-κB site from HIV-1 LTR (lane 5) (20).

We also generated a mutant hPTX3 NF-κB oligonucleotide which did not contain a NF-κB-binding site. This mutated oligonucleotide was unable to compete for the binding of the
upon TNFα induction). As expected, also the double mutant −180 m NF-κB/Sp 1-CAT is not responsive to TNFα and IL-1β stimulation, although it retains a basal activity. On the other hand, the AP-1 mutant −180 m AP-1-CAT shows full cytokine responsiveness (3.8 and 2.7 mean fold induction with TNFα and IL-1β, respectively), but a much lower basal level of CAT expression (Fig. 2).

To quantify the observed stimulations with respect to canonical NF-κB sites, we made use in the same experimental setting, of an artificial CAT reporter construct containing four tandem NF-κB sites derived from IL-6 promoter (24) and cloned in a pBl CAT 2 vector (13). This reporter gave a mean fold induction of 4.5 ± 0.35 upon TNFα stimulation relatively to untreated cells (data not shown), therefore of comparable entity to those observed with the PTX3 constructs.

**Cotransfection with Expression Vectors**—To further substantiate that the NF-κB site is indeed the main functional responsive element, we cotransfected 8387 cells with the −180-CAT and with the −180 m NF-κB-CAT constructs as reporters together with p50 and p65 NF-κB expression vectors (17) either alone or in combination. As shown in Fig. 5, top, cotransfection of p50 alone did not modify the basal CAT activity by the construct in the wild type configuration, as compared with cells transfected with the empty vector pRSPA, while p65 and the combination of the two increased the basal activity by a factor of 3.6 (p50/p65) to 3.9 (p65 alone). The addition of TNFα was effective in all the experimental conditions tested (3, 2.6, 1.7, and 1.7 fold induction over the unstimulated cells in pRSPA, p50, and p50/p65 transfected cells respectively). On the other hand, overexpression of p65 alone, or in combination with p50, as well as addition of TNFα had no effect on the −180 m NF-κB-CAT construct carrying the mutation in the −96 NF-κB site (Fig. 5, bottom). These data are consistent with the hypothesis that the hPTX3 natural promoter contains a functional NF-κB site.

Under the same experimental conditions, the control reporter plasmid containing four NF-κB binding sites from the IL-6 promoter was induced by p50/p65 overexpression by 5.9-fold, while no induction was detectable against a pSV2 CAT reporter plasmid utilized as a negative control (not containing
PTX3 belongs structurally to the family of the classical acute phase protein pentraxins, which include CRP and SAP, in several animal species. Both genes are characteristically induced by IL-6 in combination with IL-1 and TNFα, mainly if not exclusively, in hepatocytes (25–31). PTX3 was the first cloned member of the newly emerging group of “long pentraxins,” (5–9) because they show a long amino-terminal domain fused to the carboxy-terminal pentraxin domain (corresponding to most of the classical pentraxin sequence). The significance of this genetic acquisition is far from being understood, but all the long pentraxins do not show a liver-restricted expression pattern and seem to be expressed in a much wider spectrum of organs, such as the brain and the testis (6–9). Furthermore, hPTX3 was shown to be transcribed after exposure to IL-1β, TNFα, and the bacterial product LPS (i.e. all prototypical proinflammatory signals) but not by IL-6, in several different cell types, including endothelial cells, fibroblasts, hepatocytes, and monocytes (1, 10). Furthermore, PTX3 expression induced by IL-1β is not modified in endothelial cells and hepatocytes by concomitant exposure to IL-6 (data not shown). The same gene was cloned in TNFα-stimulated fibroblasts, named TSG-14 (2), and demonstrated to be directly induced by TNFαs (32, 33).

Inducibility by IL-1β and TNFα, but not by IL-6, may correlate well with the demonstrated role of NF-κB (for review, see Refs. 34–36) and, furthermore, with the presence of only one NF-IL 6 binding site (37, 38) and with the absence of APRF elements (39–41) in the human promoter. Both elements, in fact, have been demonstrated to be necessary in multiple copies for IL-6 inducibility (42).

The murine gene (82% identical at the amino acid level) shows a similar exon/intron organization and is localized on a syntenic chromosomal region (4). It is induced in vitro only in peritoneal macrophages, in some fibroblasts, and in very few endothelial cell lines, but not in hepatocytes; on the other hand, it was induced in vivo in several muscular tissues after LPS i.v. injection (an acute phase experimental model), but not in the liver (4, 32). In addition, in situ hybridization studies have indicated that in the heart and in the thigh, the endothelial cells were the most abundant producer cell type (4).

The alignment between the human and the murine promoters shows a high overall degree of conservation, including few hypothetical binding sites for transcription factors, in particular the NF-κB site which is here demonstrated as functionally important for the hPTX3 gene. What are the structural reasons for the differences in the expression of PTX3 between humans and mice is still unclear.

The reported lack of consensus sites for hepatic transcription factors in the murine promoter (and in the human promoter as well) may account in part for the absence of induction in the liver (another obvious difference with the classical CRP and SAP genes (3), but the positive elements required for its inducibility in the endothelial cells of the muscular district have yet to be elucidated. On the other hand, recent work with transgenic animals has shown, in the case of CRP, that the precise characterization of the functional elements required for the in vivo “acute phase” inducibility may require a complex interaction between 5’ and 3’ elements (43), which was unexpected on the basis of previous in vitro studies (27–29, 31).

We have described the functional role of the NF-κB site in the promoter of the hPTX3 gene for TNFα inducibility in fibroblasts. We can only speculate at the moment, on the basis of the large amount of published data, that this same site may be relevant also for LPS inducibility of the gene in fibroblasts as well as in other cell types. NF-κB may interact with other factors as suggested by others (44–48), particularly in view of the presence of AP-1 and Sp1 sites in close proximity to the

**FIG. 5.** Effect of NF-κB p50 and p65 overexpression on hPTX3 wild-type and mutant promoter. 8387 cells were cotransfected with the −180-CAT (top) or the −180 m NF-κB-CAT construct (bottom) and with expression vectors containing NF-κB p50 and p65 cDNAs driven by the Rous sarcoma virus promoter. Fold induction refers to the CAT activity of the different CAT constructs measured in presence of NF-κB-containing plasmids relative to those measured with the empty pRSPA vector only (to which a value of 1.0 was assigned). White and gray bars, untreated and TNFα-treated cells, respectively.

a NF-κB element) when cotransfected with p50 and p65 in combination (data not shown).

**DISCUSSION**

In this report, we characterize the promoter of the human PTX3 gene (hPTX3). A genomic fragment of 1317 bp, located 5’ to the transcriptional start site, responds to TNFα and IL-1β stimulation in transiently transfected human 8387 fibroblasts but not in human hepatoma Hep 3B cells, as measured by transfection and CAT assays (more than 2-fold induction) and by RNase protection analysis (3–6-fold induction). Deletion mutants show that the 180 bp more proximal to the start site are sufficient for TNFα- and IL-1β-inducible transcriptional activity. On the contrary, the last 74 bp are unresponsive. In the intervening 106 bp we show that a classical NF-κB binding site is present and furthermore that p50/p50 homodimers and p50/p65 heterodimers can bind to this element after incubation with nuclear extracts from 8387 fibroblasts. TNFα exposure increases this NF-κB activity, while the minimal construct carrying an inactivating mutation of this site loses the TNFα inducibility in the same cells. Finally, we confirmed the hypothesis that NF-κB proteins are functionally active on the hPTX3 promoter by cotransfection with p50 and p65 NF-κB expression vectors. On the contrary, Sp1 and AP-1 do not seem to play a major role for the cytokine inducibility of the gene.

These data show for the first time that a classical NF-κB complex can functionally interact with the “long pentraxin” hPTX3 promoter in human fibroblasts after exposure to TNFα. The different methods utilized indicate a 2–5-fold transcriptional induction of the gene, which is in agreement with the observed increase in nuclear runoff experiments on isolated monocytes (10).
NF-κB site, and of the fact that they both have been reported to interact functionally with NF-κB complexes (44, 48). Indeed, the Sp1 mutant shows a reduced TNFα and IL-1β inducibility, while the AP-1 mutant is fully responsive to cytokines, although its basal level of expression is significantly reduced. Further work will be required to directly address the possible interplay of different transcription complexes on the hPTX3 promoter.

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