Human macrophage inflammatory protein-3α/CCL20/LARC/Exodus/SCYA20 is transcriptionally upregulated by tumor necrosis factor-α via a non-standard NF-κB site

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Abstract The 5′-flanking sequences of the human macrophage inflammatory protein-3α/CCL20 gene were cloned and transfected into G-361 human melanoma cells in a luciferase reporter construct. Tumor necrosis factor-α (TNF-α) treatment stimulated luciferase expression, and promoter truncations demonstrated that TNF-α inducibility is conferred by a region between nt −111 and −77, which contains a non-standard nuclear factor-κB (NF-κB) binding site. The requirement for NF-κB was demonstrated as follows: (i) mutations in this NF-κB site abrogated TNF-α responsiveness; (ii) TNF-α activated a construct containing two copies of the CCL20 NF-κB binding site; (iii) overexpression of NF-κB p65 activated the CCL20 promoter; (iv) NF-κB from nuclear extracts of TNF-α-stimulated cells bound specifically to this NF-κB site.

Key words: CCL20; Macrophage inflammatory protein-3α; Tumor necrosis factor-α; Chemokine; Nuclear factor-κB; Transcriptional; Promoter

1. Introduction

Chemokines comprise a large group of closely related proteins which play important roles in inflammation and immune response regulation. Due to a conserved pair of cysteine residues, chemokines are subdivided into two main groups, the CC and CXC chemokines. Other chemokine subfamilies have also been identified, the C and CX_3C chemokines. Chemokines can attract various different cell types, including effector cells, into areas of inflammation. They also regulate immune homeostasis by coordinating lymphocyte and dendritic cell (DC) trafficking, and are involved in the regulation of hematopoiesis and angiogenesis [1,2].

Human macrophage inflammatory protein-3α (MIP-3α)/CCL20 [3], also called liver and activation-induced chemokine (LARC) [4], Exodus [5] or SCYA20, is a CC chemokine which attracts memory T lymphocytes and immature DCs [6–9]. CCL20 binds to the chemokine receptor CCR6, which is expressed on immature DCs and memory T lymphocytes, but is also found on B lymphocytes [10–17]. CCL20 mRNA is highly expressed in lung, liver and inflamed tissues, such as tonsil, appendix and lesional psoriatic skin [3–5,7,10]. The cellular source of CCL20 is mainly of epithelial origin, such as keratinocytes, but it is also expressed by other cell types, including endothelial cells, fibroblasts, and monocytes [3–5,7,18,19]. CCL20 is released upon stimulation of cells with various pro-inflammatory cytokines, such as interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), IL-17 and interferon-γ (IFN-γ), but also by other stimuli, including lipopolysaccharide, 12-*O*-tetradecanoylphorbol-13-acetate (PMA) and viral infection [7,18].

The mechanism by which CCL20 expression is regulated by pro-inflammatory cytokines such as TNF-α has not been analyzed in detail so far, although evidence exists for both transcriptional and post-transcriptional regulation [18,20]. However, TNF-α is known to frequently act via the transcription factor nuclear factor-κB (NF-κB), which is therefore likely to be involved in regulation of CCL20 expression [20]. NF-κB is a transcription factor complex containing the proteins p50 (NF-κB1), p52 (NF-κB2), p65 (RelA), c-Rel and RelB [21]. Various homo- or heterodimers can bind to NF-κB recognition sites located in regulatory regions of many genes, but the most common complex is the p50/p65 heterodimer. Activation of NF-κB is associated with phosphorylation and proteolytic degradation of the inhibitory protein IκB through activation of the IκB kinase complex [22,23]. Since NF-κB plays a pivotal role in inflammatory and immune responses, we investigated whether this transcription factor is involved in regulation of CCL20 expression. We have cloned and functionally characterized the CCL20 promoter, and show that stimulation with TNF-α can cause NF-κB-dependent activation of the CCL20 promoter and have further identified an element which is bound and activated by NF-κB.

2. Materials and methods

2.1. Reagents

Recombinant human TNF-α was purchased from Genzyme. For experiments using HT-29 cells, recombinant human TNF-α from R&D Systems was used.

2.2. Plasmids

The 5′-flanking region of the human CCL20 gene (nt −626 to +58 relative to the transcription start) was cloned by PCR from human genomic DNA isolated from human peripheral blood leukocytes. The

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Abbreviations: IL-1, interleukin-1; TNF-α, tumor necrosis factor-α; PMA, 12-*O*-tetradecanoylphorbol-13-acetate; IL-8, interleukin-8; EMSA, electrophoretic mobility shift assay

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primers were designed according to the genomic sequence using the primer (sense) 5′-CATCATCATGGTACCAAATCAAGGTGAAG-CTGAGGTTTGAGC-3′ and the primer (antisense) 5′-CATCAT-CATCTCGAGGGTTTTTAGCTCAAAGAACAGATCTGC-3′ (restriction sites for cloning are underlined). The amplified PCR product was digested with *Kpn*I and *Xho*I and cloned into the *Kpn*I and *Xho*I sites of the pGL2-basic vector (Promega). Serial 5′-truncations of the CCL20 promoter were created by PCR using the original fragment as template. The following primers were used: primer 5′-CATCATGGTACCTGTGTGGGGCTGACC-3′ (S4: nt −358 to +58 relative to the transcription start); primer 5′-CATCATGG-TACCGGATGAAAGTCTTTTCTG-3′ (S5: nt −288 to +58 relative to the transcription start); primer 5′-CATCATGGTACCTGGGGC-CAGTTGATC-3′ (S6: nt −111 to +58 relative to the transcription start) and primer 5′-CATCATGGTACCGGCAACACGCCTTCTG-3′ (S7: nt −77 to +58 relative to the transcription start). These primers were used in combination with the antisense primer for amplification. The fragments were subcloned into the *Kpn*I and *Xho*I sites of the pGL2-basic vector. The truncated interleukin-8 (IL-8) promoter-luciferase construct (nt −101 to +40 relative to the transcription start) has been described previously [24].

For introduction of mutations into the putative CCL20 NF-κB binding site primer 5′-CATCATGGTACCTGGGGCCAGTTGAT-CAATGGGGAAggggCCATG-3′ was used (mutated nucleotides are written in small letters). To alter the putative CCL20 NF-κB binding site into the NF-κB binding site of the IL-8 promoter, the following primer was used: 5′-CATCATGGTACCTGGGGCCAGTTGAT-CAATGG**GGAATTTCC**CATG-3′ (the IL-8 NF-κB binding site is written in bold).

These primers were used in combination with the antisense primer for amplification and fragments were cloned into the *Kpn*I and *Xho*I sites of pGL2-basic vector.

The construct tkLUC contains 155 bp of the thymidine kinase (tk) promoter of the herpes simplex virus I cloned into pGL2-basic vector [25]. The construct 2×(CCL20)NF-κB tkLUC contains two copies of the sequence CCL20-NF-κB: 5′-GATCAATGGGGAAAACCCCA-TGTGGCAACACG-3′ upstream of tkLUC in pGL2-basic vector. The construct pRL-tk was constructed by cloning the 155 bp of the tk promoter into the *Bgl*II and *Hin*dIII sites of the pRL-basic vector (Promega). All constructs were confirmed by sequencing.

The pRcCMV-p65 expression plasmid was kindly provided by W.C. Greene (Gladstone Institute of Virology and Immunology, San Francisco, CA, USA).

2.3. Cell culture, transient transfections and luciferase assays

The human melanoma cell line G-361 was cultivated in minimal essential medium+5% fetal calf serum (FCS) and passaged twice per week. Transfections were performed as described previously [24]. Luciferase activity in cell lysates was determined using the luciferase assay system (Promega) and recorded in a Microbeta Wallac Jet luminometer.

The human embryonic kidney cell line HEK293 was cultivated in RPMI 1640 supplemented with 10% FCS. For transfections 2.4×10^5 cells were seeded into each well of a six well plate and transfected with 1.7 μg reporter gene construct, 0.34 μg pcDNA3.1 vector or p65 expression plasmid together with 100 ng pRL-tk and 1.7 μl lipofectamine for 5 h at 37°C in FCS-free RPMI. For assaying firefly and renilla luciferase expression in the same samples, the dual luciferase assay system (Promega) was used.

The human colon adenocarcinoma cell line HT-29 was cultivated in Dulbecco's modified Eagle's medium supplemented with 10% FCS.

2.4. Reverse transcription-PCR (RT-PCR)

RT-PCR was performed according to standard protocols. For amplification the following primers were used: CCL20 sense: 5′-GAACTGGGTACTCAACACTGAGCAG-3′, CCL20 antisense: 5′-TTTACTGAGGAGACGCAC-3′, IL-8 sense: 5′-ATGACTTCCAA-GCTGGCCGTGGCT-3′, IL-8 antisense: 5′-TCTCAGCCCTCTT-CAAAAACTTCTC-3′, β-actin sense: 5′-ATGGGTCAGAAGGA-TTCCTATGTG-3′, β-actin antisense: 5′-CTTCATGAGGTAGTC-AGTCAGGTC-3′.

PCR was performed at 94°C for 2 min for denaturation, then 94°C for 20 s, 58°C for 20 s, 72°C for 30 s (for detection of CCL20 and IL-8 cDNAs 38 cycles and for detection of β-actin 30 cycles were performed) and a final extension at 72°C for 5 min using a Perkin-Elmer 9700 thermal cycler.

2.5. Electrophoretic mobility shift assays (EMSAs)

Nuclear extracts and EMSA experiments were prepared from G-361 cells and HT-29 cells as described previously [24]. For EMSA experiments, the following oligonucleotides were used: CCL20-NF-κB: 5′-GATCAATGGGGAAAACCCCATGTGGCAACACG-3′, CCL20-NF-κB mut: 5′-GATCAATGGGGAAggggCCATGTGG-CAACACG-3′, HIV NF-κB: 5′-GATCTTGTTACAAGGGACTT-TCCGCG-3′.

For supershift analysis, goat anti-p50 and rabbit anti-p65 (Santa Cruz) were used. 2 μl of each antibody was used per sample in EMSA analysis.

3. Results

3.1. Cloning of the 5′-flanking sequences of the CCL20 gene and functional characterization in G-361 cells

Expression of CCL20 has been shown to be induced by various pro-inflammatory cytokines, such as IL-1, TNF-α, IFN-γ and IL-17 [7,18] and stimuli such as PMA [4] and viral infection [19] in various cell lines and primary cells. Some cell lines respond to combinations of cytokines, such as IL-1 and TNF-α, or TNF-α and IL-17, by synergistic enhancement of CCL20 expression [7,18]. Since little is known about the transcriptional regulation of this chemokine, we cloned the 5′-flanking sequences of the CCL20 gene and analyzed the effect of TNF-α on activation of the CCL20 promoter.

The genomic sequence of the CCL20 gene is now available in Ensembl (clones AC027560 and AC073065). Meanwhile, the genomic structure of the CCL20 gene was also published by Nelson et al. [26]. We analyzed the 5′-flanking region of the CCL20 gene for potential transcription factor binding sites and demonstrate the presence of a putative TATA- and CAAT-box, as well as potential binding sites for various transcription factors, including NF-κB (Fig. 1). Nelson et al. suggested the transcription start to be located 81 bases upstream of the initiating AUG on the basis of the promoter analysis program Proscan [26]. However, Hieshima et al. analyzed the transcription start by 3′-rapid amplification of cDNA ends PCR using RNA derived from PMA-stimulated U937 cells and screening of a cDNA library from human liver. They indicated the transcription start to be located 58 bp upstream of the start codon AUG [4], which we have adopted for the current study. We cloned the CCL20 promoter by PCR from human genomic DNA and subcloned the PCR fragment into the pGL2-basic vector, to drive expression of the firefly luciferase coding region.

For transient transfection experiments we chose the human melanoma cell line G-361, which has been utilized by us in the past for transient transfection experiments using the IL-8 promoter, driving NF-κB-dependent reporter gene expression [24,25]. Although levels of endogenous CCL20 produced by G-361 cells are very low (data not shown), this cell line has proven to be useful for promoter analysis and responds well to stimulation with TNF-α.

The CCL20 promoter (nt −626 to +58)-luciferase construct was transiently transfected into the human melanoma cell line G-361. Stimulation of transfected cells with TNF-α (200 U/ml) for 24 h resulted in a 6-fold enhancement of luciferase activity. Various 5′-truncations of the promoter were constructed and cloned into the pGL2-basic vector. The truncated CCL20 promoter constructs S4 (nt −358 to +58), S5 (nt −288 to +58) and S6 (nt −111 to +58) were transiently transfected into G-361 cells. Stimulation of transfected cells with TNF-α (200 U/ml) for 24 h resulted in a 6–10-fold stim-

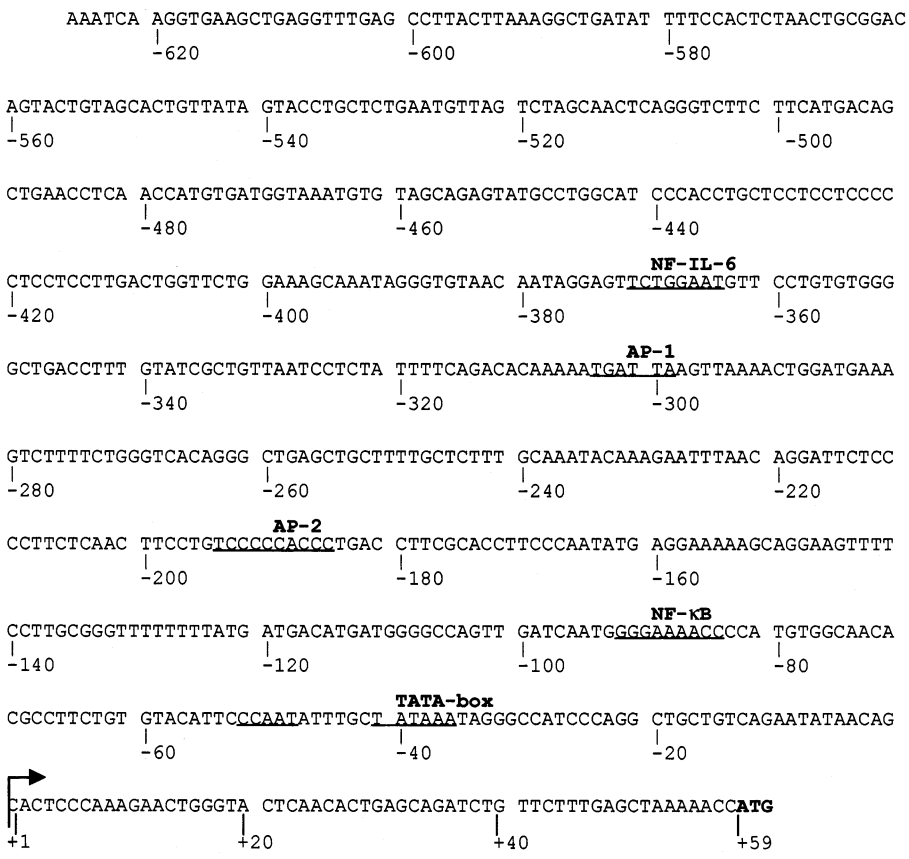


Fig. 1. Schematic representation of the 5′-flanking sequences of the CCL20 gene. The putative transcription start is marked as +1 and indicated by an arrow. The start codon ATG at position +59 is written in bold. The TATA-box and other potential binding sites for transcription factors are underlined.

ulation of luciferase activity (Fig. 2a). The activation of the CCL20 promoter reporter gene constructs by TNF-α was compared with that of a truncated IL-8 promoter (−101 to +40)-luciferase construct, which contains a functional NF-κB binding site and is activated in G-361 cells. TNF-α induces a 25-fold activation of the IL-8 promoter, which was higher compared to the 6-fold activation of the short CCL20 promoter construct S6 (Fig. 2a).

When a further 34 bp were removed from the 5′-end of the short CCL20 promoter construct S6 (nt −111 to +58) resulting in construct S7 (nt −77 to +58), the TNF-α inducibility was abrogated, indicating the presence of regulatory sequences within this region (Fig. 2a). Analysis of the CCL20 promoter sequence showed that the sequence 5′-GGGGAAAACCCC-3′ located between nt −93 and −82 represents a putative binding site for NF-κB. Pro-inflammatory cytokines, such as IL-1 and TNF, are known activators of NF-κB, which is involved in regulation of various chemokine genes [27]. Regulation of CCL20 expression through activation of NF-κB has been suggested by Izadpanah et al., who infected the human colon adenocarcinoma cell line HT-29 with a recombinant adenovirus expressing a mutant IκBα protein, which acts as a superrepressor of NF-κB activation. They showed that stimulation of infected cells with different inflammatory stimuli causes a significant reduction in CCL20 release [20]. To determine whether NF-κB is directly involved in the activation of the CCL20 promoter by TNF-α, we introduced mutations into the truncated CCL20 promoter (S6, nt −111 to +58). We transfected G-361 cells with the construct S6 containing mutations in the putative NF-κB binding site (CCL20 S6 NF-κB mutated) and saw a loss of responsiveness to TNF-α, suggesting that this site does indeed represent a binding site for NF-κB. In contrast, replacement of the CCL20 NF-κB binding site by the NF-κB binding site of the IL-8 promoter (CCL20 S6 NF-κB IL-8) resulted in the same levels of inducibility by TNF-α as the wild-type construct S6 (Fig. 2b).

Two copies of the element containing the putative NF-κB binding site were cloned upstream of a 155 bp fragment of the herpes simplex virus I tk promoter, to determine whether TNF-α can activate via this site in a different promoter context. Transfection of G-361 cells with a reporter gene construct containing only the tk promoter (tkLUC) and treatment with TNF-α for 24 h did not enhance luciferase expression. In contrast, transfection with the construct containing two copies of the CCL20 NF-κB sequences fused to the tk promoter (2×(CCL20)NF-κB tkLUC) resulted in a 5-fold induction of luciferase expression after TNF-α treatment (Fig. 2c).

3.2. *The NF-κB subunit p65 (RelA) transactivates the CCL20 promoter*

To analyze whether NF-κB can physically bind and transactivate the CCL20 promoter in a different cell system, HEK293 cells were cotransfected with various CCL20 promoter constructs together with an expression plasmid for

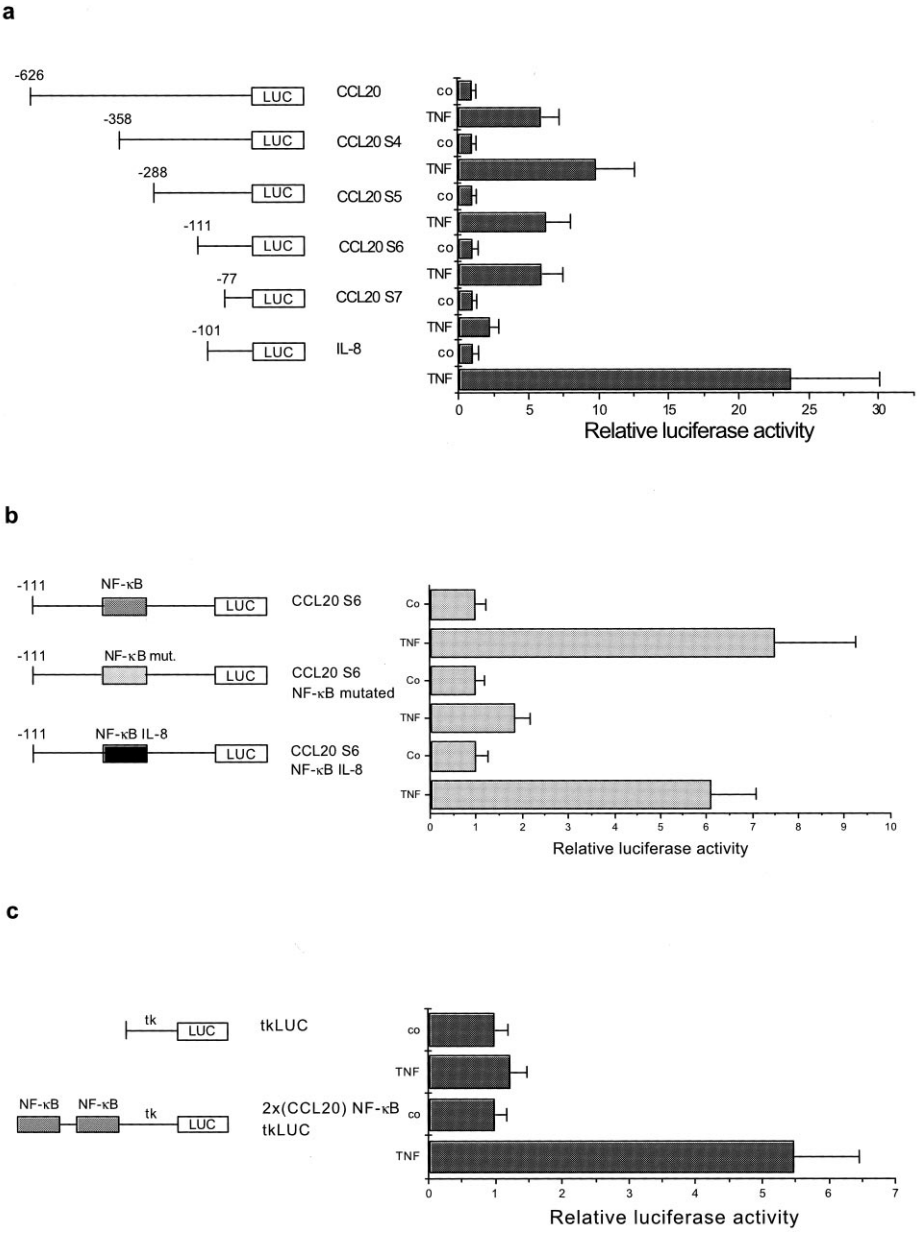


Fig. 2. a: The CCL20 promoter is activated by TNF-α in the human melanoma cell line G-361. The CCL20 promoter (nt −626 to +58 relative to the transcription start), serial 5′-truncations thereof (S4: nt −358 to +58; S5: nt −288 to +58; S6: nt −111 to +58; S7: nt −77 to +58 relative to the transcription start) and the truncated IL-8 promoter (nt −101 to +40 relative to the transcription start) were cloned upstream of the firefly luciferase coding region into the pGL2-basic vector. Transient transfections of G-361 cells and further 24 h stimulation with recombinant human TNF-α (200 U/ml) were performed. Relative luciferase activities compared to untreated controls are shown as means ± S.D. from five independent experiments. b: G-361 cells were transiently transfected with the truncated CCL20 promoter construct S6 (nt −111 to +58), construct S6 containing a mutated NF-κB binding site (CCL20 S6 NF-κB mutated), or construct S6 containing the NF-κB binding site of the IL-8 promoter (CCL20 S6 NF-κB IL-8). Relative luciferase activities after a 24 h stimulation with TNF-α compared to untreated controls are shown as means ± S.D. from eight independent experiments. c: G-361 cells were transiently transfected with either the construct tkLUC or a construct containing two copies of the CCL20 NF-κB binding site upstream of the tk promoter (2×(CCL20)NF-κB tkLUC) and stimulated with TNF-α for 24 h. Relative luciferase activities compared to untreated controls are shown as means ± S.D. from five independent experiments.

the NF-κB subunit p65 (RelA). Cotransfection of p65 together with the minimal CCL20 promoter (S6, nt −111 to +58) showed a robust 300-fold activation of this promoter by overexpression of p65. In comparison, overexpression of p65 showed only little activation of the CCL20 construct S7 (nt −77 to +31) which lacks the NF-κB binding site (Fig. 3a). This minor effect may result from some interference of overexpressed p65 with the internal control vector pRL-tk.

Similar results were obtained when the p65 expression plasmid was cotransfected with 2×(CCL20)NF-κB tkLUC. Overexpression of p65 resulted in a 50-fold increase in luciferase activity, but had little effect when cotransfected with tkLUC (Fig. 3b).

3.3. NF-κB binds to a site located in the CCL20 promoter

Nuclear extracts were prepared from G-361 cells, either un-

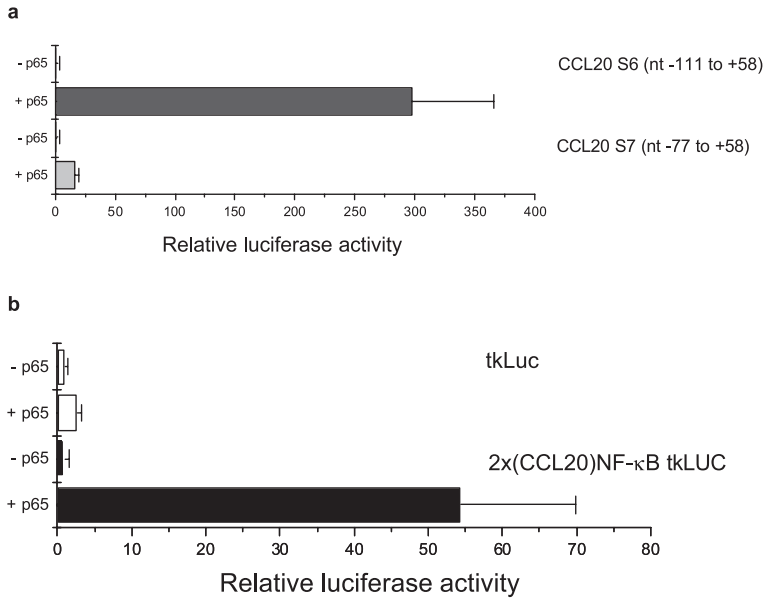


Fig. 3. The minimal CCL20 promoter is activated by overexpression of p65 in HEK293 cells. a: HEK293 cells were transiently cotransfected with the CCL20 promoter construct S6 (nt −111 to +58) (dark gray bars) or S7 (nt −77 to +58) (light gray bars) together with either the pcDNA3.1 vector or the p65 expression plasmid. The pRL-tk vector was used as internal control for transfection efficiency. Firefly luciferase activities were normalized to renilla luciferase activities. Relative luciferase activities from p65-transfected cells compared to vector-transfected cells are shown as means ± S.D. from four independent experiments. b: HEK293 cells were cotransfected with the construct tkLUC (white bars) or 2×(CCL20)NF-κB tkLUC (black bars) together with either the pcDNA3.1 vector or the p65 expression plasmid. The pRL-tk vector was used as internal control for transfection efficiency. Firefly luciferase activities were normalized to renilla luciferase activities. Relative luciferase activities compared to vector-transfected cells are shown as means ± S.D. from four independent experiments.

stimulated or stimulated with TNF-α (200 U/ml). EMSAs, using a radiolabelled oligonucleotide representing the NF-κB-binding site of the CCL20 promoter, demonstrated the presence of binding activity in nuclear extracts from TNF-α-stimulated G-361 cells. Competition experiments using excess of unlabelled oligonucleotide demonstrated that this binding activity was specific. When an excess unlabelled oligonucleotide containing mutations in the NF-κB binding site was used, no competition of the binding complex was seen. However, the complex was competed by an excess of unlabelled oligonucleotide representing the NF-κB binding site of the HIV LTR, suggesting that this binding activity is indeed NF-κB. Supershift analysis using antibodies directed against the NF-κB subunits p50 and p65 demonstrated that the complex contains both of these proteins (Fig. 4a).

This conclusion was further supported by performing EMSA analysis with a radiolabelled oligonucleotide representing the NF-κB binding site of the HIV LTR and nuclear extracts from TNF-α-stimulated G-361 cells. This complex migrated at the same speed as the complex formed on the CCL20 NF-κB binding site and was specifically competed by excess of the oligonucleotide representing the CCL20 NF-κB binding site but not with the oligonucleotide containing the mutated CCL20 NF-κB binding site (Fig. 4b).

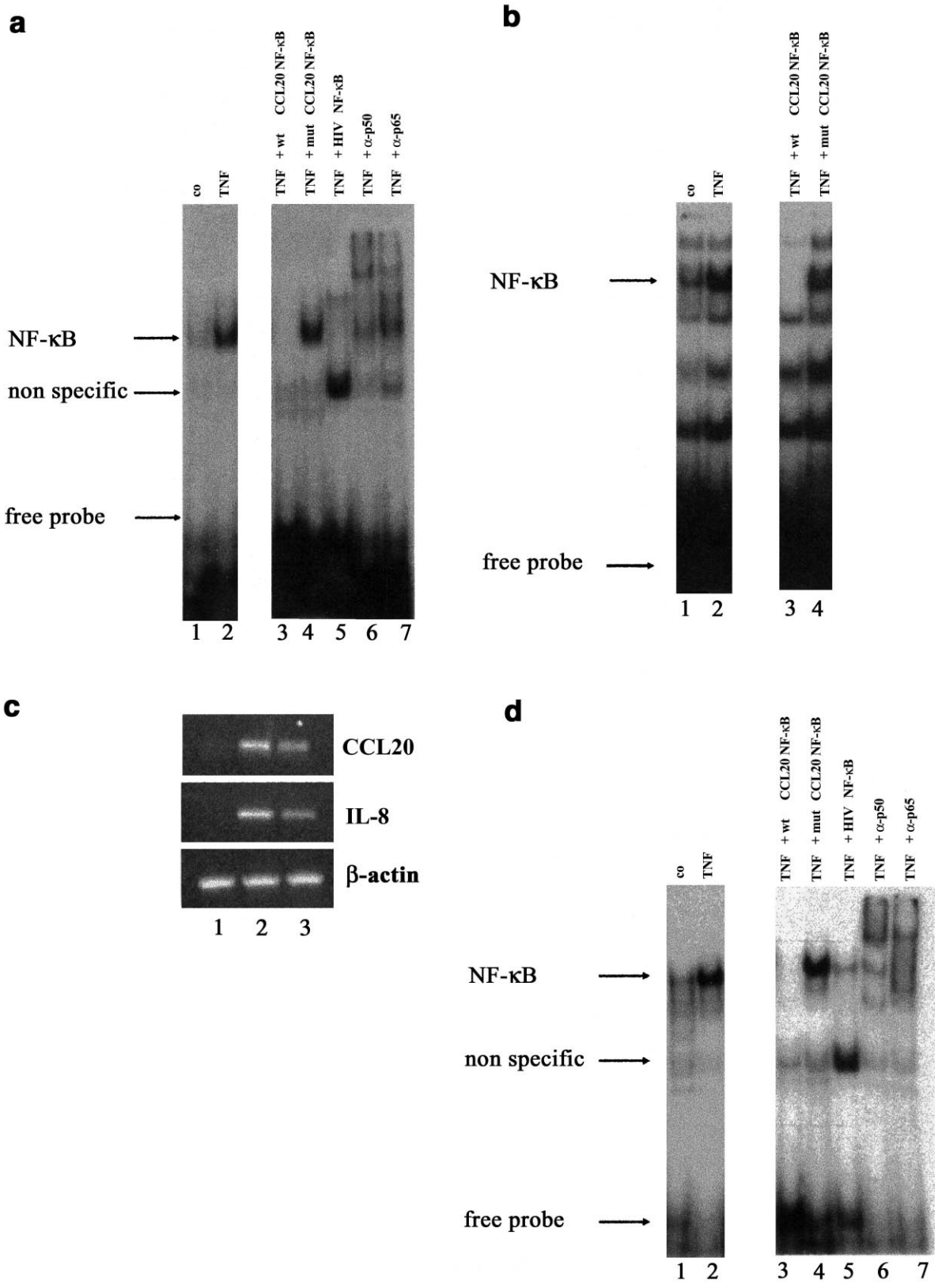
Similar experiments were performed with nuclear extracts from TNF-α-stimulated HT-29 cells, which have been analyzed by Izadpanah et al. for expression of CCL20 expression [20]. To determine whether CCL20 is regulated in HT-29 cells under our experimental conditions, we performed RT-PCR with RNA from HT-29 cells stimulated with TNF-α (10 ng/ml; R&D Systems) for 3 and 6 h using primers recognizing the CCL20 cDNA. The expression of CCL20 transcripts was compared with that of IL-8, which is known to be induced by pro-inflammatory stimuli. Expression of both CCL20 and IL-8 mRNA was induced by TNF-α in HT-29 cells (Fig. 4c).

EMSA analysis from nuclear extracts of TNF-α-stimulated HT-29 cells showed a specific complex binding to the NF-κB binding site of the CCL20 promoter containing the NF-κB subunits p50 and p65. This result further confirmed that TNF-α induced activation of NF-κB in both cell lines tested, which bound to its cognate site located in the CCL20 promoter (Fig. 4d).

4. Discussion

Taken together, our results demonstrate the clear involvement of NF-κB in regulation of CCL20 expression. Although this NF-κB binding site represents a non-standard binding site for NF-κB, a similar binding site has been shown to exist in antisense orientation in the promoter of the mouse c-myc gene [28].

Activation of the CCL20 promoter by TNF-α was 2.5–5-fold lower than activation of the IL-8 promoter in G-361 cells. In general, CCL20 expression has been shown to be 10–100-fold lower than expression of IL-8 by different cell lines and primary cells [19], which may be at least partially explained by this difference in promoter activation. However, when the NF-κB binding site of the CCL20 promoter was replaced by the IL-8 NF-κB binding site, no difference in activation was seen compared to the wild-type CCL20 promoter construct S6 (Fig. 2b), indicating that the difference in response between the CCL20 and the IL-8 promoter cannot be explained by a difference in the primary sequence of the NF-κB binding site,



but may be due to a different promoter context or involvement of sequences flanking the NF-κB binding site. Although NF-κB can bind and activate the CCL20 promoter, it has yet to be determined whether CCL20 regulation by pro-inflammatory cytokines occurs at the transcriptional level in other cell systems. Nakayama et al. concluded that induction of CCL20 expression by IL-1α in cultured human primary keratinocytes is NF-κB-independent, since dexamethasone and FK506 were unable to downregulate CCL20 expression in these cells [18]. It is also likely that in some cell types pro-

Fig. 4. NF-κB binds to a site in the CCL20 promoter. a: EMSA was performed with 10 μg of nuclear extracts from G-361 cells using a radiolabelled oligonucleotide representing the NF-κB binding site of the CCL20 promoter. Left panel: binding activity in untreated cells (lane 1) and G-361 cells treated with TNF-α for 4 h (lane 2); right panel: competition and supershift analysis from nuclear extracts of TNF-α-stimulated G-361 cells; lane 3: competition with 100× molar excess of wild-type CCL20 NF-κB oligonucleotide; lane 4: competition with 100× molar excess of mutated CCL20 NF-κB oligonucleotide; lane 5: competition with 100× molar excess of an oligonucleotide representing the NF-κB binding site of the HIV LTR; lane 6: supershift with anti-p50; lane 7: supershift with anti-p65. b: EMSA was performed with 10 μg of nuclear extracts from G-361 cells using a radiolabelled oligonucleotide representing the NF-κB binding site of the HIV LTR. Left panel: binding activity in untreated (lane 1) and G-361 cells treated with TNF-α for 1 h (lane 2); right panel: competition analysis using extracts from TNF-α-stimulated G-361 cells; lane 3: competition with 100× molar excess of wild-type CCL20 NF-κB oligonucleotide; lane 4: competition with 100× molar excess of mutated CCL20 NF-κB oligonucleotide. c: RT-PCR analysis of CCL20, IL-8 and β-actin mRNA expression in HT-29 cells (lane 1: untreated; lane 2: 3 h TNF-α; lane 3: 6 h TNF-α). Amplified product size is 321 bp for CCL20, 291 bp for IL-8 and 434 bp for β-actin mRNA. d: EMSA was performed with 10 μg of nuclear extracts from HT-29 cells using a radiolabelled oligonucleotide representing the NF-κB binding site of the CCL20 promoter. Left panel: untreated HT-29 cells (lane 1), and HT-29 cells stimulated with TNF-α for 4 h (lane 2); right panel: competition and supershift analysis from nuclear extracts of TNF-α-stimulated HT-29 cells; lane 3: competition with 100× molar excess of wild-type CCL20 NF-κB oligonucleotide; lane 4: competition with 100× molar excess of mutated CCL20 NF-κB oligonucleotide; lane 5: competition with 100× molar excess of an oligonucleotide representing the NF-κB binding site of the HIV LTR; lane 6: supershift with anti-p50; lane 7: supershift with anti-p65.
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inflammatory cytokines regulate CCL20 expression at the post-transcriptional level. In this context, the 3′-untranslated region of the CCL20 mRNA contains three copies of the destabilization motif AUUUA, which is associated with rapid mRNA degradation [4].

In this report we describe the cloning and characterization of the promoter of the human CCL20 gene and demonstrated its activation by NF-κB. Further analysis of the promoter should help to gain more insight into the regulation of this chemokine in different cell systems.

After initial submission of this manuscript, a publication by Fujiie et al. appeared, showing the cloning of the mouse LARC/MIP-3α/CCL20 promoter. The murine promoter contains an NF-κB binding site with a sequence identical to the human gene. Transfection of Caco-2 or HEK293 cells with a promoter-luciferase construct and stimulation with the pro-inflammatory cytokines IL-1α or TNF-α identified this NF-κB binding site as a key element in the regulation of this promoter [29]. These data largely confirm our observations on the human CCL20 promoter.

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