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PGE_2 enhanced TNFα-mediated IL-8 induction in monocytic cell lines and PBMC

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ABSTRACT

Background & purpose: Recent studies suggested a role of prostaglandin E_2 (PGE_2) in the expression of the chemokine IL-8 by monocytes. The function of EP4 receptor for TNFα-induced IL-8 expression was studied in monocytic cell lines.
Experimental approach: IL-8 mRNA and protein induction as well as IL-8 promoter activity and transcription factor activation were assessed in monocytic cell lines, primary blood mononuclear cells (PBMC) and transgenic HEK293 cells expressing the EP4 receptor.
Key results: In monocytic cell lines THP-1, MonoMac and U937 PGE_2 had only a marginal impact on IL-8 induction but strongly enhanced TNFα-induced IL-8 mRNA and protein synthesis. Similarly, in PBMC IL-8 mRNA induction was larger by simultaneous stimulation with TNFα and PGE_2 than by either stimulus alone. The EP4 receptor subtype was the most abundant EP receptor in all three cell lines and in PBMC. Stimulation of THP-1 cells with an EP4 specific agonist enhanced TNFα-induced IL-8 mRNA and protein formation to the same extent as PGE_2. In HEK293 cells expressing EP4, but not in wild type HEK293 cells lacking EP4, PGE_2 enhanced TNFα-induced IL-8 protein and mRNA synthesis. In THP-1 cells, the enhancement of TNFα-mediated IL-8 mRNA induction by PGE_2 was mimicked by a PKA-activator. Furthermore in these cells PGE_2 induced expression of transcription factor C/EBPß, enhanced NF-κB activation by TNFα and inhibited TNFα-mediated AP-1 activation. PGE_2 and TNFα synergistically activated transcription factor CREB, induced C/EBPß expression and enhanced the activity of an IL-8 promoter fragment containing -223 bp upstream of the transcription start site.
Conclusions and implications: These findings suggest that a combined stimulation of TNFα and PGE_2/EP4 signal chains in monocytic cells leads to maximal IL-8 promoter activity, as well as IL-8 mRNA and protein induction, by activating the PKA/CREB/C/EBPß as well as NF-κB signal chains.

1. Introduction

IL-8 is a chemokine that is released by various cell types in response to pro-inflammatory stimuli such as TNFα and IL-1ß [1]. IL-8 has been shown to have a strong chemotactic potential for leukocytes leading to extravasation and tissue invasion, but it is also involved in firm adhesion of leukocytes to endothelial cells [2,3]. In monocytes IL-8-dependent activation is mediated by the binding of IL-8 to its specific Gi-coupled IL-8 receptor CXCR2 localized on the cell surface [4]. Stimulation of the CXCR2 receptor leads to the activation of the ß2-integrins LFA-1 and Mac-1, which interact with I-CAM on the surface of the endothelial cell leading to firm adhesion [5]. IL-8 is assumed to play a pivotal role in the recruitment of inflammatory cells to the site of disease, for example in atherosclerosis, rheumatic arthritis [6], ulcerative colitis and Crohn's disease [7] and psoriasis [8].

In addition to cytokines and chemokines, prostaglandins are elevated at the sites of inflammation [9]. There is increasing evidence that prostaglandins and their specific receptors are involved in the fine tuning of the inflammatory response. In particular, PGE_2 has been shown to modulate the inflammatory response. Thus low doses of Aspirin, which prevent prostaglandin synthesis by inhibiting COX-1 and COX-2 activity, inhibited TNFα-induced IL-8 synthesis in human umbilical vein endothelial cells (HUVEC) as well as the TNFα-stimulated adhesion of monocytic U937 cells [10]. At higher concentrations

Abbreviations: COX, Cyclooxygenase; CXCR, CXC motif chemokin receptor; GAPDH, Glycerol aldehyde 3-phosphate dehydrogenase; G protein, guanine nucleotide-binding protein; EP1-4, Prostaglandin E receptor subtype 1-4; HEK-cells, Human embryonal kidney cells; IL-8, Interleukin-8; IL-1, Interleukin-1; LPS, Lipopolysaccharid; MAPK, Mitogen activated kinase; NF-κB, Nuclear factor-kappa B; PGE_2, Prostaglandin E; PKA, Protein kinase A; TNFα, Tumor Necrosis Factor α; TNFα-R, Tumor Necrosis Factor α receptor

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Table 1
Oligonucleotide primers used for realtime qPCR and generation of IL-8prom- fragments.

Gene	Forward	Reverse
GAPDH	5′-TGATGACATCAAGAAGGTGG	5′-TTACTCCTTGGAGGCCATGT
IL-8	5′-CAGTTTTGCCAAGGAGTGCTAA	5′-AACTTCTCCACAACCCTCTGC
EP1	5′-TCGCTTCGGCCTCCACCTTCTTTG	5′-CGTTGGGCCTCTGGTTGTGCTTAG
EP2	5′-CGAGACGCGACAGTGGCTTCC	5′-CGAGACGCGGCGCTGGTAGA
EP3	5′-CGGGGCTACGGAGGGGATGC	5′-ATGGCGCTGGCGATGAACAACGAG
EP4	5′-TCGCGCAAGGAGCAGAAGGAGACG	5′-GGACGGTGGCGAGAATGAGGAAGG
IL-8prom -223	5′-GCGGACGCGTGAAAACTTTCGTCATACTCCG	5′-GCGCTCGAGGAGACAGCAGAGCACACAAGCTTC
IL-8prom -208	5′- GCGGACGCGTACTCCGTATTTGATAAGGAAC	5′-GCGCTCGAGGAGACAGCAGAGCACACAAGCTTC
IL-8prom -165	5′- GCGGACGCGTGGTTTGCCCTGAGGGGATGGGCCAT	5′-GCGCTCGAGGAGACAGCAGAGCACACAAGCTTC
IL-8prom -129	5′- GCGGACGCGTCGTGGAATTTCCTCTGACATA	5′-GCGCTCGAGGAGACAGCAGAGCACACAAGCTTC
IL-8prom -112	5′- GCGGACGCGTCATAATGAAAAGATGAGGGTG	5′-GCGCTCGAGGAGACAGCAGAGCACACAAGCTTC
IL-8prom -223-ΔCEBP	5′- AGGGGATGGGCCATCCGTGGAATTTCCTCT	5′-AGAGGAAATTCCACGGATGGCCCATCCCCT
IL-8prom -223-ΔNF-κB	5′-ATCAGTTGCAAATCGCATAATGAAAAGATG	5′-CATCTTTTCATTATGCGATTTGCAACTGAT

Accession numbers for the genes were: GAPDH (AB062273), IL-8 (AK311874), EP1 (L22647), EP2 (NM_000956), EP3 (E15918) and EP4 (NM_000958).

aspirin also blocked the TNFα-stimulated migration of U937 cells to HUVEC [10].

Proteins involved in PGE_2 synthesis and signaling have been shown to be increased in inflammation. Thus, the expression of COX-2, mPGES-1 and PGE_2 receptors (EP-R) was increased in inflammatory regions of atherosclerotic plaques of patients with carotid stenosis and the proteins were co-localized in the plaque cells [11] PGE_2, which is the most abundant prostaglandin found in inflamed sites acts by binding to four specific G protein coupled prostaglandin E_2 receptors called EP1 through EP4 receptor. EP1 couples to Gq and Ca^{2+}-signaling [12]. EP2 and EP4 are coupled to Gs. Activation of these receptors led to an increase in cAMP and activation of protein kinase A [13]. In addition EP4, but not the EP2 receptor, can stimulate PI3-kinase which subsequently leads to phosphorylation and activation of Akt kinase [14]. The G protein coupling of the EP3 is more varied. Different C-terminal isoforms of this receptor exist, which signal over a decrease in cAMP (Gi-coupling) and/or an increase in IP3 and Ca^{2+} (Gq-coupling). The aim of the current study was to analyze the role of PGE_2 as a modulator of TNFα-mediated IL-8 expression in human monocytes.

Our data indicate that PGE_2 may enhance the TNFα-elicited production of IL-8 in macrophages by an activation of CREB, C/EBPβ and NF-κB through a signal chain downstream of the EP4 receptor.

2. Materials and methods

2.1. Materials

All chemicals were purchased from commercial sources indicated throughout the text. Oligonucleotides were custom-synthesized by Eurofins Operon (Ebersberg, Germany) or Biolegio (Nijmegen, Netherlands). Antibodies used were: CHOP #2895, C/EBPß #3087, CREB #4820 and pCREB(Ser133) #4276, Cell Signaling; GAPDH sc-25778; c-Jun, #9165, Cell signaling and p-c-Jun sc-16312, Santa Cruz).

2.2. Cell culture und treatment

Human monocytic cell lines THP-1, MonoMac and U937 were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated FCS and antibiotics. HEK293 cells were cultured in DMEM containing 10% FCS and antibiotics. HEK293 cells stably expressing human EP4 were established as described previously [15] and maintained in HEK293 culture medium supplemented with 0.5 mg/ml G-418 as the selection marker.

2.3. Isolation and stimulation of human primary blood mononuclear cells (PBMC)

Blood samples from ten clinically healthy human volunteers were obtained by venepuncture and collected into sterile sodium heparin containing vacuette tubes (Sarstedt, Germany). Ethical clearance to use human subjects was obtained from the designated health facility. Written consent was obtained from each person upon information of the use of blood samples. The study was approved by the institutional ethics committee at the, University of Potsdam. PBMC were isolated from whole blood using Ficoll (1077, GE Healthcare) gradient centrifugation. Identical volumes of blood and PBS were mixed and layered over Ficoll solution. After centrifugation (1332*g*, 20 min, 12 °C), PBMC were collected from the interface, washed twice with PBS and re-suspended in RPMI 1640 medium supplemented with 10% heat-inactivated FCS and antibiotics to a final concentration of 1×10^6 cells/ml. PBMC were seeded into 35 mm diameter cell culture plates and incubated with 50 ng/ml TNFα, 1 μM PGE_2 or both substances for 20 h. PBMC were then collected by centrifugation, washed with PBS and frozen in liquid nitrogen.

2.4. Real time RT-PCR

Cells were stimulated with PGE_2 and/or TNFα for 20 h and washed with PBS. Total RNA was isolated from treated cells using GeneMatrix Universal RNA kit (EURx, Poland) or Peqgold total RNA kit (Peqlab, Germany). 1–5 μg total RNA were reverse transcribed into cDNA using a mixture of oligo dT and random nucleotide primers and an M-MuLV Reverse Transcriptase (Thermo Scientific, Germany). Hot start real-time PCR for the quantification of each transcript was carried using

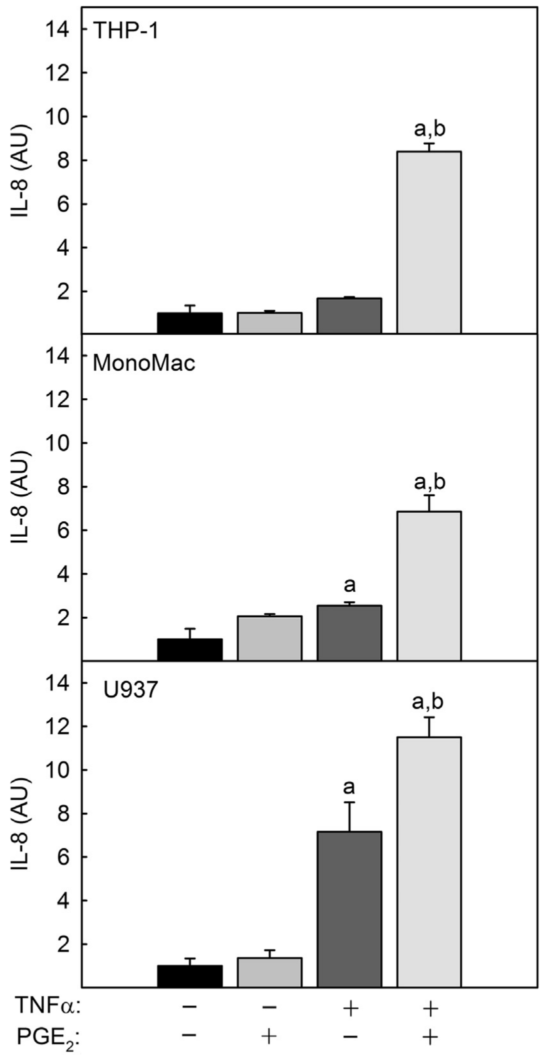

Fig. 1. PGE_2 and TNFα-induced IL-8 protein synthesis in monocytic cell lines. THP-1, MonoMac and U937 cells were stimulated with 1 µM PGE_2 and/or 50 ng/ml TNFα for 20 h. Released IL-8 in the medium was measured by ELISA. Data shown are means ± S.E.M. of at least three independent experiments performed in triplicate. Statistics: Student's *t*-test for unpaired samples. a: significantly higher than control, b: significantly higher than TNFα ($p < 0.05$).

2 × Maxima SybrGreen qPCR mix (Thermo Scientific), 0.25 µM of each primer and 2.5–5 µl of cDNA, which was diluted 1:10. PCR was performed with an initial enzyme activation step at 95 °C for 10 min, followed by 50 cycles of denaturation at 95 °C for 30 sec, annealing at 57 °C for 30 sec and extension at 72 °C for 30 sec in a real-time DNA thermal cycler (iCycler™, 20 µl reaction volume or CFX96™, 10 µl reaction volume, BIO-RAD; Munich). The oligonucleotides used are listed in Table 1. The expression level was calculated as an n-fold induction of the gene of interest (int) in treated versus control cells with GAPDH (gap) as a reference gene. The calculation is based on the differences in the threshold cycles between control (c) and treated (t) groups according to the formula: fold induction = $2^{(c-t)int}/2^{(c-t)gap}$. For the calculation of EP-R copy numbers plasmids with cloned cDNAs coding for EP-R and GAPDH were used as template to prepare standard curves with defined copy numbers.

2.5. Il-8 ELISA

Cells were stimulated with PGE_2 and/or TNFα for 20 h. After the incubation supernatants were collected and processed for IL-8 quantification by sandwich ELISA as described previously [16].

2.6. Generation of IL-8 promoter reporter gene constructs

An IL-8 promoter fragment up to position -223 upstream of the IL-8 Exon 1 (IL-8prom-223) was generated from human genomic DNA by PCR using the primers listed in Table 1, cleaved with MluI and XhoI and cloned in the MluI/XhoI site of the Firefly luciferase vector pGL3-basic. Truncated IL-8prom-promoter constructs missing putative binding site for transcription factors CREB, CHOP/AP-1, C/EBP and NF-κB were generated by PCR using pGL3-basic-IL8prom-223 as a template and the forward primers listed in Table 1. pGL3-basic-IL8prom-223 also serves as a template to generate IL-8prom deletion constructs missing the putative binding sites for C/EBP and NF-κB. Deletions were introduced in the 5′- and 3′- promoter fragments using the primers listed in Table 1 and promoter fragments were fused by PCR using primers IL-8prom-223 forward and reverse. All IL-8 promoter fragments were cloned into the MluI/XhoI site of pGL3-basic.

2.7. Cell transfection and luciferase reporter gene assay

THP-1 cells were transfected with IL-8prom reporter gene plasmids or pNL1.2-NF-κB-Luc using lipofection with GeneXplus (ATCC) according to the manufacturer's instructions. 20 h after transfection, cells were treated with 1 µM PGE_2 and/or 50 ng/ml TNFα for 8 h (promoter activation) or 20 h (NF-κB activation). At the end of the experiment, cells were lysed in 100 µl passive lysis buffer reagent (Promega) and firefly- or NanoLuc luciferase activity was measured in 50 µl of cell lysate using the Fluostar Optima (BMG Labtech, Offenburg, Germany).

2.8. Western blot analysis

THP-1 cells were stimulated with 1 µM PGE_2, 50 ng TNFα or a combination of both substances for the time indicated. Cells were lysed in lysis buffer (20 mM Tris pH 7.4, 150 mM NaCl, 1 mM ethylene diamine tetraacetic acid (EDTA), 1 mM ethylene glycol tetraacetic acid (EGTA, 1% (v/v) Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM ß-glycerolphosphate, 50 mM NaF, protease inhibitors and 1 mM sodium orthovanadate), homogenized by sonication and insoluble material was removed by centrifugation (10,000g, 15 min, 4 °C). Protein content was determined. Proteins were resolved by SDS-PAGE and transferred to a polyvinylidene difluoride (PVDF) membrane. Membranes were blocked in 5% non-fat dry milk in 20 mM Tris, 136 mM NaCl and 0,1% (v/v) Tween (TBS/Tween) for 1 h at room temperature and incubated with the first antibody in TBS/Tween containing 5% bovine serum albumin overnight at 4 °C and a horseradish-peroxidase-conjugated anti-rabbit or anti-mouse IgG for 2 h at room temperature. Visualization of immune complexes was performed using a chemoluminescence reagent.

2.9. Statistical analysis

To correct for differences in sensitivity of different cell charges towards the stimuli, values were normalized to average inducibility defined as the mean of values obtained for control, PGE_2, TNFα and TNFα + PGE_2 treated cells. Results were analysed by Student's *t*-test as indicated in the figure legends.

3. Results

3.1. PGE_2 and TNFα induced IL-8 protein expression in monocytic cell lines

THP-1, MonoMac and U937 cells were cultured for 20 h in the presence of either 1 µM PGE_2, 50 ng/ml TNFα, or both. IL-8 protein in the supernatant of cells was quantified by ELISA. TNFα significantly induced IL-8 accumulation in the supernatant of all three monocytic cell lines. The strongest IL-8 induction was observed in U937 cells (8-fold) (Fig. 1). Whereas PGE_2 alone did not induce IL-8 expression, it enhanced the TNFα-dependent IL-8 induction in THP-1 (5-fold),

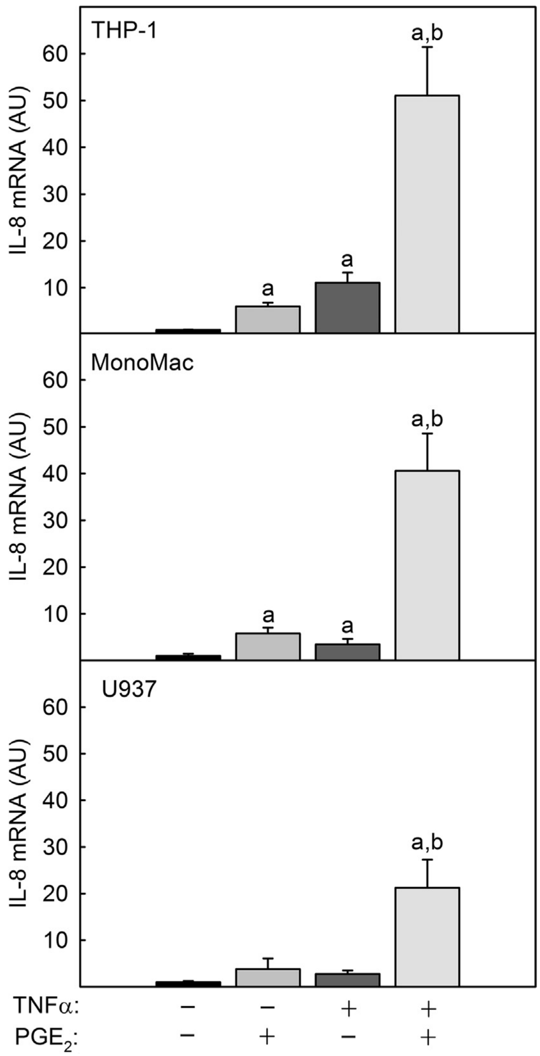

Fig. 2. PGE_2 and TNFα mediated IL-8 mRNA induction in monocytic cell lines. THP-1, MonoMac and U937 cells were stimulated with 1 µM PGE_2 and/or 50 ng/ml TNFα for 20 h. IL-8 mRNA content was measured by qPCR as described in the method section with GAPDH as reference gene. Data shown are means ± S.E.M. of four independent experiments performed in triplicate. Statistics: Student's *t*-test for unpaired samples. a: significantly higher than control, b: significantly higher than TNFα ($p < 0.05$).

MonoMac (3-fold) and U937 cells (2-fold). Thus, independent of the sensitivity to either individual stimulus, maximal IL-8 expression was achieved in all three cell lines by simultaneous stimulation with a combination of TNFα and PGE_2.

3.2. PGE_2 enhanced TNFα-induced IL-8 mRNA in monocytic cell lines

Previous studies showed that IL-8 expression is predominantly regulated on the transcriptional level [1]. THP-1, MonoMac and U937 cells were treated as above and the IL-8 mRNA was quantified by RT-qPCR. The induction pattern of IL-8 mRNA was comparable in all monocytic cell lines analyzed, however, the extent of the induction by the different conditions varied between the cell lines (Fig. 2). In THP-1 cells, PGE_2 induced IL-8 mRNA to a lesser extent than TNFα (PGE_2: 6-fold, TNFα: 11-fold). By contrast, in MonoMac cells the IL-8 induction by PGE_2 was stronger than the TNFα-dependent induction (PGE_2: 5.8-fold, TNFα: 3.5-fold). In U937 cells PGE_2 and TNFα caused a similar, statistically non-significant induction (PGE_2: 3.8-fold, TNFα: 2.8-fold). In accordance with the protein data, maximal IL-8 mRNA induction was observed in all three cell lines when the cells were stimulated with a combination of PGE_2 and TNFα (THP-1: 51-fold, MonoMac: 40.6-fold and U937: 23.3-fold). In addition stimulation of THP-1 cells with IL-6 instead of TNFα did not induce IL-8 mRNA mRNA either in the absence or presence of PGE_2 (not shown). The results indicate that the maximal induction of IL-8 protein expression observed after simultaneous stimulation by PGE_2 and TNFα was a result of maximal IL-8 mRNA induction in the monocytic cell lines under this condition.

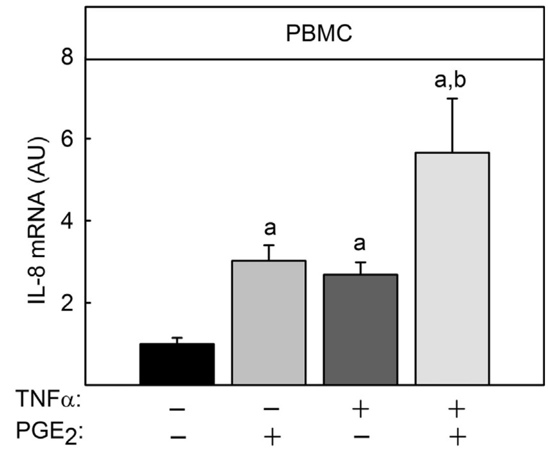

Fig. 3. PGE_2 and TNFα mediated IL-8 mRNA induction in PBMC. PBMC were isolated from blood of healthy patients and then stimulated with 1 µM PGE_2 and/or 50 ng/ml TNFα for 20 h. IL-8 mRNA content was measured by qPCR as described in the method section with GAPDH as reference gene. Data shown are means ± S.E.M. of seven independent experiments performed in triplicate. Statistics: Student's *t*-test for unpaired samples. a: significantly higher than control, b: significantly higher than TNFα ($p < 0.05$).

3.3. PGE_2 enhanced TNFα-induced IL-8 mRNA in PBMC

To analyze if PGE_2 also enhanced TNFα-induced IL-8 mRNA in primary human monocytes, primary blood mononuclear cells (PBMC) were isolated from six different patients and stimulated by PGE_2 and/or TNFα for 20 h and IL-8 mRNA expression was analyzed (Fig. 3). PGE_2 and TNFα induced IL-8 mRNA to a comparable extent (PGE_2: 3- fold; TNFα: 2.7-fold). As observed in monocytic cell lines IL-8 mRNA induction was strongest if cells were stimulated with a combination of TNFα and PGE_2 (5.7-fold). Although the maximal induction of IL-8 mRNA was higher in monocytic cell lines, it appears that the general pattern of the regulation of IL-8 mRNA expression by PGE_2 and TNFα was conserved between PBMC and monocytic cell lines.

3.4. Monocytic cell lines predominantly expressed EP4 subtype

PGE_2 acts upon a family of four different G-protein-coupled receptors called EP1 to EP4. To analyze which EP-R subtype might be involved in the modulation of IL-8 expression by PGE_2 in the monocytic cell lines and PBMC, EP-R mRNA in these cells was quantified by real-time RT-PCR. To estimate exact EP-R mRNA copy numbers standard curves with plasmids containing defined copy numbers of EP-R or GAPDH cDNA were prepared. EP4 mRNA was the most abundant in all cell lines analyzed and also the most abundant in PBMC (Table 2). The highest EP4 mRNA expression was found in THP-1 cells, displaying twice the EP4 mRNA expression than in MonoMac, PBMC and 50-fold higher than in U937 cells. EP1, EP2- and EP3 mRNAs expression levels were very low in all monocytic cell lines and near the minimum detection limit. By contrast PBMC showed higher EP2-R mRNA expression than the cell lines. It was therefore most likely that the PGE_2-dependent modulation of IL-8 expression was mediated via the EP4 receptor.

Table 2
EP-R mRNA profile in THP-1, MonoMac, U937, PBMC, HEK and HEK-EP4 cells.

Cells	EP1 (EP1 mRNA × 1000/GAPDH mRNA)	EP2 (EP2 mRNA × 1000/GAPDH mRNA)	EP3 (EP3 mRNA × 1000/GAPDH mRNA)	EP4 (EP4 mRNA × 1000/GAPDH mRNA)
THP-1	0,23 ± 0,04	1,22 ± 0,39	0,38 ± 0,06	130, 27 ± 23,76
MonoMac	0,04 ± 0,001	0,95 ± 0,67	1,58 ± 0,81	64,51 ± 40,08
U937	0,002 ± 0	0,56 ± 0,12	0,04 ± 0,02	3,43 ± 1,24
PBMC	0,044 ± 0, 022	3,3 ± 0,69	0,236 ± 0,15	37,32 ± 19,46
HEK	0,01 ± 0	0,23 ± 0,14	0,03 ± 0,01	0,20 ± 0,11
HEK-EP4	0,02 ± 0,01	0,2 ± 0,11	0,01 ± 0	313 ± 91

Monocytic cell lines, PBMC, HEK and HEK-EP4 cells were cultured for 20 h. EP-R mRNA and GAPDH mRNA was measured by real-time RT-qPCR as described in *Methods*. Plasmids (10^2 – 10^8 copies) containing EP-R or GAPDH cDNAs were used for preparing standard curves for the calculation of EP-R or GAPDH mRNA copy numbers. Data represent the mean ± SEM of at least three independent RNA preparations. EP-R mRNA contents are expressed as copy number EP-R mRNA × 1000/ copy number GAPDH mRNA.

3.5. EP4 agonist stimulated basal and amplified TNFα-mediated IL-8 mRNA induction in THP-1 cells

Since the pattern of IL-8 protein and mRNA induction by TNFα and PGE_2 as well EP-R expression was similar in all monocytic cell lines used all further experiments were performed with THP-1 cells only. To analyze if specific activation of the EP4 can mimic the effects of PGE_2 on IL-8 mRNA induction, THP-1 cells were cultured for 24 h with 0.1 μM of specific EP1-4-R agonists (EP-Ag) alone or in combination with 50 ng/ml TNFα. At this agonist concentration, the EP receptor activation was specific and maximal [17]. IL-8 mRNA induction level was quantified by real time RT-PCR. PGE_2 enhanced IL-8 formation about 6.3-fold (Fig. 4). While EP1 did not impact the basal IL-8 expression, EP2 (2.8-fold), EP3 (2.4-fold) and EP4 (3.4-fold) agonists increased IL-8 expression, albeit to a lesser extent than PGE_2. TNFα caused a 3.8-fold induction of IL-8 mRNA. This induction was significantly enhanced only by PGE_2 (15.6-fold) and the EP4 agonist (16.3-fold).

3.6. Induction of IL-8 mRNA and protein by PGE_2 and TNFα in HEK293 cells overexpressing EP4

To test the hypothesis that PGE_2 exerts its effects on IL-8 expression

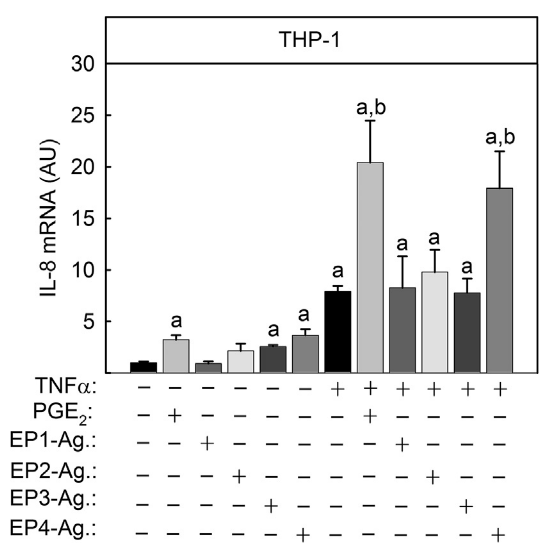

Fig. 4. EP-R Agonist and TNFα mediated IL-8 mRNA induction in THP-1 cells. THP-1 cells were stimulated with 1 μM PGE_2 or 0.1 μM of EP-R specific agonists and/or 50 ng/ml TNFα for 20 h. IL-8 mRNA content was measured by qPCR as described in the method section with GAPDH as reference gene. Data shown are means ± S.E.M. of four independent experiments performed in triplicate. Statistics: Student's *t*-test for unpaired samples. a: significantly higher than control, b: significantly higher than TNFα ($p < 0.05$).

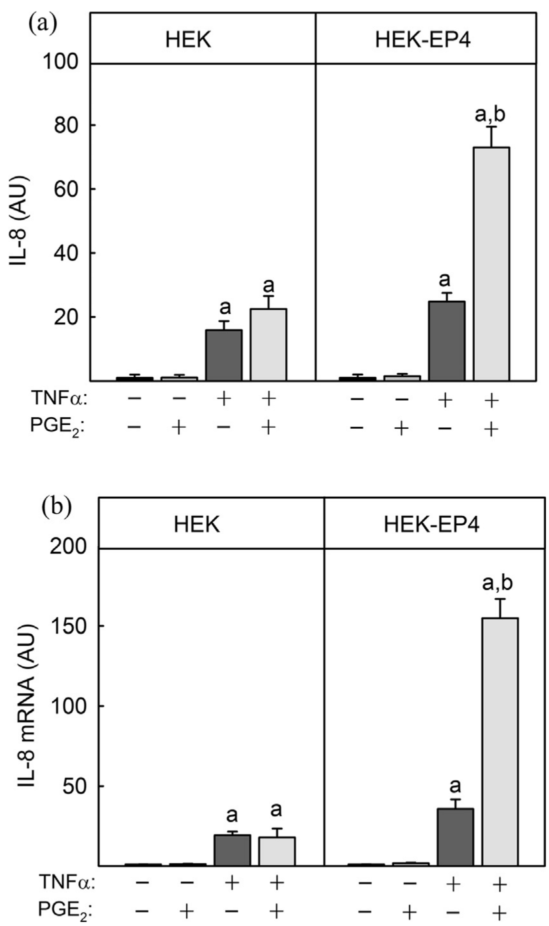

Fig. 5. PGE_2 and TNFα mediated IL-8 protein and mRNA induction in HEK and HEK-EP4 cells. HEK and HEK-EP4 cells were stimulated with 1 μM PGE_2 and/or 50 ng/ml TNFα for 20 h. (A) Released IL-8 in the medium was measured by ELISA. (B) IL-8 mRNA content was measured by qPCR as described in the method section with GAPDH as reference gene. Data shown were means ± S.E.M. of three to four independent experiments performed in triplicate. Statistics: Student's *t*-test for unpaired samples. a: significantly higher than control, b: significantly higher than TNFα ($p < 0.05$).

via EP4, wildtype HEK293 cells (HEK) or HEK293 cells stably overexpressing the human EP4 (HEK-EP4) were stimulated with PGE_2 and/ or 50 ng/ml TNFα, and IL-8 protein and mRNA induction was measured. PGE_2-stimulation of HEK293 cells, which express all EP-R at a very low level (Table 2), or HEK-EP4 cells had no effect on IL-8 protein or mRNA induction (Fig. 5A and B). TNFα induced IL-8 protein and mRNA in HEK or HEK-EP4 cells to a similar extent (protein: HEK 15.9-fold, HEK-EP4 24.8-fold; mRNA: HEK 19.2-fold, HEK-EP4 35.7-fold).

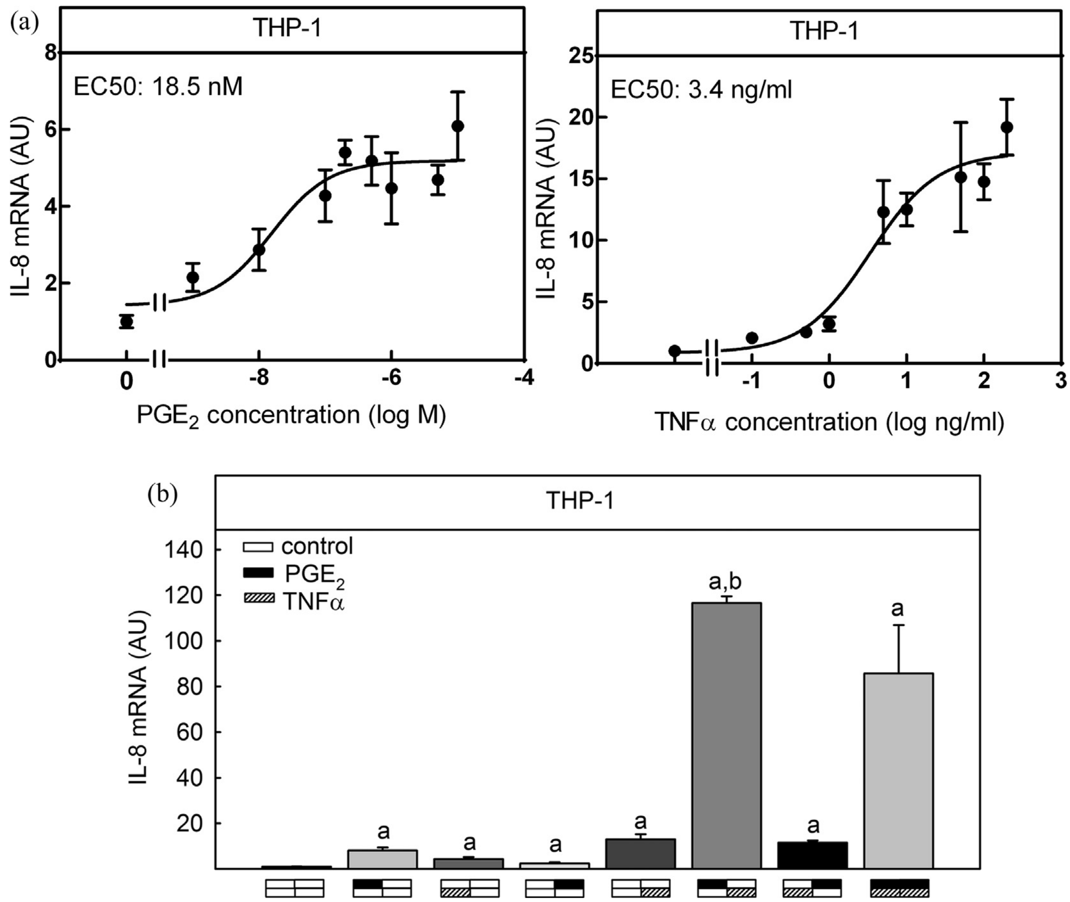

Fig. 6. Dose and time-dependent IL-8 mRNA induction by PGE_2 and TNFα in THP-1 cells. (A) THP-1 cells were stimulated with 0.001–20 µM PGE_2 or 0.1–200 ng/ml TNFα for 20 h. (B) THP-1 cells were stimulated with medium, 1 µM PGE_2 and/or 50 ng/ml TNFα for the first 10 h as indicated graphically. Cells were collected by centrifugation, re-suspended in fresh medium and stimulated for the second 10 h as indicated. IL-8 mRNA content was measured by qPCR as described in the method section with GAPDH as reference gene. Data shown are means ± S.E.M. of at least three independent experiments performed in triplicate. Statistics: Student's *t*-test for unpaired samples. a: significantly higher than control, b: significantly higher than TNFα + PGE_2 for 20 h ($p < 0.05$).

PGE_2 significantly amplified TNFα-induced IL-8 expression in HEK-EP4 cells but not in HEK cells (protein: 73-fold; mRNA: 154.8-fold). Together these experiments show that the effect of PGE_2 on TNFα-induced, but not basal, IL-8 expression in THP-1 cells could be mimicked in HEK cells by EP4 overexpression.

3.7. Dose- and time-dependent induction of IL-8 mRNA by PGE_2 and TNFα in THP-1 cells

To get more insight into the mechanism by which PGE_2 and TNFα induce maximal IL-8 expression THP-1 cells were stimulated with different concentrations and time-patterns of PGE_2 and TNFα. TNFα and PGE_2 dose dependently induced IL-8 mRNA with an EC50 of 3.4 ng/ml (TNFα) and 18.5 nM (PGE_2) (Fig. 6A). At 50 ng/ml TNFα and 1 µM PGE_2 IL-8 mRNA induction was maximal (PGE_2: 4.5-fold and TNFα: 15-fold) (Fig. 6A). Since the IL-8 induction by simultaneous stimulation with a combination of saturating concentrations of TNFα and PGE_2 (50 ng/ml and 1 µM, respectively) was around 50- to 80-fold (Figs. 2 and 6B) and thus larger than the sum of the individual maximal inductions, it seems that this induction was more than additive.

To analyze if the maximal IL-8 mRNA induction by the combination of PGE_2 and TNFα required the simultaneous presence of both agonists over the entire induction period, the induction period was divided into two subsequent stimulation periods of 10 h with a medium change in between (Fig. 6B). If THP-1 cells were stimulated with PGE_2 or TNFα alone for the first and the last 10 h IL-8 mRNA induction was rather low (4–13-fold). If cells were stimulated with TNFα only during the first 10 h and subsequently stimulated with PGE_2 the induction of the IL-8 mRNA was not stronger than with either stimulus alone. By contrast, if cells were stimulated with PGE_2 only during the first 10 h and were then subsequently stimulated for 10 h with TNFα alone, IL-8 mRNA induction was significantly higher than with either stimulus alone (116-fold induction) and even outperformed the induction observed if both stimuli were present simultaneously over the entire 20 h period (85-fold induction). These results indicated that PGE_2 sensitized the cells for the subsequent stimulation with TNFα.

3.8. Signal chains involved in PGE_2-mediated enhancement of TNFα-stimulated IL-8 induction

EP4 couples to Gs and stimulates adenylate cyclase and cAMP formation. In addition activated EP4 can form a complex with β-arrestin which leads to activation of Src and subsequent activation of PI3K kinase by transactivation of the EGF receptor [18]. EP4-activation also enhanced EP1-mediated activation of the transcription factor NF-κB in a Src-dependent manner [19].

IL-8 expression is tightly controlled by a core promoter 223 bp upstream of the transcription start point, which contains binding sites for transcription factors CREB, CHOP, AP-1, C/EBP and NF-κB (Fig. 7A). Activation/induction of these transcription factors by PGE_2 and TNFα was analyzed in THP-1 cells. PKA/CREB: To evaluate the role of the cAMP/PKA signaling pathway in the TNFα/PGE_2-mediated IL-8 regulation, THP-1 cells were stimulated with the cell permeable PKA activator 6-Bnz-cAMP, PGE_2, and/or TNFα and IL-8 mRNA was measured.

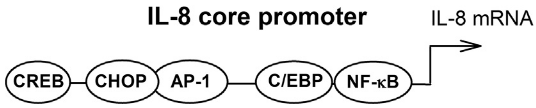

Fig. 7A. Transcription factor binding sites in the IL-8 core promoter.

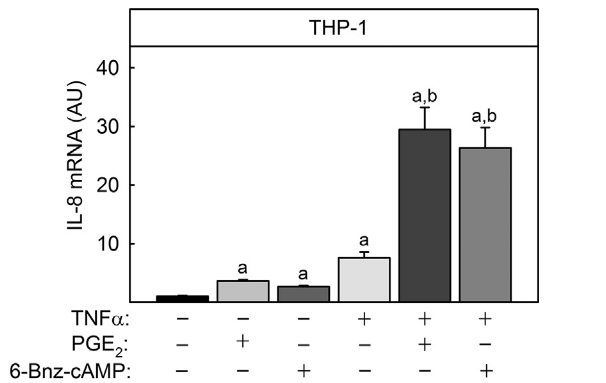

Fig. 7B. PGE_2, 6-Bnz-cAMP and TNFα mediated IL-8 mRNA induction in THP-1 cells. THP-1 cells were stimulated with 1 µM PGE_2, 100 µM 6-Bnz-cAMP and/or 50 ng/ml TNFα for 20 h. IL-8. mRNA content was measured by qPCR as described in the method section with GAPDH as reference gene. Data shown are means ± S.E.M. of four independent experiments performed in triplicate. Statistics: Student's *t*-test for unpaired samples. a: significantly higher than control, b: significantly higher than TNFα ($p < 0.05$).

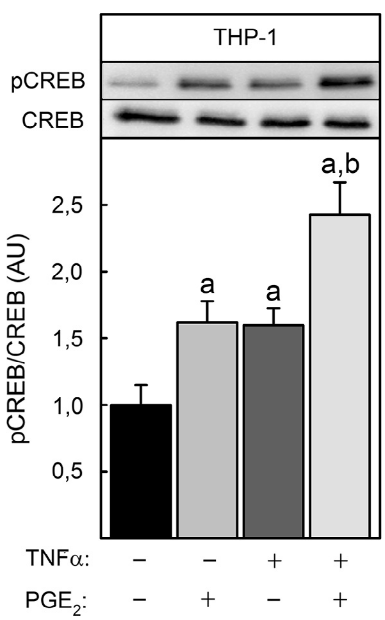

Fig. 7C. PGE_2 and TNFα mediated CREB phosphorylation in THP-1 cells. THP-1 cells were stimulated with 1 µM PGE_2 and/or 50 ng/ml TNFα for 5 min. Proteins were extracted from cells with lysis buffer containing fluoride and vanadate to inhibit phosphatases. Phosphorylated and total CREB was determined by Western blot using specific antibodies, peroxidase-coupled secondary antibodies and a luminogenic substrate. Band intensity was quantified luminometrically and expressed as ratio between phosphorylated and total protein. Data shown were means ± S.E.M. of four independent experiments performed in triplicate. Statistics: Student's *t*-test for unpaired samples. a: significantly higher than control, b: significantly higher than TNFα ($p < 0.05$).

PGE_2, 6-Bnz-cAMP and TNFα alone induced IL-8 mRNA slightly but significantly in THP-1 cells (Fig. 7B). 6-Bnz-cAMP enhanced TNFα-mediated IL-8 mRNA induction to the same extent as PGE_2. These results show that in THP-1 cells both the IL-8 induction by PGE_2 alone and the PGE_2-mediated enhancement of TNFα-mediated IL-8 mRNA induction are PKA-dependent. Since transcription factor cAMP-regulated-binding protein (CREB) is a major target of protein kinase A, PGE_2- and TNFα-dependent CREB phosphorylation was analyzed in THP-1 cells by western blot with pCREB (Ser-133) specific antibodies. Stimulation with PGE_2 or TNFα induced a significant and comparable CREB phosphorylation in THP-1 cells, which reached a maximum after simultaneous stimulation with both substances (Fig. 7C). CHOP: CHOP activity is mainly regulated on the expression level [20], therefore the regulation of CHOP protein levels by TNFα and PGE_2 was measured in THP-1 cells by Western blot. PGE_2 and TNFα alone did not influence CHOP expression, however, they reduced CHOP expression slightly, but not significantly when cells were stimulated with both substances at the same time (Fig. 7D) Activation of IL-8 expression by the transcription factor CHOP therefore seems unlikely. AP-1: AP-1 is a heterodimer formed by the two subunits c-Jun and c-Fos. Its activity is mainly regulated by transcriptional induction and/or a c-Jun N-terminal kinase (JNK) dependent phosphorylation of the c-Jun subunit. Therefore, the expression as well as the phosphorylation of c-Jun after the stimulation of THP-1 cells with PGE_2 and TNFα was analyzed by Western blot with c-Jun and phospho-c-Jun specific antibodies. Expression and phosphorylation of c-Jun war markedly increased by stimulation with TNFα (c-Jun: 2.5-fold, p-c-Jun: 3.5-fold, Fig. 7D). By contrast PGE_2 stimulation alone did not induce c-Jun expression or phosphorylation, but significantly inhibited c-Jun phosphorylation induced by TNFα (Fig. 7D). C/EBP: Like transcription factor CHOP, the activity of C/EBP is mainly regulated on the expression level [21]. So the regulation of C/EPB expression by PGE_2 and TNFα was analyzed by Western blot with C/EBPß specific antibodies. Both PGE_2 and TNFα significantly induced C/EBPß expression slightly and to a comparable extent (1.5 and 1.3 fold). C/EBPß expression was maximal when THP-1 cells were stimulated with both substances at the same time (2.5-fold, Fig. 7D). Since PGE_2 and TNFα regulated C/EBPß expression with the same pattern as CREB-phosphorylation it is likely that expression of C/EBPß was a result of CREB-activation. CREB phosphorylation and the subsequent C/EBPß expression were previously described in macrophages stimulated by PGE_2 through the activation of EP2 and EP4 receptors [22]. NF-κB: In the majority of cells, IL-8 transcription is mainly controlled by the transcription factor NF-κB [1] To analyze if the stimulation of the EP4 by PGE_2 activates NF-κB or modulates TNFα-induced NF-κB activation, THP-1 cells were transfected with the NF-κB-controlled Nanoluc luciferase reporter gene plasmid and the subsequent NF-κB activation was quantified by the determination of luciferase activity in extracts of cells stimulated with PGE_2, TNFα or both substances for 24 h. Stimulation of THP-1 cells by PGE_2 alone did not activate NF-κB, in contrast to TNFα which led to a 1.5-fold NF-κB activation (Fig. 7E). TNFα-dependent NF-κB was significantly enhanced by PGE_2 (1.8-fold). These results show that induction of IL-8 expression by PGE_2 and TNFα may be a result of a complex activation of PKA - CREB - C/EBP, AP-1 and NFκB signal chains in THP-1 cells.

3.9. Activation of the IL-8 promoter by PGE_2 and TNFα in THP-1 cells

To analyze if PGE_2-stimulated IL-8 mRNA induction was a consequence of IL-8 promoter activation, THP-1 cells were transfected with a reporter gene construct expressing the reporter gene under the control of an IL-8 promoter fragment spanning 223 bp upstream of the putative IL-8 transcription start point. This basal IL-8 promoter fragment contains binding sites for the transcription factors CREB, CHOP, AP-1, C/EBP and NF-κB. 16 h after transfection, cells were treated with 1 µM PGE_2 or 50 ng/ml TNFα for 8 h and firefly luciferase activity under the control of the IL-8-223 promoter was measured. Both PGE_2 and TNFα activated the IL-8 promoter in THP-1 cells significantly and to a comparable extent (PGE_2 and TNFα: 1.6-fold) (Fig. 8A). In regard to IL-8 mRNA and protein induction, the maximal activation of the IL-8-223 promoter was observed when THP-1 cells were stimulated with a combination of TNFα and PGE_2 (3.3-fold, Fig. 8A). Truncation of the

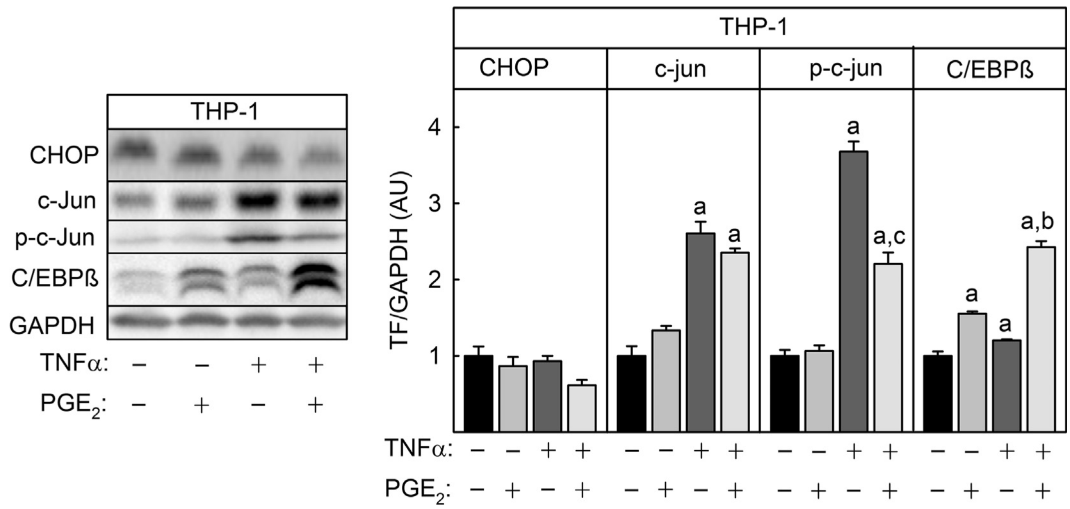

Fig. 7D. PGE_2 and TNFα mediated activation of transcription factors CHOP-, c-Jun and C/EBPß in THP-1 cells. THP-1 cells were stimulated with 1 µM PGE_2 and/or 50 ng/ml TNFα for 20 h. Proteins were extracted from cells with lysis buffer and expression of CHOP, c-Jun/p-c-Jund and C/EBPß was measured by Western Blot as described in Fig. 5C using specific antibodies for the transcription factors and GAPDH as a housekeeping protein. Transcription factor protein was expressed as ratio between transcription factor and GAPDH. Data shown were means ± S.E.M. of three independent experiments performed in triplicate. Statistics: Student's *t*-test for unpaired samples. a: significantly higher than control, b: significantly higher than TNFα, c: significantly lower than TNFα ($p < 0.05$).

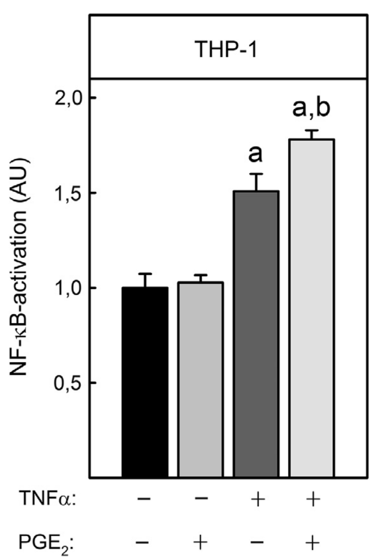

Fig. 7E. PGE_2 and TNFα mediated NF-κB activation in THP-1 cells. THP-1 cells were transfected with a NF-κB-NanoLuc-luciferase reporter gene plasmid. Cells were than stimulated with 1 µM PGE_2 and/or 50 ng/ml TNFα for 24 h. NanoLuc luciferase activity was measured in lysates as described in the method section. Values are means ± S.E.M. of seven independent experiments performed in triplicate. Statistics: Student's *t*-test for unpaired samples. a: significantly higher than control, b: significantly higher than TNFα ($p < 0.05$).

CREB binding site neither affected the regulation of the promoter activity by PGE_2, TNFα, nor the combination of both, indicating that a direct activation of the promoter by a PGE_2-dependent activation of CREB at this site is not of major relevance. As neither TNFα nor PGE_2 activated CHOP, this transcription factor does not seem to be relevant for IL-8 regulation in THP cells under the conditions chosen. In regard to the activation of c-Jun by TNFα, but not by PGE_2, truncation of the AP-1 site abolished the activation of the promoter by TNFα alone but left the activation by PGE_2 unaffected. The combination of PGE_2 and TNFα caused a stronger activation of the construct lacking the AP-1 binding site than either substance alone. Thus, it is likely that AP-1 is essential to stabilize the complex of the additional transcription factors at the promoter site, but is itself not involved in the PGE_2- and TNFα-dependent regulation of the promoter activity. Concerning the PGE_2- and TNFα-dependent induction of C/EBP (Fig. 7D), truncation of the C/EBP binding site largely abolished the PGE_2 and TNFα-dependent activation of the reporter gene construct while the remaining NF-κB site alone permitted a reporter gene activation by the combination of PGE_2 and TNFα. A residual promoter also lacking this site was no longer regulated by the combination of PGE_2 and TNFα.

The importance of the C/EBP and NF-κB site was further confirmed using IL-8prom-223 deletion constructs. Deletion of the C/EBP site largely decreased the PGE_2 and TNFα-dependent promoter activation and deletion of the NF-κB site completely abolished promoter activation (Fig. 8B).

It appears that both PGE_2 and TNFα can activate the promoter by an induction of C/EBP. The extent of the induction of C/EBP by PGE_2, TNFα or the combination paralleled the extent of the activation of the promoter construct. In addition, TNFα may trigger the activation of NF-κB. This activation appears to be enhanced by PGE_2.

4. Discussion

This current study showed that simultaneous activation of TNFα-R and EP4 signal chains were necessary for maximal IL-8 protein and/or mRNA induction in the monocytic cell lines THP-1, MonoMac and U937 and in PBMCs. In THP-1 cells IL-8 formation was most likely induced by induction of C/EBPß and NF-κB.

4.1. Role of prostaglandin E_2 in IL-8 induction

IL-8 has been shown to be induced by many different stimuli. TNFα and IL-1ß are known to be highly potent stimulators of NF-κB-dependent IL-8 expression in various cell types. TNFα and IL-1ß also induced COX-2 as well as microsomal PGE synthase 1, both key regulatory enzymes in PGE_2 synthesis from arachidonic acid [23]. As a consequence, the concentration of prostaglandin PGE_2 is elevated in inflamed tissues. The actions of PGE_2 are mediated by the binding to 4 different G protein coupled receptors, EP1 to EP4, which activate different G-proteins and signal chains. EP1 couples to Gq and Ca^{2+}-signaling [12] EP2 and EP4 couple to Gs and EP3 to Gi [13]. The role of PGE_2 in inflammation is controversial. A number of studies describe anti-inflammatory properties of PGE_2, including the suppression of T-cell induction [24] and the prevention of natural killer cell activation [25]. In human macrophages

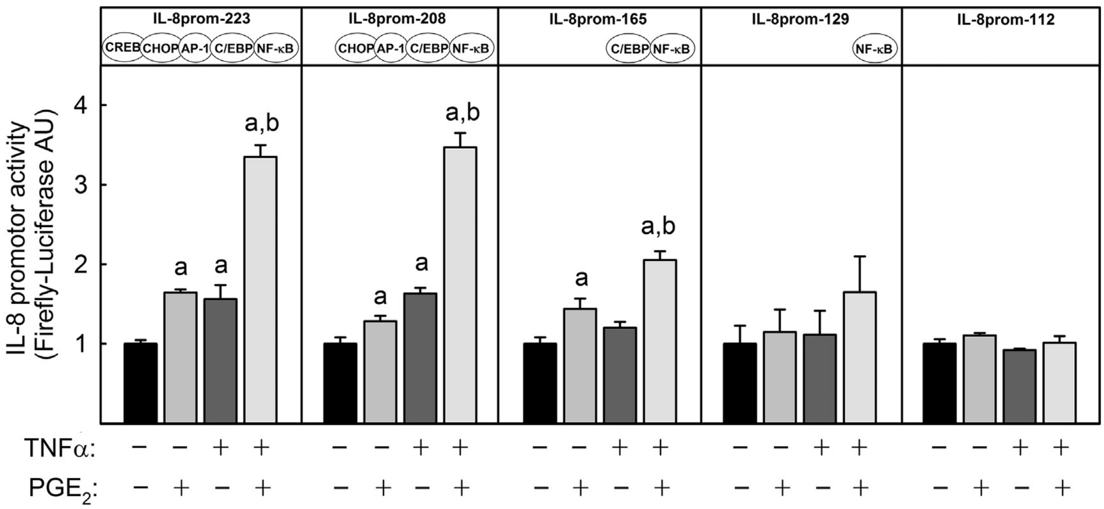

Fig. 8A. PGE_2 and TNFα mediated IL-8prom-223 or truncated promoter activation in THP-1 cells. THP-1 cells were transfected with IL-8 promoter reporter gene plasmids harbouring the IL-8prom-223 core or truncated promoters. After 24 h THP-1 cells were stimulated with 1 µM PGE_2 and/or 50 ng/ml TNFα for 8 h. Firely luciferase activity was measured in lysates as described in the method section. Data shown are means ± S.E.M. of at least four independent experiments performed in triplicate. Statistics: Student's *t*-test for unpaired samples. a: significantly higher than control, b: significantly higher than TNFα ($p < 0.05$).

PGE_2 suppressed LPS-elicited formation of the chemokines IL-8, MIP-1α, MIP-1ß and MCP-1 by binding to EP4 [26]. In addition, PGE_2 suppressed TNFα-formation in mouse macrophages in a PKA-dependent manner and inhibited LPS-induced TNFα-formation in mouse Kupffer cells via Gs-coupled EP2 and EP4 [27]. PGE_2 also inhibited Thrombin-induced IL-8 production in U937 cells and LPS-induced IL-8 formation in human peripheral blood monocytes [28,29].

By contrast, PGE_2 stimulated IL-8 formation in human T-lymphocytes [30], cystic fibrosis airway epithelia cells [31] and human colonic epithelial cells [32]. Interestingly, PGE_2 was also involved in IL-8 formation induced by the peptide hormone bradykinin in human airway smooth muscle cells. In these cells the cyclooxygenase inhibitor indomethacin inhibited bradykinin-stimulated IL-8 formation, whereas exogenous PGE_2 activated the IL-8 promoter and enhanced IL-8 formation [33].

In the present study, PGE_2 had only a minor impact on IL-8 formation in the monocytic cell lines THP-1, MonoMac and U937, but significantly enhanced TNFα-mediated IL-8 formation. The stimulatory effect of PGE_2 on IL-8 production was most likely mediated by the activation of EP4 signal chain because (1) an EP4 agonist could mimic the effect of PGE_2 in THP-1 cells, (2) EP4 showed the highest expression level in all three monocytic cell lines as well as in PBMCs and (3) PGE_2 amplified the TNFα-induced IL-8 formation in HEK-EP4 but not in HEK cells. The role of the EP4 therefore seems to cooperate with TNFα signal chains to induce maximal IL-8 formation rather than to directly activate IL-8 induction. The finding that prostaglandins can enhance TNFα-induced IL-8 formation rather than directly activate IL-8 expression was also shown for PGD_2, which enhanced TNFα-stimulated IL-8 expression in THP-1 cells by a DP/cAMP-dependent signal chain [34].

In other cell types EP4 activation alone was sufficient for PGE_2-mediated IL-8 induction. In Caco-2 cells the overexpression of EP4 but not of the EP2 led to PGE_2-stimulated IL-8 formation [35]. In addition stimulation of non-transfected Caco-2 cells with an EP4 specific agonist, but not with an EP2 agonist, led to the same massive IL-8 formation as stimulation with PGE_2 [32] The fact that EP4 activation induced IL-8 expression in Caco-2 but not in monocytic cell lines or HEK293-EP4

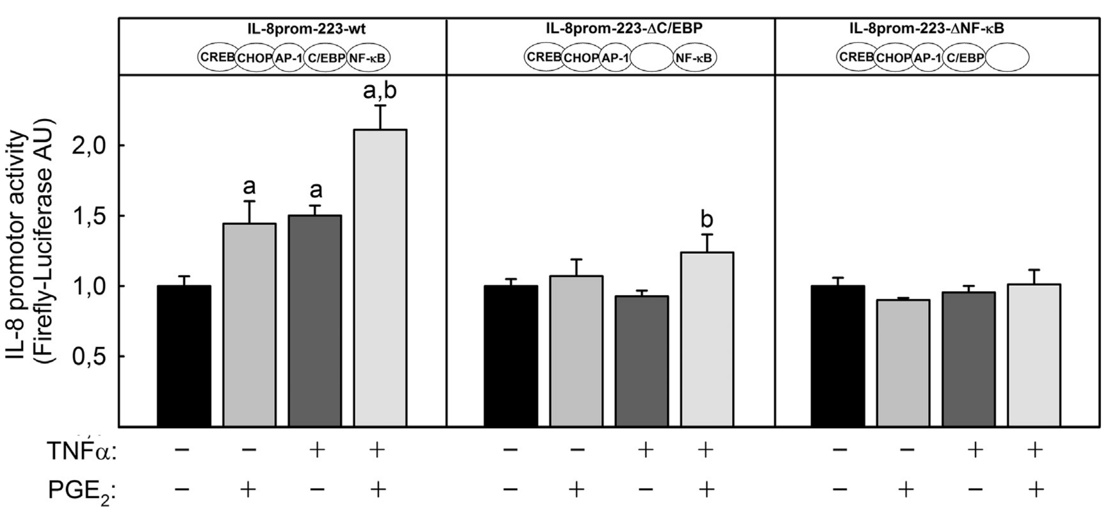

Fig. 8B. PGE_2 and TNFα mediated IL-8prom-223 or C/EBP or NF-κB deleted promoter activation in THP-1 cells. THP-1 cells were transfected with IL-8 promoter reporter gene plasmids harbouring the IL-8prom-223 core or deleted promoters lacking putative C/EBP or NF-κB sites. After 24 h THP-1 cells were stimulated with 1 µM PGE_2 and/or 50 ng/ml TNFα for 8 h. Firely luciferase activity was measured in lysates as described in the method section. Data shown are means ± S.E.M. of at least four independent experiments performed in triplicate. Statistics: Student's *t*-test for unpaired samples. a: significantly higher than control, b: significantly higher than TNFα ($p < 0.05$).

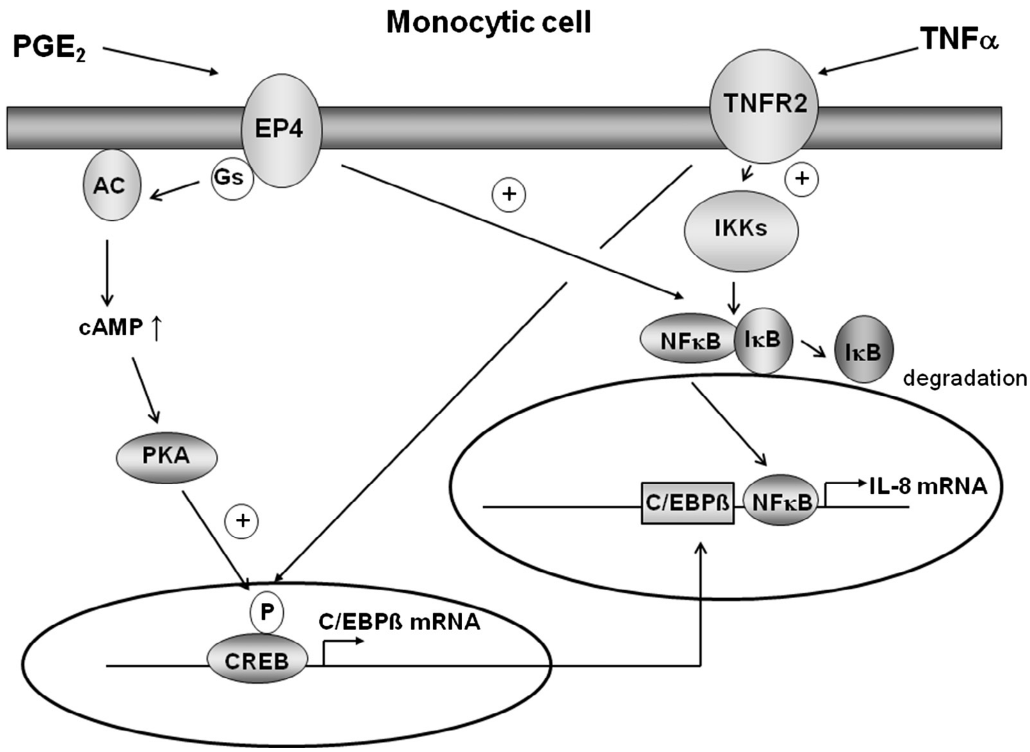

Fig. 9. Model of PGE_2 and TNFα-mediated IL-8 induction in monocytic cells. TNFα binds to the TNFR II, activates transcription factor NF-κB and induce C/EBPß expression possibly by activating of CREB. PGE_2-bound EP4 activates cAMP-dependent protein kinase A which may lead to CREB phosphorylation and subsequently induce C/EBPß expression. In addition PGE_2 enhanced NF-κB activation by TNFα. Together, the combined activation of transcription factors C/EBP and NF-κB by TNFα and PGE_2 led to maximal IL-8 expression in THP-1 cells.

cells may be due to a different EP receptor expression pattern in these cells. In contrast to monocytic cell lines and HEK cells, Caco-2 cells express a functional EP1 receptor in addition to the EP4 receptor [36]. Enhancement of IL-8 formation by the activation of the EP4 signal chain was also shown in a previous study with double transgenic HEK EP1 + EP4 cells. In these cells the activation of EP1 signal chain by a specific agonist led to IL-8 formation, which was augmented by additional stimulation of EP4 [19].

4.2. Targets of EP4-dependent signal chains in TNFα/PGE_2-stimulated IL-8 formation

IL-8 expression is mainly regulated on the transcriptional level. A core IL-8 promoter region spanning nucleotides -223 bp upstream of the putative IL-8 transcription start ATG is essential and sufficient for transcriptional regulation of the gene. The core promoter includes potential binding sides for the transcription factors CREB, CHOP, AP-1, C/EBP and NF-κB [37]. On the other hand, the NF-κB site is essential for IL-8 activation by pro-inflammatory cytokines IL-1ß and TNFα in most cell lines, the CREB, CHOP, AP-1 and C/EBP sites are not required for primary induction, but for maximal gene expression [37]. IL-8 induction by PGE_2 in cystic fibrosis cells or T-Lymphocytes was mediated by activation of the transcription factor CHOP (C/EBP homologues protein) [30]. By contrast, mutation of the CREB binding site suppressed PGE_2-mediated activation of the IL-8 promoter in Caco-2 cells expressing EP2 and EP4 [38] implicating a functional role of the transcription factor CREB in PGE_2-mediated IL-8 induction. Also, for the stimulatory effect of PGD_2 on TNFα-mediated IL-8 and MCP-1 expression in THP-1 cells, the transcription factor CREB was identified as a target of the DP signal chain since inhibition of PKA by the inhibitor H89 could block, and PKA-activation with the activator db-cAMP could mimic the PGD_2 effect [34].

Activation of CREB therefore seems to be a possible mechanism responsible for the PGE_2/EP4-mediated enhancement of TNFα-induced IL-8 induction in THP-1 cells since the PKA activator 6-Bnz-cAMP mimicked the effect of PGE_2. In accordance with such a model, stimulation of THP-1 with PGE_2 led to a significant CREB-phosphorylation at Ser-133 which evokes its transcriptional activity. Surprisingly TNFα also induced CREB phosphorylation in THP-1 cells. TNFα-induced CREB-phosphorylation was also observed in endothelial cells leading to enhanced expression of VCAM-1 [39]. In these cells TNFα-induced CREB phosphorylation was dependent on p38-MAPK activation.

However, direct binding of CREB to the IL-8 promoter as the main mechanism for PGE_2/TNFα-induced IL-8 induction seems unlikely since the truncation of the CRE half site only partially reduced promoter activation by PGE_2 and left promoter activation by TNFα + PGE_2 unaffected. A possible signal chain may therefore be a CREB dependent induction of C/EBPß expression which subsequently binds to the IL-8 promoter to induce IL-8 expression. This hypothesis is supported by the observations that a) PGE_2 and TNFα induced C/EBPß expression, b) activation of the IL-8 promoter by PGE_2 and TNFα was blunted after truncation or deletion of the C/EBP binding site in the IL-8 promoter and c) a sequential stimulation of THP-1 cells with PGE_2 in the first 10 h, which may induce C/EBPß expression, and stimulation with TNFα in last 10 h, which may activate NF-κB, led to an even larger IL-8 mRNA induction than stimulation with both substances for 20 h. A similar signal chain was also observed in murine bone derived macrophages and macrophage cell lines. In these cells, PGE_2 stimulation of EP2 and EP4 rapidly led to CREB phosphorylation and subsequently to C/EBPß expression [22]. As a result of C/EBPß induction, PGE_2 induced the expression of the anti-inflammatory genes Arg 1, IL-10 and Mrc1, which inhibited T-cell proliferation.

In contrast to PGE_2, which mainly acts via CREB and/or C/EBP activation, induction of IL-8 expression by TNFα is most likely mediated by the binding of transcription factors AP-1 and NF-κB, since TNFα alone activated AP-1 and NF-κB in THP-1 cells and the truncation of the AP-1 and NF-κB sides blunted promoter activation by TNFα alone.

Besides activating a PKA, CREB and C/EBPß signal cascade, PGE_2 and TNFα could also induce IL-8 expression by activating transcription factor NF-κB in a synergistic manner. In a previous study with double transgenic HEK-EP1 + EP4 cells, stimulation with an EP1 but not with an EP4 specific agonist activated the IL-8 minimal promoter, but stimulation of both EP1 and EP4 was necessary for maximal IL-8 promoter activation and IL-8 formation [19]. Maximal IL-8 promoter activation by EP1 + EP4 activation was achieved by maximal NF-κB activation when both receptors were stimulated. In line with IL-8 protein and mRNA as well as IL-8 promoter activity, PGE_2 also enhanced NF-κB activation in THP-1 cells. Together PGE_2 and TNFα most likely induce IL-8 expression by synergistic activation of C/EBPß expression and NF-κB activation (Fig. 9).

4.3. Potential physiological role of PGE_2 for TNFα-induced IL-8 formation in inflammatory processes

There are a number of indicators suggesting an important role for TNFα/PGE_2-induced IL-8 formation in inflammatory processes as colitis, rheumatic arthritis and atherosclerosis.

Inflammatory bowel diseases as ulcerative colitis and Crohn's disease are associated with high levels of pro-inflammatory cytokines such as TNFα and IL-1ß, and as a consequence increased levels of the chemokine IL-8 [40]. In addition, intestinal PGE_2 levels, as well as prostaglandin receptor EP4 expression are highly elevated in ulcerative colitis patients [41,42]. It is therefore most likely that IL-8 concentrations were upregulated in ulcerative colitis by a simultaneous stimulation by TNFα and PGE_2 which may lead to an enhanced infiltration of neutrophil cells to the site of inflammation and onset of the disease.

TNFα/PGE_2-stimulated IL-8 formation may also play a role in rheumatic arthritis. The development of rheumatic arthritis, which is characterized by a painful destruction of cartilage and bone, is largely regulated via excessive production of pro-inflammatory cytokines such as TNFα. In rheumatic arthritis proteinase MMP-3 and IL-8 levels are elevated in the synovial fluid. Increased MMP-3 expression leads to joint destruction [43], whereas IL-8 recruits inflammatory cells into the synovium [44]. In a SW982 synoviocyte model system, TNFα induced synthesis of IL-8 and MMP-3, and this induction was largely reduced by inhibition of cPLA2, an enzyme which liberates arachidonic acid, the precursor molecule for PGE_2 synthesis [45]. As TNFα also increased COX-2 mediated PGE_2 formation in this model, it is most likely that TNFα and PGE_2 synergistically induced IL-8 and MMP-3 formation leading to an escalation in the development of rheumatic arthritis.

Atherosclerosis is a chronic inflammatory process initiated by a diverse group of stimuli. Monocyte-derived macrophages comprise the majority of cellular components of the inflamed vascular tissue. One of the initial steps in the development of atherosclerosis is the firm adhesion of monocytes to endothelial cells leading to monocyte/macrophage differentiation and tissue invasion. This firm adhesion is initiated by TNFα-induced expression of adhesion molecules I-CAM and V-CAM on the surface of endothelial cells and chemokine, especially IL-8-induced activation of ß2-integrins on the surface of monocytes [3,5,2]. There is increasing evidence that prostaglandins may be involved in this mechanism. Long term incubation of human endothelial cells which linoleic acid, the precursor molecule of arachidonic acid and therefore PGE_2, enhanced adhesion of THP-1 cells and this enhancement was largely blocked by a neutralizing anti-IL-8 antibody [46]. COX-2, the inducible form of the key enzyme in prostaglandin synthesis, was expressed in atherosclerotic lesions but not in normal arteries [47]. Inhibition of COX-1 and COX-2 by nonsteroidal anti-inflammatory drugs like aspirin, led to a reduced progression of coronary atherosclerosis [48]. Our results indicate that TNFα and PGE_2 synergistically induced IL-8 expression in monocytic cell lines. Since both inflammatory stimuli were released in inflamed tissue it is therefore most likely that induced IL-8 expression will cause increased adhesion of monocytes to endothelial cells and promote the onset of atherosclerosis.

Conflict of interest

Authors declare that there is no conflict of interest..

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