Synergistic induction of monocyte chemoattractant protein-1 (MCP-1) by platelet-derived growth factor and interleukin-1

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Abstract Monocyte chemoattractant protein-1 (MCP-1) plays an important role in the recruitment of monocytic cells to the site of inflammation. Resting mesangial cells express barely detectable levels of MCP-1 mRNA. Treatment of rat mesangial cells with platelet products PDGF-AB, PDGF-BB or serotonin transiently induced MCP-1 expression with a maximum after 2 to 4 h and a decline to baseline after 6 to 8 h. Different kinetics were observed with interleukin-1β (IL-1β), which induced a long lasting elevation of MCP-1 mRNA for more than 20 h. Together, PDGF and IL-1β synergistically induced MCP-1 expression. The effect was most obvious after 16 to 20 h, when induction by PDGF alone had already faded, but still PDGF strongly enhanced IL-1β-induced MCP-1 mRNA expression. MCP-1 mRNA levels were regulated by changes in the stability of the mRNA: inhibition of protein synthesis by cycloheximide by itself induced MCP-1 mRNA expression and led to superinduction in the presence of PDGF. Message stabilization also contributed to the synergistic action of PDGF and IL-1β: the apparent half life of MCP-1 mRNA determined in the presence of actinomycin D was prolonged when both stimuli were added together. We could thus show that in mesangial cells different types of cytokines and growth factors synergize to enhance MCP-1, the secretion of which could lead to the recruitment of monocytic cells into the inflamed mesangium.

Key words: Monocyte chemoattractant protein (MCP-1); Interleukin-1; Platelet-derived growth factor (PDGF-AB, PDGF-BB); Mesangial cell (rat)

1. Introduction

Monocyte chemoattractant protein-1 (MCP-1) was originally characterized as a gene (JE) induced by platelet-derived growth factor (PDGF) in mouse fibroblasts [1,2]. Since then, it was shown that MCP-1 is a potent chemokine with considerable specificity for monocytes [3]. It is expressed in many different cell types besides fibroblasts such as endothelial cells, vascular smooth muscle cells, osteoblastic cells, mesangial cells or monocytes themselves.

Enhanced expression of the protein was observed in vivo in different models of inflammation and injury in which infiltration of monocytic cells was observed (e.g. [4,5]). In the kidney, MCP-1 expression was enhanced in different animal models of experimental glomerular nephritis (e.g. [6–9]). Glomerular MCP-1 was also detectable in biopsies from patients with inflammatory or proliferative glomerulonephritis [9]. The in vivo localization of MCP-1 suggested that glomerular mesangial cells might be a source of MCP-1, not excluding other cell types such as epithelial cells and infiltrating monocytes.

Platelet-derived growth factor (PDGF), the stimulus which led to the detection of MCP-1 activity, was recently shown to play a predominant role in glomerulonephritis [10,11]. In the model of anti-thymocyte antibody-induced glomerulonephritis [6], MCP-1 was found to be enhanced in an early phase, which might relate to the effect of immune complexes, but it was also elevated at a later time period, when both PDGF and interleukin-1 (IL-1) levels were known to be increased.

Given its functional role as chemoattractant, investigations have focussed on the regulation of MCP-1 expression by inflammation-related cytokines such as IL-1, tumor necrosis factor α (TNFα) or interferon γ. In mesangial cells, IL-1 and TNFα are the most potent stimuli investigated so far [12–14].

In order to get a further insight into the regulation of MCP-1 expression during glomerular inflammation, we investigated the mechanism of MCP-1 mRNA expression by PDGF and IL-1β in cultured mesangial cells.

2. Materials and methods

2.1. Materials

Cell culture media consisted of Dulbecco's modified Eagle's medium (DMEM), L-glutamine, penicillin and streptomycin (Biochrom), fetal calf serum (Gibco) and bovine insulin (Sigma). Recombinant human platelet-derived growth factors (rhPDGF-AA, -AB and -BB) were kindly provided by J. Hoppe (Biozentrum Würzburg, Germany). Recombinant human IL-1β (5×10^7 U/mg) was obtained from Boehringer.

2.2. Cell culture

Rat mesangial cells were isolated and cultured as previously described [15]. Cells were grown in DMEM (supplemented with 2 mM L-glutamine, 5 μg/ml insulin, 100 U/ml penicillin and 100 μg/ml streptomycin) containing 10% FCS. Before stimulation, the cells were growth-arrested by serum deprivation for 3 days in DMEM containing 0.5% FCS. For the experiments cells were used between passages 10 and 25.

2.3. Northern blot analysis

Analysis of mRNA expression was performed as previously described [16]. Total RNA was extracted according to the protocol of Chomczynski and Sacchi [17] with minor alterations. RNA yield usually was 30–40 μg/Petri dish.

Separation of total RNA (10 μg/lane) was achieved by use of 1.2% agarose gels containing 2% (v/v) formaldehyde with 1 × MOPS as gel/running buffer. Separated RNA was transferred to nylon membranes by capillary blotting. Hybridization was performed with cDNA probes labeled with [^{32}P]dCTP using the Megaprime random prime labeling kit (Amersham). A cDNA probe specific for GAPDH was obtained as reverse transcribed DNA amplified by polymerase chain reaction as described [18]. A cDNA probe specific for rat MCP-1 was kindly provided by Dr. T. Yoshimura (NCI, Frederick, MD) [19]. A cDNA probe for the inducible cyclooxygenase (PGHS-2) was kindly provided by D. DeWitt (Michigan State University, MI) [20].

DNA/RNA hybrids were detected by autoradiography using Kodak X-OMAT AR film; exposure time ranged between 1 and 6 days. Quantitative analysis was performed by densitometric scanning of the au-

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toradiographs (Bioprofil, Fröbel, Germany). All values were corrected for differences of RNA loading by calculating the ratio of MCP-1 or PGHS-2 to GAPDH expression or ethidium bromide fluorescence of 28S rRNA.

3. Results

3.1. MCP-1 mRNA expression in mesangial cells: induction by PDGF isoforms and serotonin

Basal levels of MCP-1 mRNA were very low in growth-arrested and cycling mesangial cells. Ongoing transcriptional activity became evident, when the cells were incubated in the presence of the inhibitor of protein synthesis cycloheximide (CHX; Fig. 1). CHX (10 μg/ml) rapidly enhanced the steady state levels of MCP-1 mRNA within 1 to 2 h. Elevated levels then persisted for several h. PDGF-AB and -BB in the concentration range of 5 to 50 ng/ml induced MCP-1 mRNA (Fig. 2), whereas PDGF-AA was without effect. No significant difference was observed between the two active isoforms. Induction of MPC-1 mRNA expression was transient, peaked at about 2 to 4 h and declined thereafter. Examples are shown in Figs. 1 and Fig. 4. In the presence of CHX, PDGF led to increasing superinduction of MCP-1 mRNA during the period measured (up to 6 h). Qualitatively the same results were obtained when serotonin (5-HT), another factor released from activated platelets, was used as stimulus (data not shown).

3.2. Effect of IL-1β plus PDGF on MCP-1 mRNA expression

As compared with PDGF or 5-HT, IL-1β induction of MCP-1 mRNA followed different kinetics. A continuous increase in mRNA levels was observed for 6 to 8 h and these increased levels persisted for at least 24 h (Figs. 3, 4). IL-1β was a potent inducer of MCP-1 mRNA in cycling and growth-arrested mesangial cells in the presence and absence of serum. PDGF in contrast, was more effective in serum-reduced cultures (0.5% serum).

Coincubation of mesangial cells with IL-1β and PDGF resulted in an enhanced MCP-1 mRNA expression. During the early phase, MCP-1 mRNA elevation was just additive. At later time points, when the induction of MCP-1 mRNA by PDGF declined, the elevated levels in cells coincubated with PDGF and IL-1β persisted (Fig. 3). Even after 16 h, when PDGF by itself no longer had any effect on MCP-1 mRNA levels, coincubation still led to enhanced MCP-1 mRNA levels (Fig. 4). A similar interaction was also observed with 5-HT and IL-1β: a synergistic increase in MCP-1 mRNA induction was observed after 16 h, at a time when serotonin by itself was without effect (data not shown). Induction of MCP-1 mRNA by TNFα, another inflammatory cytokine, followed similar kinetics as observed with IL-1β. Coincubation of mesangial cells with TNFα and PDGF synergistically enhanced MCP-1 mRNA expression (Fig. 5).

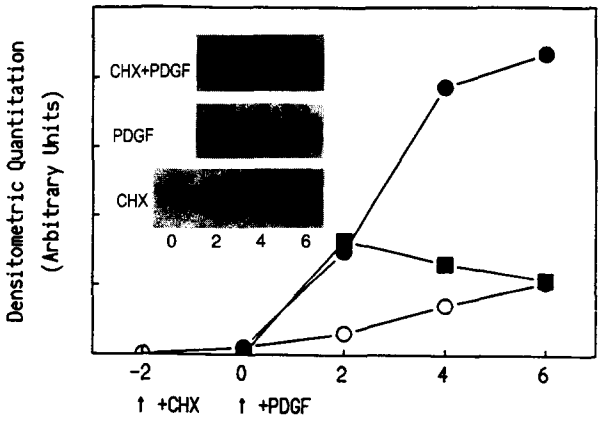

Fig. 1. Induction of MCP-1 mRNA by PDGF and CHX mesangial cells were incubated with CHX (○, 10 μg/ml), PDGF-AB (■, 20 ng/ml) or both compounds (●) for the times (h) indicated. Northern blot analyses were performed as described. Data shown are representative of three independent experiments.

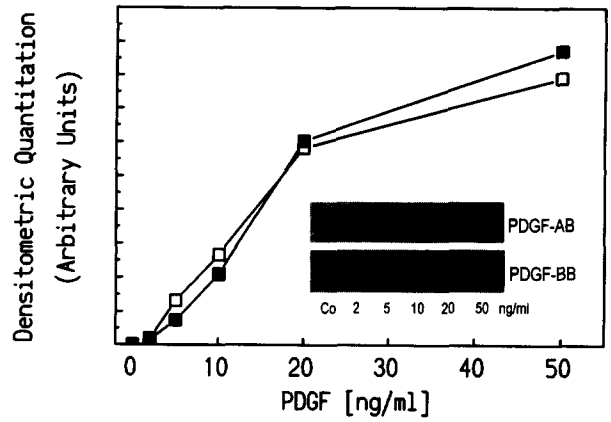

Fig. 2. Concentration-dependent induction of MCP-1 mRNA by PDGF-AB and -BB mesangial cells were incubated with PDGF-AB (■) or PDGF-BB (□) at the concentrations indicated for 2 h. Northern blot analysis was performed as described.

In order to investigate whether PDGF or IL-1β induced global upregulation of inducible genes related to inflammation we determined the mRNA expression of cyclooxygenase-2 (prostaglandin G/H synthase, PGHS-2). The mRNA of this enzyme was upregulated by PDGF, but not by IL-1β. When PDGF was added together with IL-1β, cyclooxygenase expression was not further enhanced (Fig. 5).

3.3. MCP-1 mRNA stability

mRNA stability was determined in the presence of the inhibitor of transcription actinomycin D (10 μg/ml). Coincubation of mesangial cells with PDGF and IL-1β increased the apparent half life of MCP-1 compared with the half life in the presence of either stimulus alone (Fig. 6).

4. Discussion

Recruitment of monocytic cells from the circulation to inflamed or injured tissue sites such as in glomerulonephritis has been observed in many forms of glomerular disease. Among other factors, MCP-1, which is secreted from activated mesangial cells, has been postulated to be involved as a chemoattractant of monocytes to the glomerulus [22]. Thus far, interleukin-1 (IL-1), tumor necrosis factor α (TNFα) [12,13], thrombin [23], interferon γ [24] and immune complexes [25] have been shown to induce MCP-1 mRNA expression in mesangial cells. Since PDGF seems to be critically involved in the pathogenesis of various types of glomerular inflammation [10,11], we investigated the effect of PDGF alone and in combination with IL-1β on the expression of MCP-1 mRNA.

MCP-1 mRNA levels were very low in unstimulated mesangial cells, but were significantly enhanced in the presence of cycloheximide (CHX). Induction by CHX is generally interpreted as mRNA stabilization due to the reduced synthesis of RNases. mRNA stabilization by CHX is typical for immediate early genes, the expression of which is independent of de novo protein synthesis. MCP-1 was originally identified as an immediate early gene in fibroblasts and CHX-dependent MCP-1 mRNA expression was also observed in smooth muscle cells and endothelial cells [26,27]. Regulation by CHX, however, seems to be cell-specific, because in peripheral blood monocytes it caused inhibition of MCP-1 mRNA expression [26,27].

Induction of MCP-1 mRNA by PDGF-AB, -BB or serotonin (5-HT) and IL-1β or TNFα followed different kinetics: prolonged expression was observed by incubation with IL-1β or TNFα, whereas induction of MCP-1 by PDGF-AB and -BB or 5-HT was transient. The apparent half lives of MCP-1 mRNA determined in the presence of the inhibitor of transcription, actinomycin D, seemed to contradict these kinetics: The apparent half life in the presence of IL-1β was rather shorter than in the presence of PDGF. IL-1β thus appeared to affect mRNA transcription. With respect to PDGF, additional mechanisms seem to be operative in the absence of actinomycin D, which counteract the mRNA stabilization observed in the presence of actinomycin D. A similar effect was previously observed in 5-HT-stimulated mesangial cells, where the apparent half life of the inducible form of cyclooxygenase exceeded the transient expression [28]. Because of the low expression in unstimulated cells, we could not determine the half life of MCP-1 mRNA in the absence of any stimulation. In vascular smooth muscle cells, which have many characteristics in common with mesangial cells, the apparent half life of MCP-1 mRNA was ~1 h and was prolonged by PDGF or angiotensin II [29].

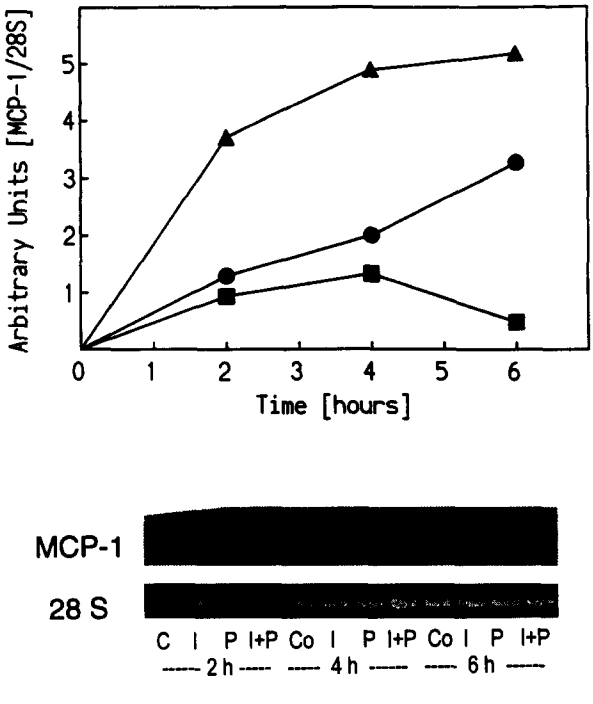

Fig. 3. Time course of PDGF- and IL-1β-mediated MCP-1 mRNA induction mesangial cells were incubated with IL-1β (I, ● 2 U/ml), PDGF-BB (P, ■, 20 ng/ml) or both stimuli (I+P, ▲) for the times indicated. No MCP-1 mRNA signal was detected in untreated cells (Co). Data shown are representative of four experiments with similar results.

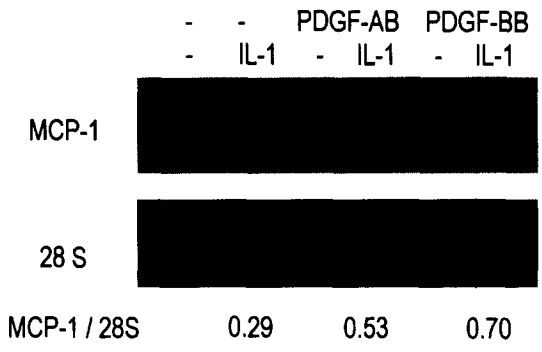

Fig. 4. Synergistic induction of MCP-1 mRNA by PDGF and IL-1β mesangial cells were incubated with PDGF-AB or -BB (20 ng/ml each) in the presence or absence of IL-1β (2 U/ml) for 16 h. Data are representative of three experiments.

Induction of MCP-1 mRNA was synergistic, when PDGF and IL-1β were added together. This setting might reflect the situation in vivo more closely, when both cytokines, which may be secrected by activated mesangial cells or by infiltrating platelets and monocytes, are present together in the inflamed glomerulus. Synergism was also observed with PDGF and TNFα, a cytokine which shares many properties with IL-1β. The synergism was reflected at the level of mRNA stabilization, which was enhanced, when PDGF and IL-1β acted together. Synergism between PDGF and IL-1β was not a general phenomenon, as other proteins associated with inflammation such as the inducible cyclooxygenase-2, were not synergistically induced by PDGF and IL-1β.

We could thus show that two cytokines, which are involved in glomerular inflammatory reactions and which act via different intracellular signalling pathways, cooperate to enhance MCP-1 expression and thus might promote infiltration of monocytic cells into inflamed tissues.

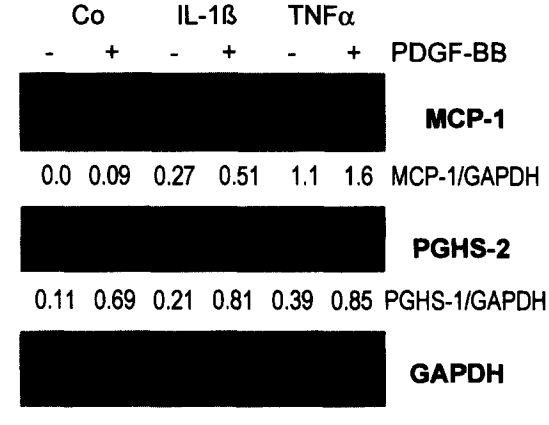

Fig. 5. Specificity of synergistic MCP-1 mRNA induction mesangial cells were incubated with IL-1β (2 U/ml), TNFα (5 ng/ml) with or without PDGF-BB (20 ng/ml) for 2 h. Northern blot analysis was performed as described with cDNAs specific for MCP-1, cyclooxygenase-2 (prostaglandin G/H synthase-2, PGHS-2) and GAPDH.

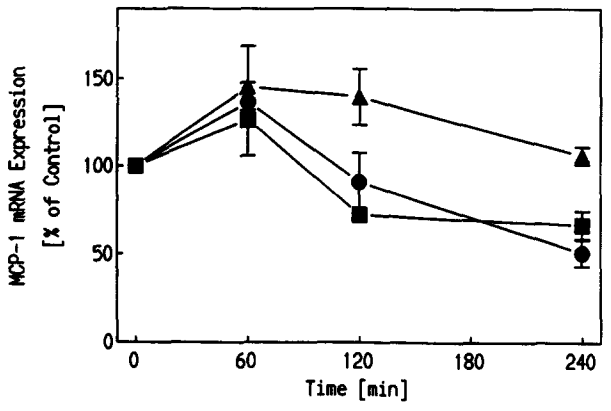

Fig. 6. Apparent half life of MCP-1 mRNA mesangial cells were incubated with IL-1β (●, 2 U/ml), PDGF-AB (■, 20 ng/ml) or both stimuli (▲) for 2 h. Transcription was then inhibited by addition of actinomycin D (10 μg/ml) and cells were further incubated for the times indicated. Northern blot analyses were quantitated by densitometry. Data are mean ± S.E.M. of three to five experiments.

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