 

# Ciglitazone Inhibits Plasmin-Induced Proinflammatory Monocyte Activation via Modulation of p38 MAP Kinase Activity

Tatiana Syrovets, Almut Schüle, Marina Jendrach, Berthold Büchele, Thomas Simmet

Department of Pharmacology of Natural Products and Clinical Pharmacology, University of Ulm, Germany

**Keywords**

Monocytes/macrophages, cytokines, chemotaxis, transcription factors, p38 MAPK

**Summary**

Plasmin triggers chemotaxis and NF-κB- and AP-1-mediated proinflammatory gene expression in human peripheral monocytes (PM). Compared with macrophages and dendritic cells, PM express mainly the peroxisome proliferator-activated receptor (PPAR) γ and traces of PPARα as detected by semiquantitative RT-PCR and immunoblotting. The PPARγ agonist ciglitazone, but not the PPARα agonist clofibric acid, concentration-dependently inhibited the plasmin-, but not the FMLP-induced PM chemotaxis. Similarly, release of interleukin (IL)-1α, IL-1β and tumor necrosis factor (TNF)-α from plasmin-stimulated PM was concentration-dependently inhibited by ciglitazone, but not by clofibric acid, while the LPS-induced TNF-α release remained unaffected by any of both PPAR agonists. Ciglitazone activates PPARγ as shown by a novel surface plasmon resonance analysis and inhibits the plasmin-induced activation of NF-κB and AP-1. It also inhibits p38 MAPK phosphorylation essential for the plasmin-induced PM chemotaxis and gene activation. Thus, activation of PPARγ by ciglitazone may allow controling of the plasmin-mediated recruitment and activation of PM at sites of inflammation.

## Introduction

Apart from lysis of blood clots, plasmin has been linked to additional functions, particularly in inflammation and atherogenesis. Consistent with the enhanced expression of fibrinolytic genes in atherosclerotic lesions (1-3), increased fibrinolytic activity has also been described in coronary endarterectomy specimens (4). The pathophysiological significance of these findings was confirmed in plasminogen-deficient mice that demonstrated impaired development of atherosclerotic lesions as well as severely compromised PM recruitment to sites of inflammation (5-7). Further in line with a role of plasmin in inflammation, urokinase-type plasminogen activator expression was induced in inflamed synovial tissues in rheumatoid arthritis and elevated levels of $\alpha_2$-antiplasmin-plasmin complexes have been detected in synovial fluid from affected joints (8, 9). Moreover, inflammatory reactions are generally linked to local contact activation known to trigger plasmin-mediated PM activation (10).

On the cellular level, we have previously shown that plasmin induces proinflammatory activation of PM in terms of leukotriene lipid mediator release, chemotaxis, and cytokine induction (10-13). Although in PM, as in other cells, the molecular identity of the plasmin-activated receptor has not been identified yet, signaling proceeds independent from inositol 1,4,5 trisphosphate in a pertussis toxin-sensitive and cyclic guanosin monophosphate-dependent manner (11, 12). In PM plasmin triggered concentration-dependent expression of IL-1α, IL-1β, TNF-α and tissue factor. Consistent with induction at the level of transcription, plasmin-mediated PM stimulation led to activation of the transcription factors AP-1 and NF-κB suggesting an overall proinflammatory gene activation (13).

The mitogen-activated protein kinase (MAPK) pathways encompassing the stress-activated kinases (SAPK), JNK, and p38 are widespread signal transduction mechanisms. The MAPK p38 can be strongly activated by environmental stresses and cytokines (14). Importantly, p38 was identified as the receptor for experimental pyridinyl-imidazole antiinflammatory drugs that suppress cytokine release (15). Increasing evidence suggests that p38 activation may not only be crucial for LPS-mediated induction of cytokines in PM or macrophages (14), but may also play a significant role in the signaling by other stimuli (16, 17).

Peroxisome proliferator-activated receptors (PPARs) belong to the nuclear receptor superfamily. PPARs function as ligand-activated transcription factors that modulate expression of target genes by binding to specific response elements (PPRE) (18, 19). PPAR ligands include hypolipidemic drugs such as fibrates and insulin-sensitizing compounds such as thiazolidinediones. Recent findings suggested that PPARs might have a role in the regulation of inflammation (19). PPARγ ligands have been reported to inhibit the phorbol myristate acetate-induced TNF-α release by PM, but not that triggered by LPS (20). However, this observation was not confirmed by others (21). Indeed, the putative regulatory role of PPARγ in the regulation of PM or macrophage cell activation and the production of proinflammatory cytokines remains controversial (20, 22-24); some authors even reported that undifferentiated PM would not express PPARγ, but only PPARα (25). In addition, it cannot be ruled out that PPARγ agonists might target other receptors or mechanisms as it has been reported for 15-deoxy-$\Delta^{12,14}$-$PGJ_2$ that can exert at least part of its function through direct inhibition of IκB kinase rather than activation of PPARγ (26-29).

Nevertheless, fibrates and thiazolidinediones as agonists for PPARα and PPARγ, respectively, are widely used for the treatment of hyperlipidemia and diabetes (19). Because of the putative anti-inflammatory effects of these drugs, we have investigated the modulatory effects of PPAR agonists on the plasmin-mediated activation of PM.

Correspondence to: Dr. Thomas Simmet, Department of Pharmacology of Natural Products and Clinical Pharmacology, University of Ulm, Helmholtzstr. 20, D-89081 Ulm, Germany – Tel.: 0049 731 5002 4280; Fax: 0049 731 5002 4299; E-mail: thomas.simmet@medizin.uni-ulm.de

## Materials and Methods

*Materials*

Plasmin (Lot 354915 and 352817, specific activity 14.83 Committee on Thrombolytic Agents (CTA) U/mg) was from Fluka (Deisenhofen, Germany). Polyclonal antibodies (Ab) against PPARα and PPARγ were from Santa Cruz Biotechnology (Santa Cruz, CA), rabbit anti-PPARγ Ab used for surface plasmon resonance (SPR) was from Alexis (San Diego, CA), rabbit Abs against p38 MAPK and P-p38 were from New England Biolabs (Beverly, MA). Ciglitazone was from Biomol (Plymouth Meeting, PA), clofibric acid from Tocris Cookson (Avonmouth, UK). Anti-CD14 and anti-CD41 Abs were purchased from Immunotech (Marseille, France). Histopaque 1077, FMLP and LPS (Escherichia coli serotype 055:B5) were obtained from Sigma. Percoll was from Pharmacia Biotech (Uppsala, Sweden). Oligo$(dT)_{25}$ were from Dynal (Oslo, Norway). Oligo(dT) and random primers were from Life Technologies (Karlsruhe, Germany). MACS anti-CD14 magnetic micro beads were from Miltenyi Biotec (Auburn, CA). M-CSF and IL-4 were purchased from R&D Systems (Minneapolis, MN). GM-CSF was from Sandoz (East Hanover, NJ) and neutravidin from Pierce (Rockford, IL). Other chemicals were of analytical grade; all reagents were LPS-free as measured by the Limulus amoebocyte lysate assay (Sigma).

*Monocyte Preparation and Cytokine Measurements*

PM were isolated by plasma/Percoll gradient centrifugations (12, 13). Preparations with ≥ 94% $CD14^+$ cells were used. Contaminating cells were lymphocytes. FACS analysis with anti-CD41 mAb did not reveal any platelets associated with PM. For PPAR analysis by RT-PCR and immunoblot, PM were additionally purified with anti-CD14 magnetic microbeads yielding > 98% $CD14^+$ cells. Some experiments conducted without the additional purification step yielded essentially the same results.

For cytokine measurements PM were incubated for 1 h in lysine-free RPMI 1640 in the presence or absence of PPAR ligands; then 100 ng/ml LPS or 0.43 CTA U/ml plasmin was added and incubations continued for another 8 h. Controls received appropriate amounts of DMSO. The PPAR agonists did not affect the plasmin activity as tested with SS-2251 (Chromogenix, Milano, Italy). Cytokines were assayed in cell-free supernatants by ELISAs specific for IL-1α (Cytimmune, College Park, MD), IL-1β (Biosource, Camarillo, CA) and TNF-α (R&D Systems, Minneapolis, MN). Detection limits were 0.8 pg/ml for IL-1α, 0.31 pg/ml for IL-1β, and 4.4 pg/ml for TNF-α.

*Differentiation of Monocytes into Macrophages and Dendritic Cells*

PBMC were isolated from buffy coats by Histopaque 1077 gradient centrifugation. Adherent PM were differentiated into macrophages with 15 ng/ml M-CSF for 8 days (30, 31). FACS analysis showed downregulation of CD14 and upregulation of CD68. Dendritic cells were differentiated from adherent PM in the presence of 1000 U/ml GM-CSF and 25 ng/ml IL-4 for 6 days (30, 31). FACS analysis demonstrated that cells expressed CD1a but not CD83 (32).

*Semiquantitative RT-PCR*

mRNA isolated with Oligo$(dT)_{25}$ magnetic beads was analyzed with primers specific for IL-1α, IL-1β, TNF-α and HLA(B) as internal standard (13). Primers for PPARα: 5'-CCA GTA TTT AGG AAG CTG TCC-3' (sense) and 5'-TGA AGT TCT TCA AGT AGG CCT C-3' (antisense) with an expected size of 493 bp for the PCR product; for PPARγ: 5'-GGC AAT TGA ATG TCG TGT CTG TGG AGA TAA-3' (sense) and 5'-AGC TCC AGG GCT TGT AGC AGG TTG TCT TGA-3' (antisense), with an expected size of 900 bp for the PCR product (25). PCR reactions did not reach the saturation phase. Control experiments showed no DNA contaminations. PCR products were identified by direct sequencing (Abi Prism 310, Applied Biosystems, Foster City, CA).

*Western Blot Analysis and Immunostaining*

PM, macrophages, and dendritic cells ($5 \times 10^6$ cells/sample) were analyzed as described (13).

*Surface Plasmon Resonance Analysis of PPAR Activation*

Nuclear extracts were isolated from PM ($5 \times 10^6$ cells/sample) treated with PPAR ligands for 1 h (13). Biotinylated double-stranded PPAR response element (PPRE) (AGA CCT TTG GCC CAG TTT TT) of the 3-hydroxy-3-methyl-glutaryl-CoA-synthase (HMG) promoter (33) was immobilized on a sensor chip (XanTec Analysensysteme, Münster, Germany) (34). Before measurements, nuclear extracts were transferred by gel filtration into binding buffer (20 mM HEPES, pH 7.9, 150 mM NaCl, 0.5 mM $MgCl_2$, 0.1 mM EDTA, 1.0 mM DTT, 0.05% Nonidet P 40, 0.1% BSA and 1 μg of poly[d(I·C)]). Measurements were performed with a dual channel IBIS II optical sensor device (XanTec Analysensysteme, Münster, Germany) (34). Binding of 1 μg protein of nuclear extracts to immobilized PPRE was analyzed in 40 μl binding buffer at 8° C. Binding of nuclear extracts from unstimulated PM was used as reference. In competition experiments the fluid phase contained 1 nmole PPRE (specific competition) or NF-κB RE (non-specific competition). For "supershift" experiments 1 μg of rabbit anti-PPARγ Ab (Alexis) or rabbit anti-PPARα Ab (H-98, Santa Cruz) was added 10 min before measurement.

*Migration Assays*

Cell migration was evaluated in triplicate using tissue culture-treated 24-well Transwell plates (Costar, Cambridge, MA) with polycarbonate membranes (pore size 5 μm) (12, 13, 35). PM were preincubated with PPAR activators for 20 min at 37° C and then allowed to migrate for 90 min towards 10 nM FMLP or 0.43 CTA U/ml plasmin.

*Electrophoretic Mobility Shift Assays*

Analysis of nuclear translocation of NF-κB and AP-1 was performed by EMSA as previously described (13).

*Statistical Analysis*

Data shown represent mean ± SEM where applicable. Statistical significance was calculated with Newman-Keuls test. Differences were considered significant for $p < 0.05$.

## Results

*Differential Expression of PPARα and PPARγ in Human Peripheral Monocytes and Macrophages and Dendritic Cells*

PPARα mRNA expression was highest in macrophages followed by dendritic cells, while in freshly isolated PM hardly any PPARα could be detected (Fig. 1A). When 40 amplification cycles were used, a PPARα band from PM appeared, but those from macrophages and dendritic cells were beyond the linear amplification range (results not shown). In contrast, PPARγ mRNA, expression was very strong in both macrophages and dendritic cells; in PM it was lower, but still robust.

Differential expression of PPARα and PPARγ in PM, macrophages and dendritic cells was confirmed at the level of protein expression showing essentially the same pattern as the mRNA expression (Fig. 1B). PM preparations were not preactivated as judged by the lack of TNF-α mRNA expression (data not shown). Thus, PPARγ is readily expressed in non-activated PM and its amounts are enhanced upon differentiation into either macrophages or dendritic cells. Differentiation is also accompanied by a strong upregulation of PPARα.

*Effects of PPARγ and PPARα Ligands on Monocyte Migration*

Using ciglitazone, a thiazolidinedione, and clofibric acid, a fibrate, the significance of PPARγ or PPARα activation for the plasmin-induced PM migration was investigated. As shown in Fig. 2, chemotaxis

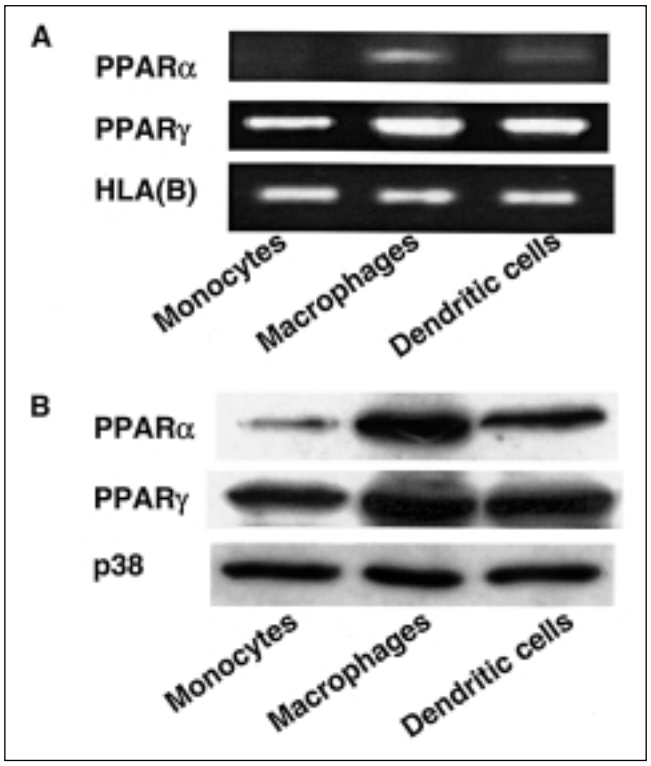


*Fig. 1* Differential expression of PPARα and PPARγ in PM, macrophages and dendritic cells. mRNA expression (A). Semiquantitative RT-PCR of mRNA extracted from freshly isolated PM or PM-derived macrophages or dendritic cells. HLA(B) was used for normalization. Protein expression (B). Western blot of PPARα and PPARγ protein expression using appropriate Abs. Expression of p38 MAP kinase was used for normalization. Representative of 3 experiments

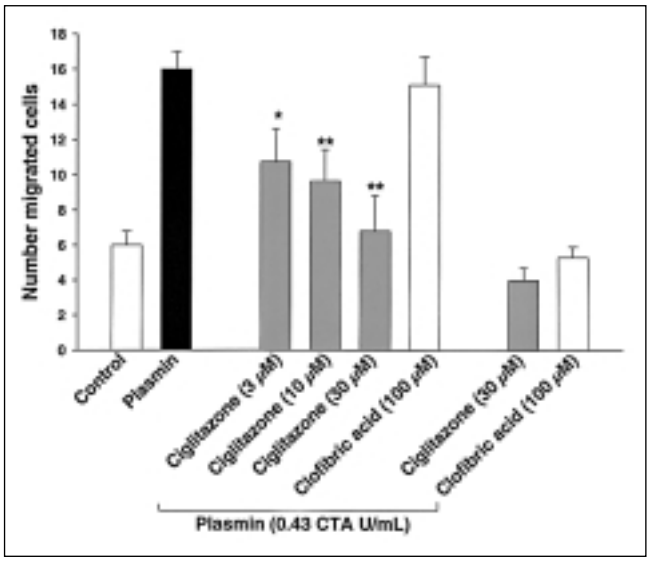


*Fig. 2* Effects of ciglitazone and clofibric acid on plasmin-induced PM migration. PM preincubated with the PPAR agonists were subsequently allowed to migrate across polycarbonate membranes towards plasmin 0.43 CTA U/ml. Mean ± SEM, n = 4 each performed in triplicate. *, $p < 0.05$, **, $p < 0.01$, versus plasmin controls

was concentration-dependently blocked by ciglitazone between 3 and 30 μM, but not by 100 μM clofibric acid. The PPAR ligands did not significantly affect random migration. In contrast to the plasmin-mediated chemotaxis, PM migration induced by FMLP 10 nM remained unaffected even by the highest concentrations of the PPAR agonists used (number of migrated cells; control: 4 ± 0.9; FMLP (10 nM): 20 ± 1.2; FMLP/ciglitazone (30 μM) 21 ± 0.9; FMLP/clofibric acid (100 μM) 19 ± 1.3; n = 4 performed in triplicate). Thus, ciglitazone significantly interferes with the plasmin-mediated, but not the FMLP-induced chemotactic signaling in PM.

### *Effects of PPARγ and PPARα Ligands on Proinflammatory Cytokine Production*

The effects of ciglitazone and clofibric acid on the plasmin-induced release of IL-1α, IL-1β and TNF-α were further examined (Fig. 3A-C). The PPARγ agonist ciglitazone concentration-dependently inhibited the release of all three cytokines assayed. Up to 100 μM the PPARα ligand clofibric acid had no effect on cytokine production. Neither the PPARγ ligand ciglitazone nor clofibric acid had any significant effect on TNF-α release triggered by LPS 100 ng/ml (control: n.d.; LPS (100 ng/ml): 100%; LPS/ciglitazone (10 μM): 110 ± 26%; LPS/clofibric acid (100 μM): 88 ± 20%, n = 4 each). Thus, treatment of PM with the PPARγ ligand ciglitazone, but not with the PPARα ligand clofibric acid, led to significant inhibition of the plasmin-induced proinflammatory cytokine production.

### *Activation of PPAR as Analyzed by Surface Plasmon Resonance*

Activation of PPAR either by the PPARγ agonist ciglitazone or the PPARα ligand clofibric acid was examined by measuring binding to PPAR response element (PPRE) by surface plasmon resonance (SPR) analysis. Treatment of PM with ciglitazone activated PPAR resulting in an increased binding of nuclear extracts (NE) to PPRE as compared to unstimulated cells (Fig. 4A, left hand panel). In order to characterize the composition of the DNA-protein complex, experiments with PPARα- and PPARγ-specific antibodies were performed. These data demonstrate that ciglitazone triggered binding of PPARγ as shown by the enhanced SPR signal (Fig. 4A, right hand panel). Binding of PPARγ could be antagonized by an excess of PPRE oligonucleotide (1 nmole) in the fluid phase, but not by unrelated NF-κB consensus oligonucleotide (1 nmole). In agreement with the faint expression of PPARα in PM, clofibric acid 100 μM stimulated barely any detectable increase in binding to PPRE (Fig. 4B).

### *Ciglitazone Inhibits Activation of NF-κB and AP-1 by Plasmin*

NF-κB and AP-1 binding sites are present in the promoter regions of many cytokine genes (36). PPARs have been reported to inhibit activation of NF-κB and may thus downregulate expression of the NF-κB-dependent cytokine genes (23, 37, 38). As analyzed by EMSA, 0.43 CTA U/ml plasmin stimulated nuclear translocation of NF-κB and activation of AP-1 that was inhibited by preincubating PM with the PPARγ ligand ciglitazone 10 μM. By contrast, the PPARα ligand clofibric acid 100 μM had no effect on the plasmin-mediated activation of nuclear factors (Fig. 5). The specificity of NF-κB and AP-1 binding was confirmed in competition experiments; a 100-fold molar excess of unlabeled NF-κB and AP-1, respectively, but not of unspecific AP-2 probe abolished binding of the nuclear extracts to their respective consensus oligonucleotides (data not shown).

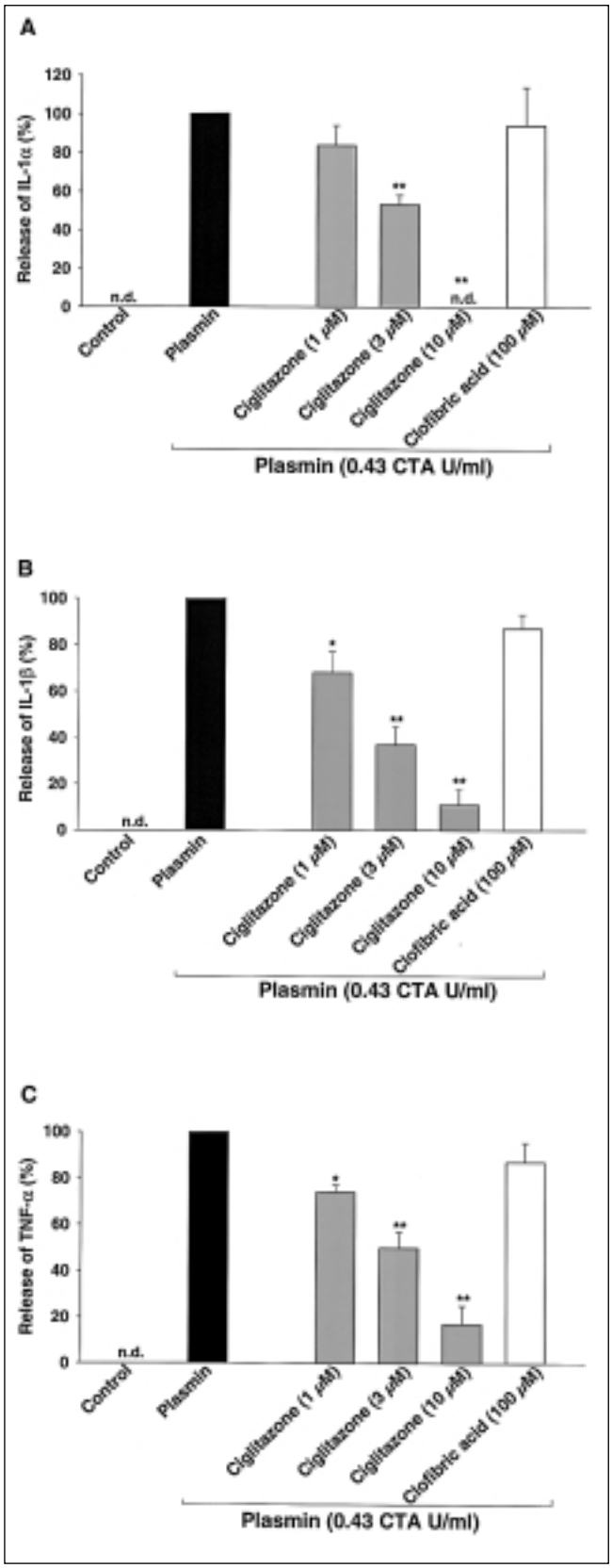


*Fig. 3* Effects of ciglitazone and clofibric acid on plasmin-mediated proinflammatory cytokine production. PM preincubated with or without the PPAR agonists were stimulated with plasmin 0.43 CTA U/ml. Supernatants were analyzed by ELISA for IL-1α (100%: 5.3 ± 1.8 ng/ml) (A), IL-1β (100%: 8.5 ± 2.5 ng/ml) (B) and TNF-α (100%: 25.5 ± 6.2 ng/ml) (C). Spontaneous release of cytokines was below the detection limit (n.d.). Mean ± SEM, n = 4 each. *, $p < 0.05$, **, $p < 0.01$ versus plasmin controls

*Ciglitazone Inhibits p38 Phosphorylation*

Previous results from our lab indicate that activation of p38 is essential for both, the plasmin-induced PM chemotaxis and the cytokine expression (39). Therefore, we investigated the effects of ciglitazone and clofibric acid on the plasmin-induced phosphorylation of p38 MAPK. Plasmin 0.43 CTA U/ml triggered p38 phosphorylation, which indeed was effectively inhibited by the PPARγ ligand ciglitazone, but not by the PPARα agonist clofibric acid (Fig. 6). Thus, the PPARγ activator ciglitazone inhibits p38 phosphorylation crucial for the plasmin-mediated proinflammatory PM activation.

## Discussion

PPARs are expressed to a different extent in various cells and tissues (18, 19). Both, differential tissue expression as well as different ligand patterns determine cell-specific functions of PPAR isotypes. PPARγ is prominently expressed in cells of the myelomonocytic lineage (40). PPARγ expression was shown in monocytic cell lines as well as in macrophages (21-24, 41), although its expression in unstimulated PM remains unclear. PPARγ is reportedly expressed in adherence-isolated PM (42) and PPARγ agonists inhibit the PMA-induced cytokine production in these cells suggesting that PPARγ is functionally active (20). However, other authors detected either none or only negligible amounts of PPARγ mRNA in PM and no immunodetectable PPARγ protein at all (21, 25). On the other hand, our observation that PM as well as other antigen-presenting cells such as macrophages and immature dendritic cells express PPARγ is in agreement with findings of Gosset et al. (43); these authors also provided evidence for a complex immune modulatory function of PPARγ activation in immature dendritic cells.

As analyzed by semiquantitative RT-PCR, PM isolated by gentle autologous plasma/Percoll density gradient centrifugation express PPARγ mRNA. PM were not preactivated as shown by the lack of proinflammatory cytokine mRNA expression (13). PPARγ expression was also verified at the protein level by immunoblotting. PPARγ was present in nuclear, but not in cytosolic extracts (results not shown).

The PPARγ activator ciglitazone was a potent inhibitor of the plasmin-triggered PM chemotaxis. By contrast, the PPARα ligand clofibric acid had no effect. Neither ciglitazone nor clofibric acid affected migration of PM to the standard chemoattractant FMLP indicating that the inhibitory effect of the PPARγ agonist is restricted to distinct chemoattractants. Compared with ciglitazone, 15-deoxy-$\Delta^{12,14}$-$PGJ_2$, another PPARγ agonist yet with lower specificity (27, 39), yielded comparable results (44). Similar to plasmin-induced chemotaxis in PM, various PPARγ, but not PPARα ligands, inhibit directed vascular smooth muscle cell migration (45, 46). On the other hand, both PPARγ and PPARα ligands were reported to inhibit the MCP-1-induced migration of the THP-1 tumor cell line by a so far unidentified mechanism (47).

Cytokine release by plasmin-stimulated PM was also concentration-dependently inhibited by ciglitazone, but not by clofibric acid. Again the inhibitory effect the PPARγ ligand ciglitazone proved to be stimulus-specific because it was not observed when TNF-α release by PM was triggered by LPS. Consistent with the effects on PM migration, similar results were obtained with the prostaglandin derivative and PPARγ activator 15-deoxy-$\Delta^{12,14}$-$PGJ_2$ (44).

In order to characterize the function of ciglitazone and clofibric acid in plasmin-stimulated PM, we examined PPAR activation by a novel SPR analysis. As expected, ciglitazone triggered activation of PPARγ as detected in NE. In accordance with the low expression of PPARα, clofibric acid had no significant PPARα-activating effect in PM. These

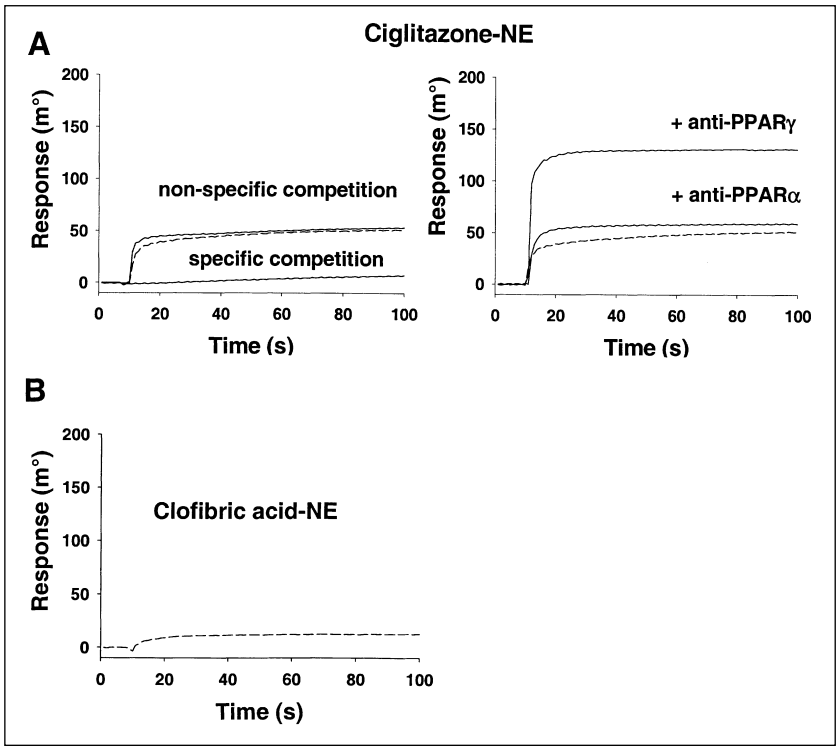


*Fig. 4* Activation of PPAR as analyzed by surface plasmon resonance. PM were incubated in the absence or presence ciglitazone 10 μM (A) or clofibric acid 100 μM (B). Differential binding of nuclear extracts (NE) from untreated versus pretreated cells was recorded. Enhanced SPR response indicates binding of the corresponding NE to PPRE (broken lines). PPRE from the HMG promoter (1 nmole) was used as specific competitor (left hand side, lower solid lines). Non-specific competitor was the NF-κB consensus sequence (1 nmole) (upper solid lines). Binding in the presence of PPARγ and PPARα Abs, each 1 μg (right hand side, solid lines). Representative of 5 experiments

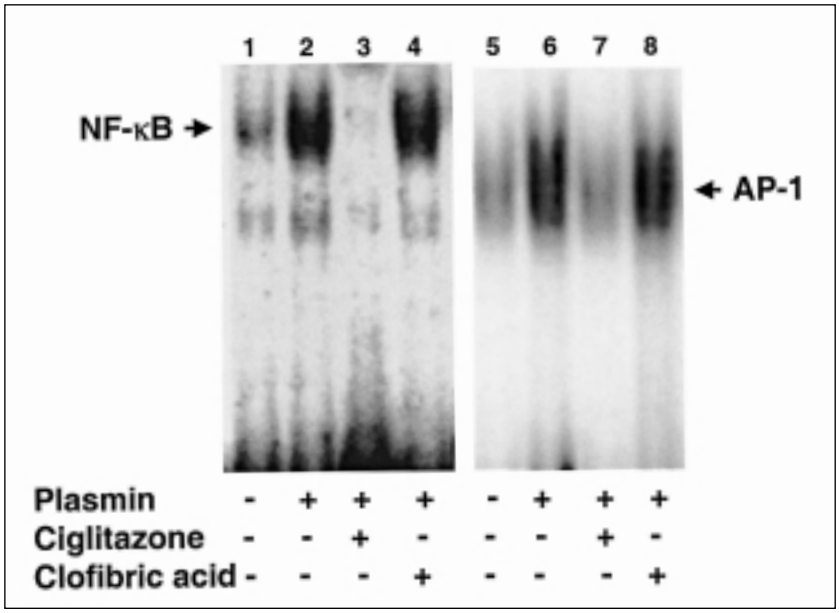


*Fig. 5* Ciglitazone inhibits the plasmin-mediated activation of NF-κB and AP-1. PM preincubated with ciglitazone 10 μM (lane 3 and 7), clofibric acid 100 μM (lane 4 and 8) or solvent (lane 1, 2, 5, 6) were stimulated with plasmin 0.43 CTA U/ml (lanes 2-4, 6-8). NE (5 μg) were subjected to EMSA with a $^{32}$P-labeled probe containing either the NF-κB or AP-1 binding site. Representative of 3 experiments

results indicate that PPARγ is expressed in PM and is functionally active.

PPARγ may regulate gene transcription by binding to PPAR response elements. Yet, it was found that PPARγ ligands could also repress activation of NF-κB-, AP-1- and STAT1-sensitive response elements in the absence of a binding site for PPARγ in the promotor region by inhibition of the transcription factors independent from DNA binding (22). Thus, activated PPARγ can physically interact with NF-κB proteins thereby inhibiting the NF-κB-dependent gene expression (23). We have shown, that plasmin triggers NF-κB signaling via IKKβ activation followed by degradation of the inhibitors IκBα and p105 (13). Indeed, the plasmin-induced NF-κB activation was inhibited by preincubating PM with the PPARγ ligand ciglitazone. Formation of the transcription inhibitory complex of NF-κB and PPARγ (23) might therefore contribute to the inhibitory effects observed. Downregulation of the plasmin-induced activation of AP-1 by ciglitazone is also consistent with the inhibitory activity of PPARγ ligands on AP-1-mediated promotor activation in macrophages (22) that may involve competition for limited amounts of essential cofactors, namely CREB-binding protein and p300 (48).

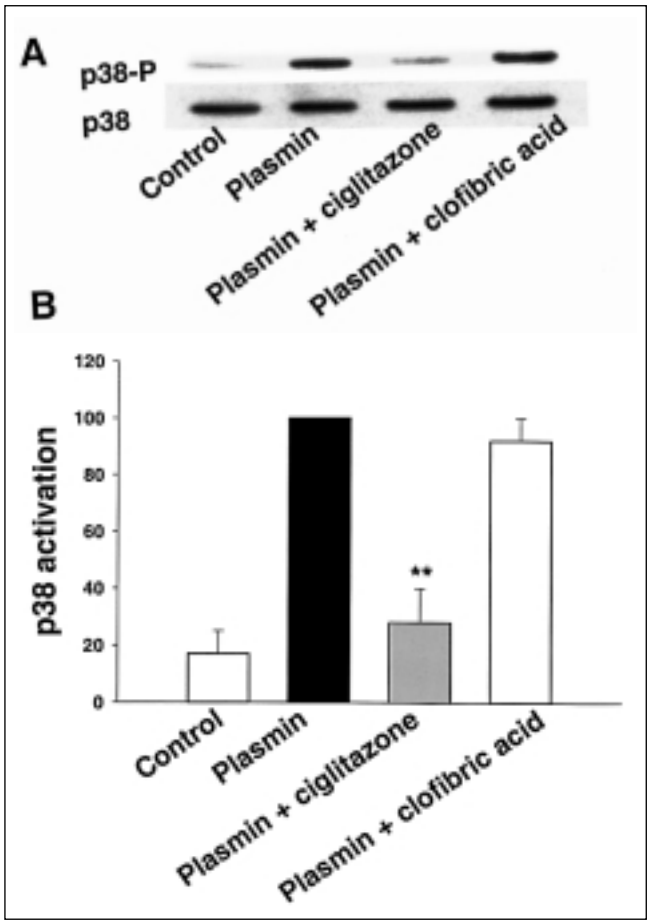


*Fig. 6* Ciglitazone inhibits the plasmin-induced activation of p38 MAPK. Freshly isolated PM were preincubated with ciglitazone 10 μM, clofibric acid 100 μM or diluent and stimulated with plasmin 0.43 CTA U/ml. Representative Western blot (A). Activation of p38 was analyzed by immunoblotting with phospho-specific p38 Ab. Equal loading was confirmed by reprobing stripped blots with Ab against p38. Quantification of p38 activation as determined by density scanning (B). Mean ± SEM, n = 4. **, $p < 0.01$ versus plasmin control

Such mechanisms would not explain how PPAR activation interferes with the chemotactic response as neither NF-κB nor AP-1 are involved in cell migration. However, in line with a recent report on the significance of p38 MAPK for the MCP-1-induced chemotaxis of THP-1 cells (49), we found that SB203580, a specific inhibitor of p38 (14), blocks the plasmin-, but not the FMLP-induced PM chemotaxis (39). Moreover, we showed that the TNF-α production by plasmin-stimulated PM, was significantly impaired by SB203580 (39) indicating that p38 signaling is also essential for the plasmin-mediated cytokine production. Both p38α and p38β isoforms were identified as receptor for SB203580 that suppresses cytokine release by blocking the p38-mediated activation of transcription factors such as AP-1. Apart from p38δ that is resistant to SB203580, PM harbour predominantly the sensitive p38α isotype (14, 50). Phosphorylation of p38 at the Thr-Gly-Tyr motif by upstream kinases such as MKK3 and MKK6 is required for activation (14). As demonstrated, phosphorylation and consequently activation of p38 was inhibited by ciglitazone. At least in endothelial cells p38 signaling has been reported to contribute to phosphorylation of heat shock protein 27 that is linked to microfilament reorganization and cell motility (14, 51); such interaction could indeed represent the missing link between p38 activation and PM chemotaxis. Thus, inhibition of p38 MAPK activation by the tested PPARγ activator is linked to the inhibitory effect on chemotaxis as well as cytokine expression in plasmin-stimulated PM.

Taken together, our data demonstrate that proinflammatory effects such as chemotaxis and proinflammatory cytokine expression in plasmin-stimulated PM can be effectively inhibited by the PPARγ ligand ciglitazone, but not by the PPARα agonist clofibric acid. Therefore, PPARγ activation might provide a pharmacotherapeutic target to control plasmin-mediated PM recruitment and activation in atherosclerosis and inflammation. That such a scenario may indeed be relevant for inflammation in vivo is supported by a recent study showing that treatment of mice suffering from TNBS-induced colitis with PPARγ agonists leads to inhibition of p38 activity and inflammatory cytokine expression, and in parallel to reduction of inflammatory lesions (52).

*Acknowledgements*

We thank Dr. Renato Colognato, Department of Pharmacology of Natural Products and Clinical Pharmacology, University of Ulm, for the macrophages and dendritic cells. The study was supported by the German Research Association, SFB 451.

## References

1. Padro T, Emeis JJ, Steins M, Schmid KW, Kienast J. Quantification of plasminogen activators and their inhibitors in the aortic vessel wall in relation to the presence and severity of atherosclerotic disease. Arterioscler Thromb Vasc Biol 1995; 15: 893-902.
2. Schneiderman J, Bordin GM, Engelberg I, Adar R, Seiffert D, Thinnes T, Bernstein EF, Dilley RB, Loskutoff DJ. Expression of fibrinolytic genes in atherosclerotic abdominal aortic aneurysm wall. A possible mechanism for aneurysm expansion. J Clin Invest 1995; 96: 639-45.
3. Kienast J, Padro T, Steins M, Li CX, Schmid KW, Hammel D, Scheld HH, van de Loo JC. Relation of urokinase-type plasminogen activator expression to presence and severity of atherosclerotic lesions in human coronary arteries. Thromb Haemost 1998; 79: 579-86.
4. Underwood MJ, De Bono DP. Increased fibrinolytic activity in the intima of atheromatous coronary arteries: protection at a price. Cardiovasc Res 1993; 27: 882-5.
5. Carmeliet P, Collen D. Molecular genetics of the fibrinolytic and coagulation systems in haemostasis, thrombogenesis, restenosis and atherosclerosis. Curr Opin Lipidol 1997; 8: 118-25.
6. Carmeliet P, Collen D. Development and disease in proteinase-deficient mice: role of the plasminogen, matrix metalloproteinase and coagulation system. Thromb Res 1998; 91: 255-85.
7. Ploplis VA, French EL, Carmeliet P, Collen D, Plow EF. Plasminogen deficiency differentially affects recruitment of inflammatory cell populations in mice. Blood 1998; 91: 2005-9.
8. Inman RD, Harpel PC. $\alpha_2$-plasmin inhibitor-plasmin complexes in synovial fluid. J Rheumatol 1986; 13: 535-7.
9. Busso N, Peclat V, So A, Sappino AP. Plasminogen activation in synovial tissues: differences between normal, osteoarthritis, and rheumatoid arthritis joints. Ann Rheum Dis 1997; 56: 550-7.
10. Weide I, Römisch J, Simmet T. Contact activation triggers stimulation of the monocyte 5-lipoxygenase pathway via plasmin. Blood 1994; 83: 1941-51.
11. Weide I, Tippler B, Syrovets T, Simmet T. Plasmin is a specific stimulus of the 5-lipoxygenase pathway of human peripheral monocytes. Thromb Haemost 1996; 76: 561-8.
12. Syrovets T, Tippler B, Rieks M, Simmet T. Plasmin is a potent and specific chemoattractant for human peripheral monocytes acting via a cyclic guanosine monophosphate-dependent pathway. Blood 1997; 89: 4574-83.
13. Syrovets T, Jendrach M, Rohwedder A, Schüle A, Simmet T. Plasmin-induced expression of cytokines and tissue factor in human monocytes

involves AP-1 and IKKβ-mediated NF-κB activation. Blood 2001; 97: 3941-50.

14. Kyriakis JM, Avruch J. Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. Physiol Rev 2001; 81: 807-69.
15. Lee JC, Laydon JT, McDonnell PC, Gallagher TF, Kumar S, Green D, McNulty D, Blumenthal MJ, Heys JR, Landvatter SW, Strickler JE, McLaughlin MM, Siemens IR, Fisher SM, Livi GP, White JR, Adams JL, Young PR. A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. Nature 1994; 372: 739-46.
16. Rezzonico R, Chicheportiche R, Imbert V, Dayer JM. Engagement of CD11b and CD11c β2 integrin by antibodies or soluble CD23 induces IL-1β production on primary human monocytes through mitogen-activated protein kinase-dependent pathways. Blood 2000; 95: 3868-77.
17. Barouch R, Kazimirsky G, Appel E, Brodie C. Nerve growth factor regulates TNF-α production in mouse macrophages via MAP kinase activation. J Leukoc Biol 2001; 69: 1019-26.
18. Escher P, Wahli W. Peroxisome proliferator-activated receptors: insight into multiple cellular functions. Mutat Res 2000; 448: 121-38.
19. Kersten S, Desvergne B, Wahli W. Roles of PPARs in health and disease. Nature 2000; 405: 421-4.
20. Jiang C, Ting AT, Seed B. PPAR-γ agonists inhibit production of monocyte inflammatory cytokines. Nature 1998; 391: 82-6.
21. Thieringer R, Fenyk-Melody JE, Le Grand CB, Shelton BA, Detmers PA, Somers EP, Carbin L, Moller DE, Wright SD, Berger J. Activation of peroxisome proliferator-activated receptor γ does not inhibit IL-6 or TNF-α responses of macrophages to lipopolysaccharide in vitro or in vivo. J Immunol 2000; 164: 1046-54.
22. Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation. Nature 1998; 391: 79-82.
23. Chung SW, Kang BY, Kim SH, Pak YK, Cho D, Trinchieri G, Kim TS. Oxidized low density lipoprotein inhibits interleukin-12 production in lipopolysaccharide-activated mouse macrophages via direct interactions between peroxisome proliferator-activated receptor-γ and nuclear factor-κB. J Biol Chem 2000; 275: 32681-7.
24. Marx N, Sukhova G, Murphy C, Libby P, Plutzky J. Macrophages in human atheroma contain PPARγ: differentiation-dependent peroxisomal proliferator-activated receptor γ (PPARγ) expression and reduction of MMP-9 activity through PPARγ activation in mononuclear phagocytes in vitro. Am J Pathol 1998; 153: 17-23.
25. Chinetti G, Griglio S, Antonucci M, Torra IP, Delerive P, Majd Z, Fruchart JC, Chapman J, Najib J, Staels B. Activation of proliferator-activated receptors α and γ induces apoptosis of human monocyte-derived macrophages. J Biol Chem 1998; 273: 25573-80.
26. Rossi A, Kapahi P, Natoli G, Takahashi T, Chen Y, Karin M, Santoro MG. Anti-inflammatory cyclopentenone prostaglandins are direct inhibitors of IκB kinase. Nature 2000; 403: 103-8.
27. Straus DS, Pascual G, Li M, Welch JS, Ricote M, Hsiang CH, Sengchanthalangsy LL, Ghosh G, Glass CK. 15-deoxy-$\Delta^{12,14}$-prostaglandin $J_2$ inhibits multiple steps in the NF-κB signaling pathway. Proc Natl Acad Sci USA 2000; 97: 4844-9.
28. Chawla A, Barak Y, Nagy L, Liao D, Tontonoz P, Evans RM. PPAR-γ dependent and independent effects on macrophage-gene expression in lipid metabolism and inflammation. Nat Med 2001; 7: 48-52.
29. Lazar MA. Progress in cardiovascular biology: PPAR for the course. Nat Med 2001; 7: 23-4.
30. Chapuis F, Rosenzwajg M, Yagello M, Ekman M, Biberfeld P, Gluckman JC. Differentiation of human dendritic cells from monocytes in vitro. Eur J Immunol 1997; 27: 431-41.
31. Smith W, Feldmann M, Londei M. Human macrophages induced in vitro by macrophage colony-stimulating factor are deficient in IL-12 production. Eur J Immunol 1998; 28: 2498-507.
32. Neumann M, Fries H, Scheicher C, Keikavoussi P, Kolb-Maurer A, Brocker E, Serfling E, Kampgen E. Differential expression of Rel/NF-κB and octamer factors is a hallmark of the generation and maturation of dendritic cells. Blood 2000; 95: 277-85.
33. Juge-Aubry C, Pernin A, Favez T, Burger AG, Wahli W, Meier CA, Desvergne B. DNA binding properties of peroxisome proliferator-activated receptor subtypes on various natural peroxisome proliferator response elements. Importance of the 5'-flanking region. J Biol Chem 1997; 272: 25252-9.
34. Syrovets T, Büchele B, Gedig E, Slupsky JR, Simmet T. Acetyl-boswellic acids are novel catalytic inhibitors of human topoisomerases I and IIα. Mol Pharmacol 2000; 58: 71-81.
35. Syrovets T, Thillet J, Chapman MJ, Simmet T. Lipoprotein(a) is a potent chemoattractant for human peripheral monocytes. Blood 1997; 90: 2027-36.
36. Tsuruta L, Arai N, Arai K. Transcriptional control of cytokine genes. Int Rev Immunol 1998; 16: 581-616.
37. Takano H, Nagai T, Asakawa M, Toyozaki T, Oka T, Komuro I, Saito T, Masuda Y. Peroxisome proliferator-activated receptor activators inhibit lipopolysaccharide-induced tumor necrosis factor-α expression in neonatal rat cardiac myocytes. Circ Res 2000; 87: 596-602.
38. Delerive P, Gervois P, Fruchart JC, Staels B. Induction of IκBα expression as a mechanism contributing to the anti-inflammatory activities of peroxisome proliferator-activated receptor-α activators. J Biol Chem 2000; 275: 36703-7.
39. Burysek L, Syrovets T, Simmet Th. Chemotaxis of human monocytes triggered by plasmin is dependent on the p38 MAP kinase signalling pathway. Naunyn-Schmiedeberg's Arch Pharmacol 2001; 363 (Suppl.): 256.
40. Tontonoz P, Nagy L. Regulation of macrophage gene expression by peroxisome proliferator-activated receptor γ: implications for cardiovascular disease. Curr Opin Lipidol 1999; 10: 485-90.
41. Shu H, Wong B, Zhou G, Li Y, Berger J, Woods JW, Wright SD, Cai TQ. Activation of PPARα or γ reduces secretion of matrix metalloproteinase 9 but not interleukin 8 from human monocytic THP-1 cells. Biochem Biophys Res Commun 2000; 267: 345-9.
42. Tontonoz P, Nagy L, Alvarez JG, Thomazy VA, Evans RM. PPARγ promotes monocyte/macrophage differentiation and uptake of oxidized LDL. Cell 1998; 93: 241-52.
43. Gosset P, Charbonnier AS, Delerive P, Fontaine J, Staels B, Pestel J, Tonnel AB, Trottein F. Peroxisome proliferator-activated receptor γ activators affect the maturation of human monocyte-derived dendritic cells. Eur J Immunol 2001; 31: 2857-65.
44. Syrovets T, Schüle A, Colognato R, Jendrach M, Kiéc B, Simmet T. Activation of PPARγ but not PPARα downregulates plasmin-induced proinflammatory activation of human monocytes. Thromb Haemost 2001; 86: P1337.
45. Goetze S, Xi XP, Kawano H, Gotlibowski T, Fleck E, Hsueh WA, Law RE. PPARγ-ligands inhibit migration mediated by multiple chemoattractants in vascular smooth muscle cells. J Cardiovasc Pharmacol 1999; 33: 798-806.
46. Marx N, Schonbeck U, Lazar MA, Libby P, Plutzky J. Peroxisome proliferator-activated receptor γ activators inhibit gene expression and migration in human vascular smooth muscle cells. Circ Res 1998; 83: 1097-103.
47. Kintscher U, Goetze S, Wakino S, Kim S, Nagpal S, Chandraratna RA, Graf K, Fleck E, Hsueh WA, Law RE. Peroxisome proliferator-activated receptor and retinoid X receptor ligands inhibit monocyte chemotactic protein-1-directed migration of monocytes. Eur J Pharmacol 2000; 401: 259-70.
48. Li M, Pascual G, Glass CK. Peroxisome proliferator-activated receptor γ-dependent repression of the inducible nitric oxide synthase gene. Mol Cell Biol 2000; 20: 4699-707.
49. Ashida N, Arai H, Yamasaki M, Kita T. Distinct signaling pathways for MCP-1-dependent integrin activation and chemotaxis. J Biol Chem 2001; 276: 16555-60.
50. Hale KK, Trollinger D, Rihanek M, Manthey CL. Differential expression and activation of p38 mitogen-activated protein kinase α, β, γ, and δ in inflammatory cell lineages. J Immunol 1999; 162: 4246-52.

51. Lambert H, Charette SJ, Bernier AF, Guimond A, Landry J. HSP27 multimerization mediated by phosphorylation-sensitive intermolecular interactions at the amino terminus. J Biol Chem 1999; 274: 9378-85.
52. Desreumaux P, Dubuquoy L, Nutten S, Peuchmaur M, Englaro W, Schoonjans K, Derijard B, Desvergne B, Wahli W, Chambon P, Leibowitz MD, Colombel JF, Auwerx J. Attenuation of colon inflammation through activators of the retinoid X receptor (RXR)/peroxisome proliferator-activated receptor γ (PPARγ) heterodimer. A basis for new therapeutic strategies. J Exp Med 2001; 193: 827-38.

Received November 30, 2001 Accepted after revision April 30, 2002