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Peroxisome proliferator-activated receptor and retinoid X receptor ligands inhibit monocyte chemotactic protein-1-directed migration of monocytes

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Abstract

Monocyte chemotactic protein-1 (MCP-1)-directed transendothelial migration of monocytes plays a key role in the development of inflammatory diseases. Infiltration of tissues by monocytes requires degradation of extracellular matrices, a process that involves matrix metalloproteinases. We studied the effects of peroxisome proliferator-activated receptor (PPAR) γ, α, and retinoid X receptor α (RXRα) ligands on MCP-1-directed migration and matrix metalloproteinase expression of a human acute monocytic leukemia cell line (THP-1). PPARγ ligands attenuated MCP-1-induced migration, with 50% inhibition (IC_{50}) at 2.8 μM for troglitazone and 4.8 μM for rosiglitazone. PPARα ligands WY-14643 (IC_{50}: 0.9 μM) and 5,8,11,14-eicosatetranoic acid (IC_{50}: 9.9 μM), and the potent RXRα ligand AGN 4204 (IC_{50}: 3.6 nM) also blocked monocyte migration. Troglitazone, rosiglitazone, or AGN 4204 inhibited phorbol 12-myristate 13-acetate (PMA)-induced matrix metalloproteinase-9 expression. PPARα activators WY-14643 and 5,8,11,14-eicosatetraynoic acid, however, had no inhibitory effect. AGN 4204 increased PMA-induced tissue inhibitor of matrix metalloproteinases-1 (TIMP-1) expression, whereas all PPAR ligands showed no effect. All PPAR and RXRα ligands blocked chemotaxis of THP-1 monocytes in the absence of a matrix barrier. This study demonstrates that activated PPARs and RXRα, block MCP-1-directed monocyte migration, mediated, at least in part, through their effects on matrix metalloproteinase-9 or TIMP-1 production, or chemotaxis.

Keywords: Peroxisome proliferator-activated receptor (PPAR); Retinoid X receptor (RXR); Inflammation; Monocyte; Migration; Matrix metalloproteinase

1. Introduction

Migration of monocytes into the vascular subendothelium occurs during pathologic inflammatory responses and plays a key role in the development of atherosclerosis, psoriasis, and rheumatoid arthritis (Ross, 1999; Simmons et al., 1987; Wahba et al., 1978). Transendothelial monocyte migration is a multifactorial mechanism that initially involves adhesion of circulating monocytes to cytokine-regulated adhesion molecules expressed on the surface of the endothelium (Bevilacqua et al., 1994). Adherent monocytes then move through adjacent endothelial cells towards specific chemokines (chemotaxis) followed by degradation of interstitial extracellular matrices (invasion) by matrix metalloproteinases (Adams and Shaw, 1994; Watanabe et al., 1993).

The locally produced C–C chemokine, monocyte chemotactic protein-1 (MCP-1) is a potent chemoattractant for monocytes. MCP-1 is secreted by activated vascular endothelial and smooth-muscle cells, and is expressed in human atherosclerotic and arthritic lesions (Nelken et al., 1991; Strieter et al., 1989; Taubman et al., 1992; Villiger et al., 1992). The important role of MCP-1 in monocyte

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extravasation is underscored by previous studies demonstrating that MCP-1-deficient mice or mice lacking its cognate chemokine receptor (CCR-2) have severe defects in monocyte recruitment to sites of inflammation (Kuziel et al., 1997; Lu et al., 1998).

Extravasation and migration of monocytes requires degradation of basal laminae and interstitial stroma, processes that involve a family of extracellular proteinases-designated matrix metalloproteinases (Campbell et al., 1991; Watanabe et al., 1993; Welgus et al., 1990). Matrix metalloproteinases are a family of endopeptidases collectively capable of degrading essentially all extracellular matrix components. Members include interstitial collagenases (matrix metalloproteinase-1), gelatinases (matrix metalloproteinase-2, matrix metalloproteinase-9), and stromelysins (matrix metalloproteinase-3) (Westermarck and Kahari, 1999). Specific matrix metalloproteinases are highly expressed and active in artherosclerotic lesions and in arthritic synovial membranes (Galis et al., 1994; Konttinen et al., 1999). The proteolytic activity of matrix metalloproteinases is controlled by specific tissue inhibitors of matrix metalloproteinases (TIMPs) (Gomez et al., 1997).

Peroxisome proliferator-activated receptors (PPARs) are a family of nuclear hormone receptors that heterodimerize with the retinoid X receptor α (RXRα) and function as transcriptional regulators of genes linked to lipid metabolism and glucose homeostasis (Desvergne and Wahli, 1999). Three PPAR isoforms have been identified: PPARα, PPARβ (or δ), and PPARγ. PPARα and PPARγ, which are both expressed in monocyte/macrophages and endothelial cells, have recently been shown to inhibit pro-inflammatory processes in these cells (Chinetti et al., 1998; Ricote et al., 1998). Mice deficient for PPARα display a prolonged response to inflammatory stimuli (Devchand et al., 1996). PPARγ is upregulated in activated macrophages, and inhibits the production of inflammatory cytokines and the secretion of nitric oxide synthase in monocyte/macrophages (Jiang et al., 1998; Ricote et al., 1998). In combination, these studies provide evidence for an important anti-inflammatory role for PPARα and γ.

The nuclear receptor RXRα exerts its transcriptional activity either as a homodimer (RXRα–RXRα) or heterodimer with other nuclear receptors, such as PPARs (RXRα–PPAR) (Kliewer et al., 1992; Mangelsdorf et al., 1992). The RXRα ligand 9-*cis*-retinoic acid has been recently shown to have anti-inflammatory effects through inhibition of monocyte migration (Zhu et al., 1999). Since this ligand can also bind to retinoic acid receptors (RAR) α, β and γ, it is unknown whether these effects are RXRα specific (Allenby et al., 1994).

The purpose of the present study was to determine the effects of PPARγ, PPARα and a potent RXRα ligand on MCP-1-directed migration and the expression of matrix metalloproteinases and TIMPs in a human acute monocytic leukemia cell line (THP-1).

2. Material and methods

2.1. Materials

Materials were obtained from the following suppliers: RPMI 1640 medium with L-glutamine from GIBCO BRL (Gaithersburg, MD, USA). Antibiotics, HEPES, phorbol 12-myristate 13-acetate (PMA), and dimethylsulfoxide (DMSO) were from Sigma (St. Louis, MO, USA). Fetal bovine serum was purchased from Irvine Scientific (Santa Ana, CA). Hybond-enhanced chemiluminescence nitrocellulose membrane, horseradish peroxidase-linked anti-rabbit and anti-mouse antibody, and enhanced chemiluminescence western blotting detection reagents were from Amersham Life Sciences (Arlington Heights, IL). Cell fixation and staining was performed using the Quik-Diff stain set from DADE (Miami, FL, USA). Recombinant human MCP-1 was purchased from R&D systems (Minneapolis, MN, USA). The mitogen-activated protein kinase (MAPK) extracellular-regulated kinase 1/2 (ERK1/ERK2) inhibitor or MAPK, ERK kinase (MEK) inhibitor PD98059 was from New England BioLabs (Beverly, MA, USA). Mouse monoclonal antibodies against matrix metalloproteinase-1, matrix metalloproteinase-2, matrix metalloproteinase-3, matrix metalloproteinase-9, tissue inhibitor of matrix metalloproteinases-1 (TIMP-1), and tissue inhibitor of matrix metalloproteinases-2 (TIMP-2) were from Oncogene Research Products (Cambridge, MA, USA). Rabbit polyclonal antibodies against phosphorylated and total ERK1/ERK2 MAPKs were purchased from New England BioLabs (Beverly, MA, USA). Troglitazone was provided by Parke Davis (Ann Arbor, MI, USA). Rosiglitazone (formerly BRL 49653) was a generous gift from SmithKline Beecham (King of Prussia, PA, USA). The RXRα ligand AGN 4204 was kindly provided by Retinoid Research, Allergan Pharmaceuticals (Irvine, CA, USA) (Johnson et al., 1999). WY-14643 and 5,8,11,14-eicosatetraynoic acid were purchased from BIOMOL (Plymouth Meeting, PA 19462, USA).

2.2. Cell culture

THP-1 cells, a human monocytic leukemia cell line, were purchased from American Type Culture Collection (Rockville, MA, USA). The cells were cultured in RPMI 1640 medium, containing 10% fetal bovine serum and L-glutamine. THP-1 cells at $0.5–1 \times 10^6$ cells/ml were stimulated for 24 h with PMA (100 nM) in RPMI 1640 medium with 0.4% fetal bovine serum. To analyze protein expression in supernatants, experiments were performed in serum-free medium. Troglitazone, rosiglitazone, WY-14643; 5,8,11,14-eicosatetraynoic acid, AGN 4204, or PD98059 were added 30 min before the addition of PMA. All compounds were dissolved in DMSO (0.0001–0.3%).

Vehicle control was always added at the highest final concentration of any ligand or inhibitor used for that specific experiment.

2.3. Migration and chemotaxis

Migration experiments were performed in transwell cell-culture chambers (Falcon #35 3047) as previously described (Goetze et al., 1999). THP-1 cells were centrifuged, washed in phosphate-buffered saline, centrifuged, and resuspended in RPMI 1640 medium containing 0.4% fetal bovine serum. 5×10^4 cells were placed on a gelatin-coated polycarbonate membrane (Falcon #35 3097) with 8-μm pores, and incubated at 37°C for 1 h, allowing the cells to attach. Cells were then pretreated with the indicated ligands, or vehicle (DMSO) for 30 min at 37°C.

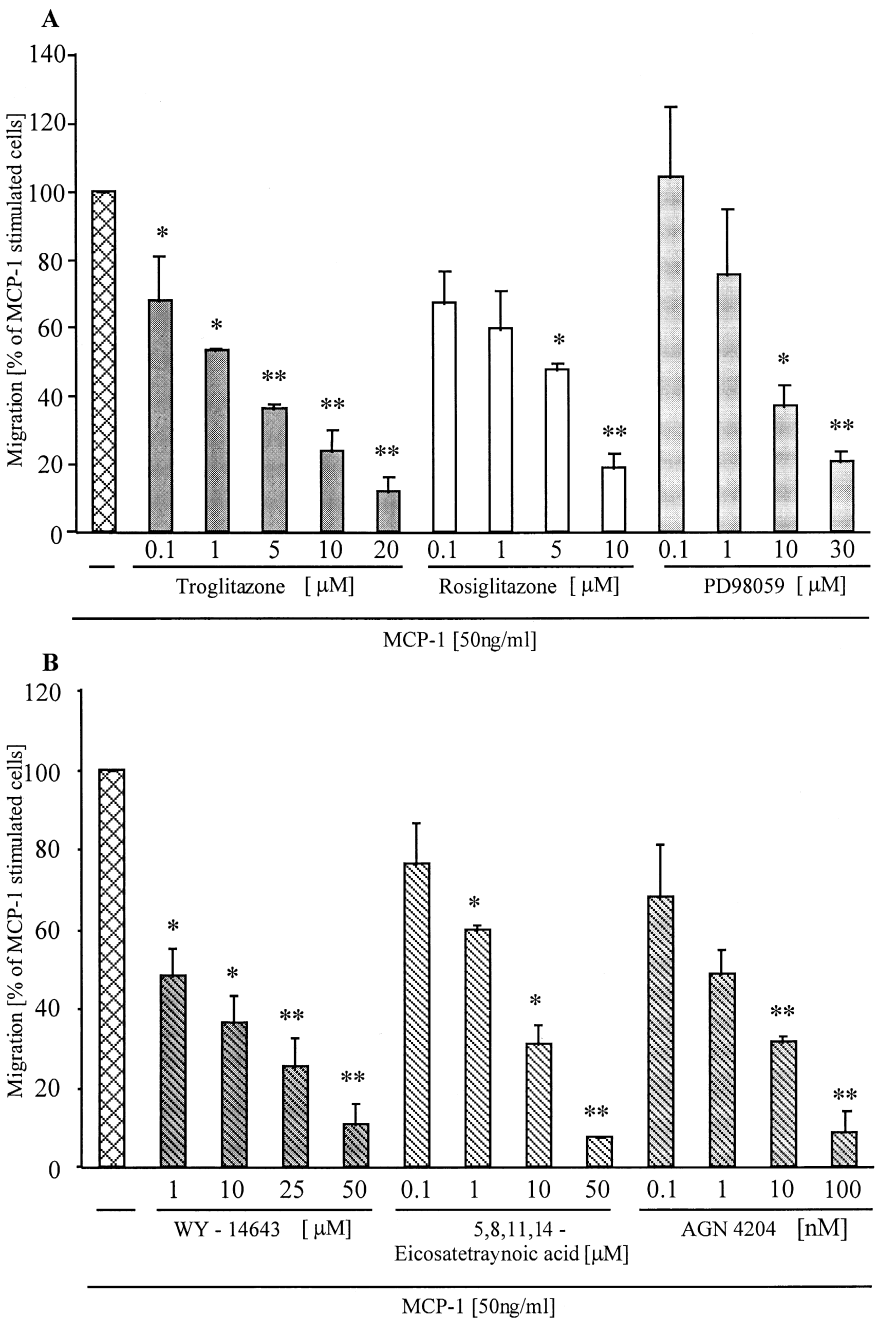

Fig. 1. PPARγ, PPARα, and RXRα ligands inhibit MCP-1 directed THP-1 monocyte migration. THP-1 monocytes were incubated with the PPARγ ligands troglitazone (0.1–20 μM), or rosiglitazone (0.1–10 μM) (A), the MEK inhibitor PD98059 (0.1–30 μM) (A), the PPARα ligands WY-14643 (1–50 μM), or 5,8,11,14-eicosatetraynoic acid (0.1–50 μM) (B), or the RXRα ligand AGN 4204 (0.1–100 nM) (B) for 30 min before addition of MCP-1 (50 ng/ml). Migration of cells is shown as percentage of vehicle (DMSO)-treated, MCP-1-stimulated cells. Experiments were repeated three times and were done in duplicate. Data are expressed as mean ± S.E.M., $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. MCP-1 + DMSO.

Migration was induced by addition of MCP-1 (50 ng/ml) to the lower compartment. After 4 h, non-migrating cells were removed with a cotton tip and the membranes were fixed and stained with Quik-Diff stain set to identify migrated cells. Chemotaxis experiments were performed for 12 h on uncoated membranes. The number of migrated cells was determined by ×200 high-power field. Experiments were performed in duplicate and were repeated at least three times.

2.4. Western immunoblotting

THP-1 cells were treated with ligands or vehicle (DMSO) 30 min before stimulation with PMA (100 nM). After 24 h, the proteins were isolated, electrophoresed, and blotted as previously described (Goetze et al., 1999). Blots were incubated with specific antibodies against matrix metalloproteinase-1, matrix metalloproteinase-2, matrix metalloproteinase-3, matrix metalloproteinase-9, TIMP-1, and TIMP-2 at a 1:100 concentration, and against phosphorylated and total ERK1/ERK2 MAPK at a 1:1000 concentration. Immunoreactive bands were visualized using horseradish peroxidase-conjugated secondary antibodies (1:1000 dilution). The peroxidase reaction was developed using an enhanced chemiluminescence detection system (Amersham). Band intensity was analyzed by densitometry.

2.5. Statistics

Analysis of variance with paired or unpaired, two-sided *t*-tests were performed for statistical analysis, as appropriate. Values of $P < 0.05$ were considered to be statistically significant. Data are expressed as mean ± standard error of the mean (S.E.M.).

3. Results

3.1. PPARγ, PPARα, and RXRα ligands inhibit MCP-1-directed THP-1 monocyte migration

MCP-1 (50 ng/ml) stimulated migration of THP-1 cells with a 7 ± 2.7-fold induction compared to unstimulated control (MCP-1: 16.9 ± 2.7 cells/high power field, control: 2.4 ± 0.6 cells/high power field, $P < 0.01$). Administration of the PPARγ ligands troglitazone or rosiglitazone led to a dose-dependent attenuation of MCP-1-induced migration (Fig. 1A), with a 50% inhibition (IC_{50}) observed at 2.8 μM (troglitazone) and 4.8 μM (rosiglitazone) (Fig. 1A). PPARα ligands WY-14643 and 5,8,11,14-eicosatetraynoic acid blocked THP-1 monocyte migration towards MCP-1 with an IC_{50} of 0.9 μM for WY-14643, and 9.9 μM for 5,8,11,14-eicosatetraynoic acid (Fig. 1B). The RXRα ligand AGN 4204 (Johnson et al., 1999) exhibited the strongest inhibitory effect on MCP-1-induced migration with an IC_{50} of 3.5 nM (Fig. 1B).

3.2. MCP-1-mediated migration of THP-1 monocytes is MAPK-dependent

The ERK1/ERK2 MAPK pathway has been shown to play an important role in the migration of other cell types

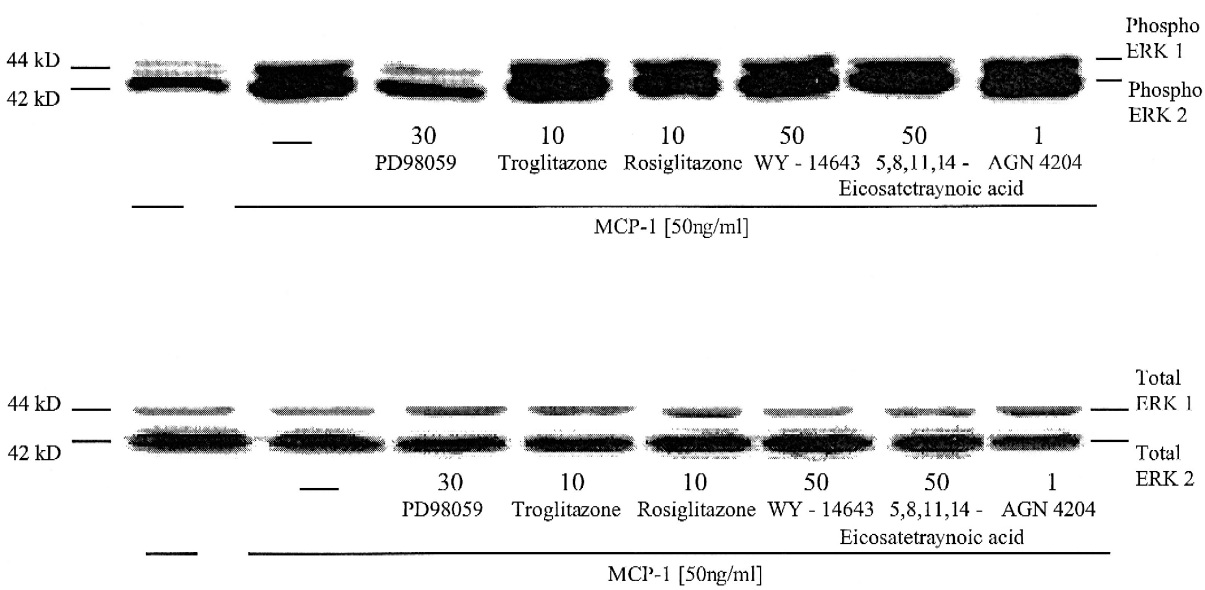

Fig. 2. PPAR and RXRα ligands have no effect on MCP-1-induced MAPK phosphorylation. THP-1 monocytes were stimulated with MCP-1 (50 ng/ml). Cells were preincubated with the MEK inhibitor PD98059 (30 μM), the PPARγ ligands troglitazone (10 μM), or rosiglitazone (10 μM), the PPARα ligands WY-14643 (50 μM), or 5,8,11,14-eicosatetraynoic acid (50 μM), or the RXRα ligand AGN 4204 (1 μM), or DMSO (−) for 30 min prior to addition of MCP-1. After 5 min, whole-cell lysates were assayed by western immunoblotting. Upper panel, representative immunoblot of three experiments with a phosphospecific ERK1/ERK2 MAPK antibody. Lower panel, representative immunoblot of three experiments with an antibody that recognizes total ERK1/ERK2 MAPK.

(Graf et al., 1997; Kundra et al., 1995). More importantly, we have previously shown that PPARγ ligands can block MAPK-dependent migration of vascular smooth-muscle cells. (Goetze et al., 1999) We, therefore, examined the

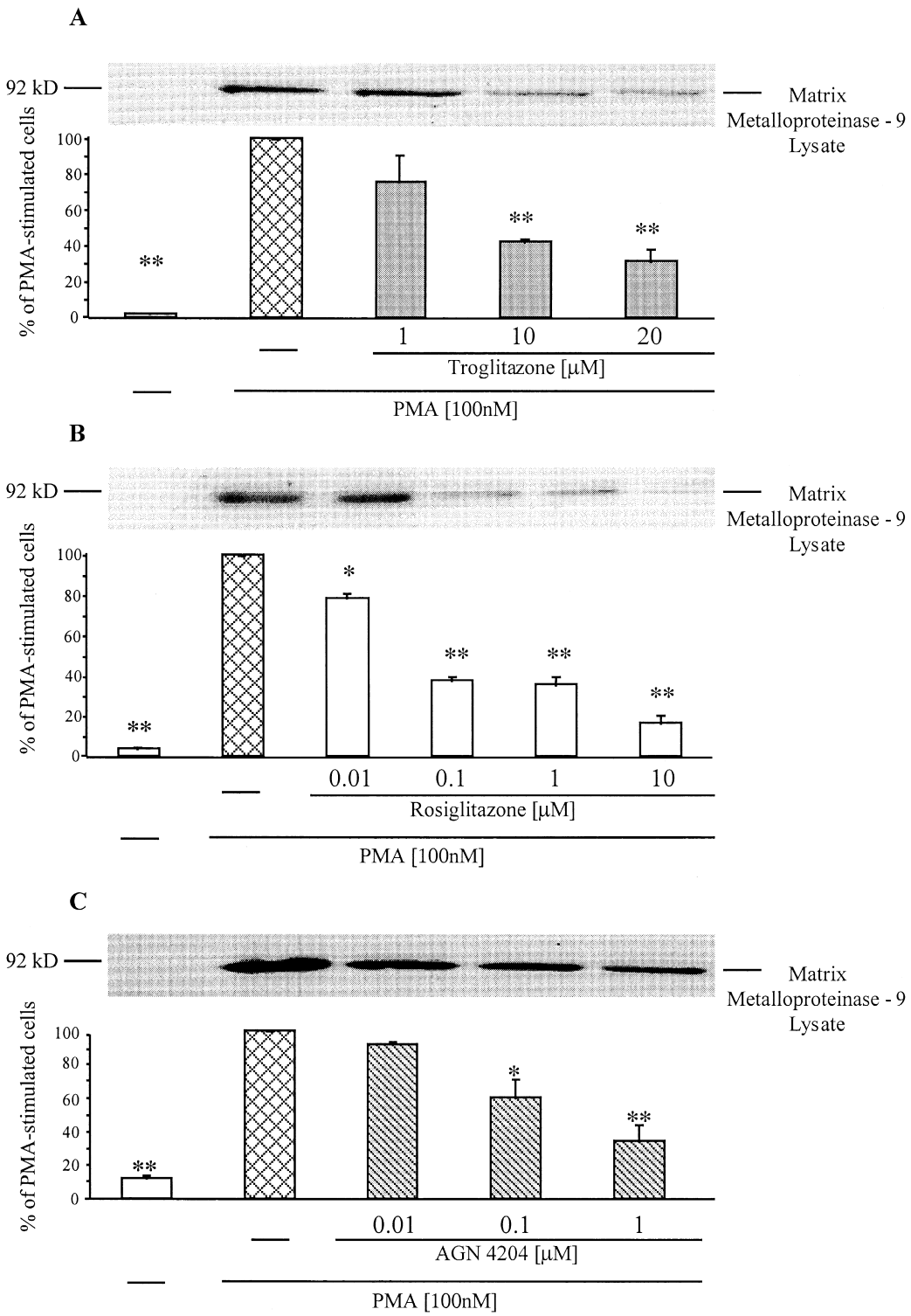

Fig. 3. PPARγ and RXRα ligands inhibit matrix metalloproteinase-9 expression in cell lysates of THP-1 monocytes. THP-1 monocytes were stimulated with PMA (100 nM). Cells were preincubated with the PPARγ ligands troglitazone (1–20 μM) (A), or rosiglitazone (0.01–10 μM) (B), or the RXRα ligand AGN 4204 (0.01–1 μM) (C), or DMSO (−) for 30 min prior to addition of PMA. After 24 h, whole-cell lysates were assayed by western immunoblotting. Upper panels, representative blots of three experiments. Lower panels, densitometric analysis of matrix metalloproteinase-9 protein levels in western blots are shown as percentage of cells incubated with PMA and DMSO. First bars indicate unstimulated THP-1 cells. Results are presented as mean ± S.E.M., $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. PMA + DMSO.

effect of blocking the ERK-MAPK pathway with the MEK inhibitor PD98059 on MCP-1-directed migration of THP-1 cells. Migration of cells towards MCP-1 was dose-dependently reduced in the presence of PD98059 (30 μM: 79.4 ± 3% inhibition, $P < 0.01$ vs. MCP-1 alone), indicating that the ERK-MAPK pathway is involved in MCP-1-mediated monocyte migration (Fig. 1A). To determine whether PPAR and RXRα ligands inhibited monocyte migration by targeting the MAPK pathway, we investigated their effect on MAPK activation. Activated, phosphorylated MAPK was assessed by immunoblotting with a phosphospecific ERK1/ERK2 MAPK antibody. The par-

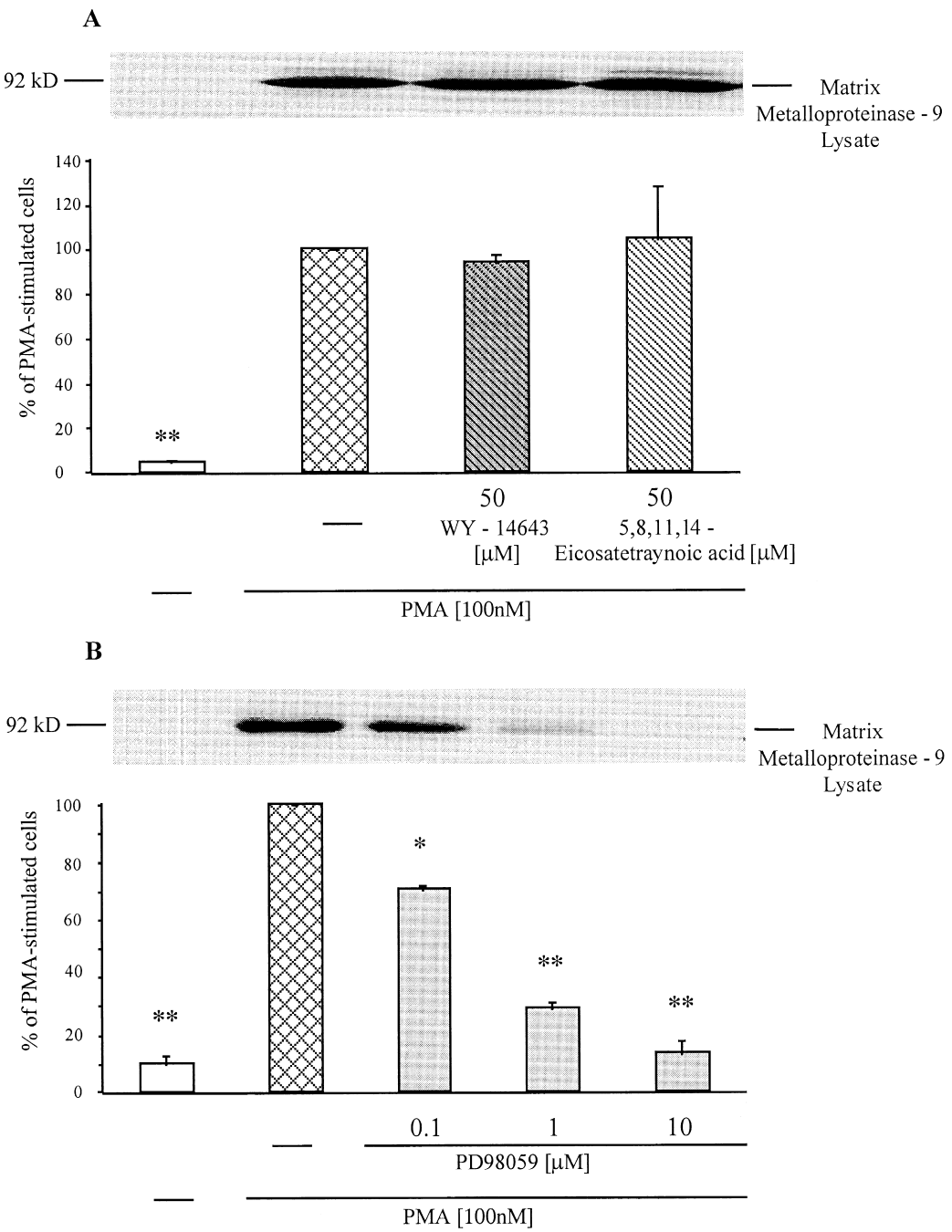

Fig. 4. PPARα ligands have no effect on matrix metalloproteinase-9 expression in cell lysates of THP-1 monocytes. PMA-stimulated matrix metalloproteinase-9 expression in THP-1 cells is MAPK dependent. THP-1 monocytes were stimulated with PMA (100 nM). Cells were preincubated with the PPARα ligands WY-14643 (50 μM), or 5,8,11,14-eicosatetraynoic acid (50 μM) (A), or the MEK inhibitor PD98059 (0.1–10 μM) (B), or DMSO (−) for 30 min prior to addition of PMA. After 24 h, whole-cell lysates were assayed by western immunoblotting. Upper panels, representative blots of three experiments. Lower panels, densitometric analysis of matrix metalloproteinase-9 protein levels in western blots are shown as percentage of cells incubated with PMA and DMSO. First bars indicate unstimulated THP-1 cells. Results are presented as mean ± S.E.M., $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. PMA + DMSO.

allel amount of total ERK1/ERK2 MAPK was determined by using an antibody that recognizes both phosphorylated and nonphosphorylated ERK1/ERK2 MAPK. MCP-1 (50 ng/ml) rapidly and transiently induced ERK-MAPK acti-

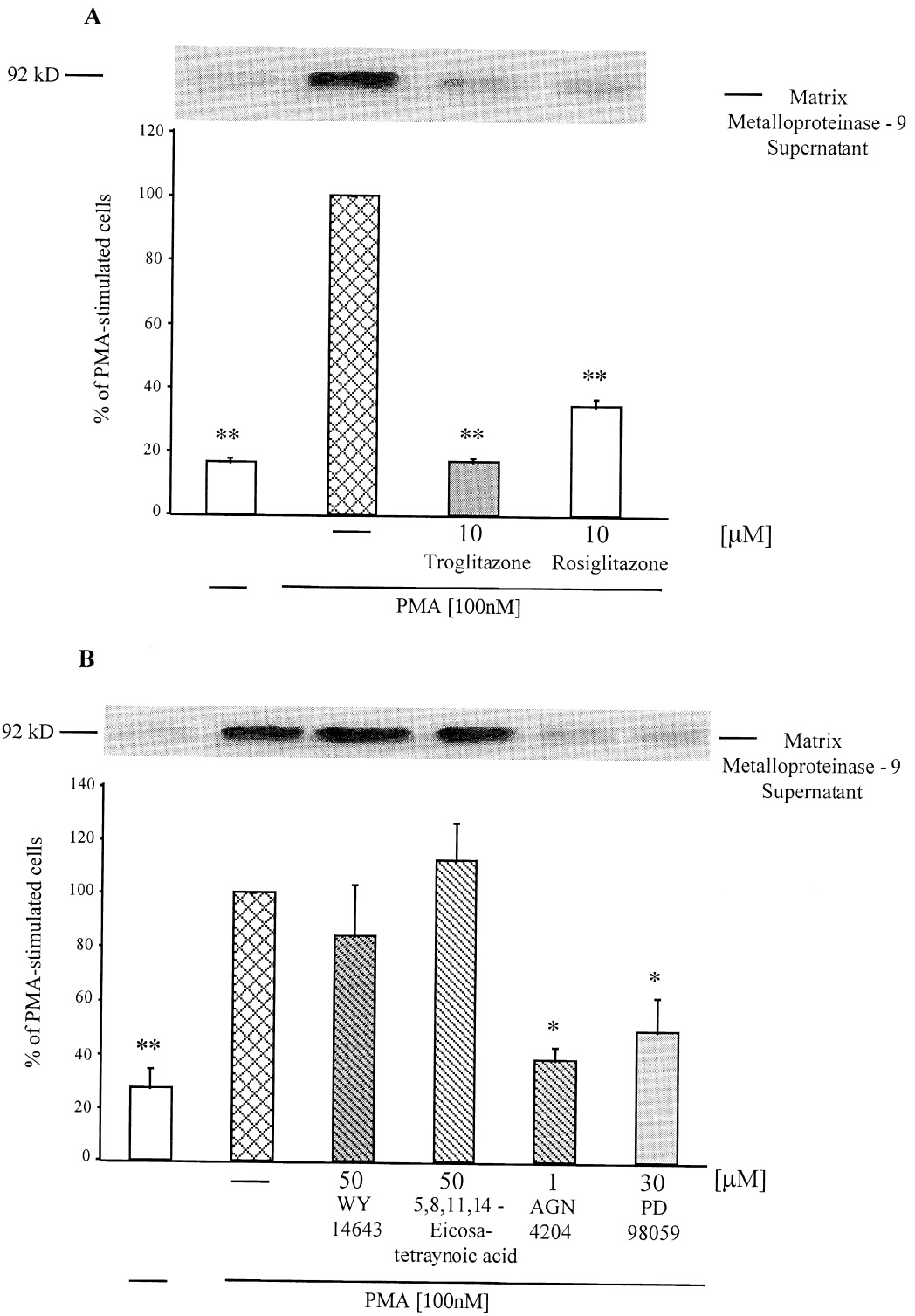

Fig. 5. PPARγ and RXRα ligands inhibit matrix metalloproteinase-9 expression in supernatants of THP-1 monocytes. THP-1 monocytes were stimulated with PMA (100 nM). Cells were preincubated with the PPARγ ligands troglitazone (10 μM), or rosiglitazone (10 μM) (A), the PPARα ligands WY-14643 (50 μM), or 5,8,11,14-eicosatetraynoic acid (50 μM) (B), the RXRα ligand AGN 4204 (1 μM) (B), or the MEK inhibitor PD98059 (30 μM) (B), or DMSO (−) for 30 min prior to addition of PMA. After 24 h, cell supernatant was collected and assayed by western immunoblotting. Upper panels, representative blots of three experiments. Lower panels, densitometric analysis of matrix metalloproteinase-9 protein levels in western blots are shown as percentage of cells incubated with PMA and DMSO. First bars indicate unstimulated THP-1 cells. Results are presented as mean ± S.E.M., $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. PMA + DMSO.

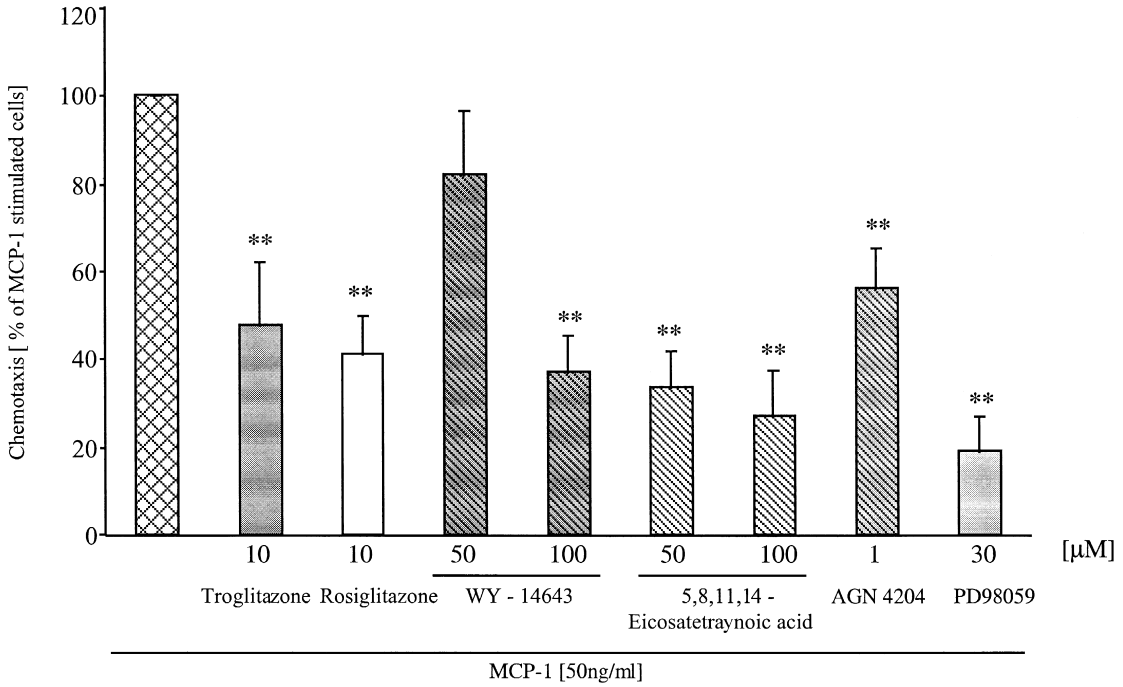

Fig. 7. PPARγ, PPARα, and RXRα ligands inhibit MCP-1-directed THP-1 monocyte chemotaxis. THP-1 monocytes were incubated with the PPARγ ligands troglitazone (10 μM), or rosiglitazone (10 μM), the PPARα ligands WY-14643 (50 or 100 μM), or 5,8,11,14-eicosatetraynoic acid (50 or 100 μM) (B), the RXRα ligand AGN 4204 (1 μM) or the MEK inhibitor PD98059 (30 μM) (A), for 30 min before addition of MCP-1 (50 ng/ml). Chemotaxis of cells through uncoated membranes is shown as percentage of vehicle (DMSO)-treated, MCP-1-stimulated cells. Experiments were repeated three times and were done in duplicate. Data are expressed as mean ± S.E.M., $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. MCP-1 + DMSO.

of RXRα and PPAR ligands on TIMP-1 and -2, important inhibitors of matrix metalloproteinase-9 activity. Interestingly, TIMP-1 and -2 levels were low in unstimulated THP-1 cells and increased after PMA stimulation. Treatment with PPARγ and PPARα ligands did not affect TIMP-1 protein levels in cell lysates or supernatants (Fig. 6). In contrast, the RXRα ligand AGN 4204 (1 μM) induced TIMP-1 expression (cell lysates: 82.7 ± 0.9% increase, $P < 0.01$ vs. PMA alone; supernatant: 161.7 ± 10.3% increase, $P < 0.01$ vs. PMA alone) (Fig. 6), identifying an additional putative target for the anti-migratory activity of RXRα. The MEK inhibitor PD98059 also attenuated PMA-induced TIMP-1 protein expression underscoring the important role of the MAPK pathway in regulating proteolysis by matrix metalloproteinases in monocytes. PMA-stimulated TIMP-2 expression in cell lysates and supernatants was not affected by any of the ligands nor by PD98059 (data not shown).

3.5. PPARγ, PPARα, and RXRα ligands inhibit MCP-1-directed THP-1 monocyte chemotaxis

Chemotaxis is the directed movement of cells along a gradient of a chemoattractant. To distinguish the effects of PPAR and RXRα ligands on MCP-1-directed chemotaxis versus migration through a matrix barrier, we measured THP-1 movement through uncoated membranes. PPARγ (troglitazone 10 μM: 47.5 ± 14.7%, and rosiglitazone 10 μM: 41 ± 9.1% of MCP-1-treated cells, $P < 0.01$ vs. MCP-1-treated cells), PPARα (WY-14643 100 μM: 37 ± 8.5% and 5,8,11,14-eicosatetraynoic acid 100 μM: 27 ± 10.5% of MCP-1-treated cells, $P < 0.01$ vs. MCP-1-treated cells), the RXRα ligand AGN 4204 (1 μM: 55.8 ± 9.5% of MCP-1-treated cells, $P < 0.01$ vs. MCP-1-treated cells), and the MAPK-pathway inhibitor PD98059 (30 μM: 19.2 ± 7.5% of MCP-1-treated cells, $P < 0.01$ vs. MCP-1-treated cells) all markedly inhibited chemotaxis of THP-1 cells towards MCP-1 (Fig. 7).

4. Discussion

Transendothelial migration of monocytes into the vascular subendothelium plays an important role in the development of inflammatory diseases (Ross, 1999; Simmons et al., 1987; Wahba et al., 1978). The chemokine MCP-1 is upregulated in the injured vessel wall where it functions as a powerful chemoattractant for monocytes adhering to the endothelium. (Lu et al., 1998; Nelken et al., 1991) PPARγ ligands are known to inhibit vascular smooth-muscle-cell migration (Goetze et al., 1999; Marx et al., 1998a). In addition to PPARγ, monocytes also express PPARα and their heterodimeric partner RXRα (Chinetti et al., 1998; Jackson et al., 1999; Yamaguchi et al., 1994). We therefore studied the effects of PPARα activators WY-14643 and 5,8,11,14-eicosatetraynoic acid, PPARγ ligands trogli-

tazone and rosiglitazone, and a potent RXRα ligand AGN 4204 on MCP-1-directed migration of THP-1 monocytes to investigate their potential as anti-migratory agents to inhibit critical early inflammatory processes in atherosclerotic diseases.

The present study demonstrates a novel anti-inflammatory effect of ligand-activated PPARs and RXRα by blocking MCP-1-directed monocyte migration. Activation of the nuclear receptors PPARα and γ, and RXRα has been suggested to regulate inflammatory processes. A role for PPARα in inflammation was supported by studies in PPARα knock-out mice demonstrating a prolonged inflammatory response in these mice (Devchand et al., 1996). PPARα ligands have been shown to inhibit endothelial VCAM-1 expression and reduce inflammatory cytokine production (Jackson et al., 1999; Poynter and Daynes, 1998). PPARγ ligands inhibit the binding of monocytes to activated endothelial cells accompanied by a blockade of endothelial VCAM-1 expression (Jackson et al., 1999). Troglitazone and other PPARγ ligands also block the endothelial production of MCP-1, a potent chemoattractant for monocytes (Murao et al., 1999; Zhu et al., 1999). Treatment of monocytes with 9-*cis*-retinoic acid, an RXR/RAR pan-agonist, has been found to inhibit MCP-1-directed monocyte migration (Zhu et al., 1999). An anti-inflammatory role for RXR is further supported by a recent study showing that cytokine production in macrophages was inhibited by an RXR-selective ligand (Na et al., 1999). Together, these studies suggest an anti-inflammatory action of ligand-activated PPARs and RXRα. In contrast, others have demonstrated that activated PPARγ can be pro-inflammatory by promoting monocyte differentiation into macrophages (Tontonoz et al., 1998). Our findings that MCP-1-directed migration of monocytes is inhibited by PPARα, PPARγ, and RXRα ligands underscores the importance of these receptors in suppressing multiple early events in tissue inflammation.

To identify potential anti-migratory mechanisms, we investigated the effects of the PPAR and RXRα ligands on monocytic metalloproteinase and TIMP expression, and chemotaxis.

The anti-migratory activity of PPARγ ligands is likely mediated through effects on monocytic matrix metalloproteinase-9 expression and chemotaxis, consistent with previous studies demonstrating that the PPARγ ligands troglitazone and 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 inhibit matrix metalloproteinase-9 expression in monocyte-derived and peritoneal macrophages (Marx et al., 1998b; Ricote et al., 1998).

Also consistent with reports in other cell types, we found no inhibitory effect of PPARα activators on monocytic matrix metalloproteinase or TIMP levels (Marx et al., 1998a). The observed blockade of migration by activated PPARα may, therefore, occur partially through an inhibition of chemotaxis. However, inhibition of chemotaxis did not likely account in full for the inhibition of migration, because PPARα anti-migration activity had a substantially lower IC_{50}. Therefore, additional anti-migratory mechanisms and the direct chemotactic target of activated PPARα remain to be determined.

The potent anti-migratory effect of the RXRα activator AGN 4204 might result from its blockade of chemotaxis and matrix metalloproteinase-9 expression, which is further enhanced by its induction of TIMP-1 expression, identifying an additional RXRα mechanism for regulating monocytic migration. Because the biological effects of RXRα can result from its heterodimer formation with other nuclear receptors (RXRα–RAR), or as an RXRα–RXRα homodimer, TIMP-1 regulation might be mediated independently from RXRα/PPARγ complexes (Mangelsdorf et al., 1992). The overall IC_{50} for the AGN 4204 effects on migration was lower than in any of the individual steps. This is likely due to RXRα regulating multiple steps in migration, including chemotaxis, matrix metalloproteinase-9 and TIMP-1 expression.

An additional finding from the present study was the demonstration that the ERK-MAPK signaling pathway participates in MCP-1-directed monocyte migration. This observation is in accord with previous studies establishing an important role for the ERK-MAPK pathway in regulating cell migration in several other cell types (Graf et al., 1997; Kundra et al., 1995). Furthermore, we found that PD98059 blocks PMA-induced monocytic matrix metalloproteinase-9 expression, demonstrating that the ERK-MAPK-dependent pathway is involved in regulating matrix metalloproteinase-9 production, which degrades the local matrix and facilitates monocyte invasion. The ERK-MAPK pathway has been previously shown to regulate growth factor-induced matrix metalloproteinase-9 expression in tumor cells and epidermal keratinocytes (Reddy et al., 1999; Zeigler et al., 1999). Expression of matrix metalloproteinase-9 is regulated in part by the activator protein-1 (AP-1) transcription factor, which is a well-established downstream target of the ERK-MAPK signaling pathway (Bond et al., 1998; Gum et al., 1997). Interestingly, activation of either PPARα, PPARγ, or RXRα can inhibit AP-1 activity (Delerive et al., 1999; Ricote et al., 1998; Salbert et al., 1993). In our study, MCP-1-induced phosphorylation of ERK-MAPK was not affected by PPAR ligands or AGN 4204. Thus, a common denominator for the anti-migratory effect of these nuclear receptors may be related to their ability to block the ERK-MAPK–AP-1-matrix metalloproteinase-9 pathway downstream of the ERK phosphorylation and activation.

In conclusion, these data provide further evidence for an anti-inflammatory action of PPARs and RXRα through their inhibition of MCP-1-directed monocyte migration, mediated, at least in part, through their effects on matrix metalloproteinase-9 or TIMP-1 production, or chemotaxis. These findings are corroborated by recent animal studies, in which thiazolidinediones, which are ligands for PPARγ, markedly reduce colonic inflammation in a mouse model

of inflammatory bowel disease (Su et al., 1999). Additionally, troglitazone has been shown to block monocyte/macrophage homing to atherosclerotic plaques in apoE-deficient mice (Pasceri et al., 2000). Therefore, pharmacologic activation of these nuclear receptors may provide novel therapeutic approaches for inflammatory diseases, such as atherosclerosis and rheumatoid arthritis.

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