Suppression of Constitutive but Not IL-1β-Inducible Expression of Monocyte Chemoattractant Protein-1 in Mesangial Cells by Retinoic Acids: Intervention in the Activator Protein-1 Pathway

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Abstract. Retinoic acid regulates a wide range of biologic processes, including inflammation. This study investigated the effect of all-trans-retinoic acid (t-RA) on the constitutive and cytokine-inducible expression of monocyte chemoattractant protein 1 (MCP-1) in rat mesangial cells. Serum-deprived mesangial cells exhibited substantial levels of MCP-1 mRNA, and the expression was markedly upregulated by interleukin-1β (IL-1β). Pretreatment with t-RA abrogated the constitutive mRNA expression but did not inhibit the IL-1β–inducible expression. The similar effects were observed by 9-cis-RA. The suppressive effect of t-RA required retinoic acid receptors. t-RA did not affect the stability of MCP-1 mRNA, indicating that its suppressive effect was at the transcriptional level. Experiments that used pharmacologic and genetic inhibitors showed that the IL-1β–inducible MCP-1 expression was dependent on nuclear factor-κB (NF-κB) and independent of activator protein 1 (AP-1). In contrast, the constitutive expression of MCP-1 was dependent on both NF-κB and AP-1. t-RA substantially inhibited the constitutive activity of AP-1 but did not inhibit NF-κB activity in mesangial cells. These data suggested that (*1*) constitutive and IL-1β–inducible expression of MCP-1 was differently regulated by AP-1 and NF-κB and (*2*) t-RA inhibited selectively the constitutive expression of MCP-1 via intervention in the AP-1 pathway.

Monocyte chemoattractant protein 1 (MCP-1) is a member of the chemokine family and specifically attracts monocytes (1). MCP-1 is produced ubiquitously by various cells, including resident glomerular cells (1–3). During glomerulonephritis, infiltration of monocytes/macrophages is a common pathologic feature (4). Expression of MCP-1 is observed in the mesangium of inflamed glomeruli (5–7), and MCP-1 synthesized by resident glomerular cells plays a role in macrophage attraction during glomerular inflammation (8). From this viewpoint, chemical inhibitors of MCP-1 may be useful for therapeutic intervention in glomerulonephritis.

The 5'-flanking region of the MCP-1 gene contains multiple 12-o-tetradecanoylphorbol-13-acetate response elements (TRE) and nuclear factor-κB (NF-κB) binding sites (9–11). It indicates potential roles of activator protein 1 (AP-1) and NF-κB in the regulation of MCP-1 expression. Indeed, activation of AP-1 is required for induction of MCP-1 by growth factors, lipopolysaccharide, and mechanical stress in osteoblastic cells, macrophages, and vascular endothelial cells, respectively (12–14). In tumor cells, fibroblasts, and mesangial cells, activation of NF-κB is essential for the induction of MCP-1 by interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and 12-o-tetradecanoylphorbol-13-acetate (9,15–17). In endothelial cells, NF-κB and AP-1 cooperatively upregulate expression of MCP-1 in response to IL-1β (18).

Retinoic acid (RA) is an active metabolite of vitamin A and regulates a wide range of biologic processes, including cell proliferation, differentiation, and morphogenesis (19). The action of RA is mediated by specific nuclear receptors, namely, retinoic acid receptors (RARα, β, γ) and retinoid X receptors (RXRα, β, γ). RA is known to function as a potent inhibitor of AP-1 (20). In glomerular cells, RA inhibits serum-induced proliferation and oxidant-initiated apoptosis via inhibition of AP-1 (21,22). Previous studies also showed that RA may function as an inhibitor of NF-κB (23). Based on these, RA might serve as an inhibitor of MCP-1 expression. In the present investigation, we examined the effect of all-trans-RA (t-RA) on the expression of MCP-1 in cultured rat mesangial cells. Our data suggested that t-RA inhibited selectively the constitutive expression of MCP-1 via intervention in the AP-1 pathway.

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Materials and Methods

Mesangial Cells

Mesangial cells (SM43) were established from isolated glomeruli of a male Sprague-Dawley rat and identified as being of the mesangial cell phenotype as described previously (24). Cells were maintained in Dulbecco's modified Eagle's medium/Ham's F-12 (Life Technologies BRL, Gaithersburg, MD) supplemented with 100 U/ml penicillin G, 100 μg/ml streptomycin, 0.25 μg/ml amphotericin B, and 10% fetal calf serum (FCS). Media containing 0.5% FCS were generally used for experiments.

Stable Transfectants

SM/JUNDN cells in which AP-1 is selectively inactivated were established by stable transfection of SM43 mesangial cells with a dominant-negative mutant of c-*jun*, TAM-67 (25). TAM-67 is a deletion mutant that lacks amino acids 3 to 122 of c-Jun (26). The protein encoded by this truncated c-*jun* gene retains the DNA binding and leucine zipper domains but lacks the transactivating domain. Overexpression of TAM-67, therefore, inhibits AP-1–mediated transactivation via blocking formation or binding of functional AP-1 complexes in a dominant-negative fashion (26). SM/JUNDN cells exhibit depressed activity of AP-1 under both constitutive and stimulated conditions (25).

SM/IκBαM cells in which NF-κB is selectively inactivated were established by overexpression of a super-repressor mutant of IκBα (IκBαM) (27), as described previously (17,22). IκBαM contains N- and C-terminal mutations and is resistant to both basal and stimulus-dependent degradation. When IκBαM is overexpressed, it functions as a dominant-negative mutant and blocks constitutive and inducible activation of NF-κB (27). SM/IκBαM cells exhibit blunted activation of NF-κB when stimulated by proinflammatory cytokines IL-1β and TNF-α (28).

As a control, mock-transfected mesangial cells SM/Neo that express *neo* alone were created, as described previously (29).

Pharmacologic Manipulations

Confluent mesangial cells cultured in the presence of 0.5% FCS for 24 h (serum-deprived cells) were treated with t-RA (0.5 to 5 μM; Sigma Immunochemicals, St. Louis, MO) or 9-cis-RA (1 to 5 μM; Sigma) for up to 24 h. Five μmol of t-RA was generally used for experiments. To examine effects of t-RA and 9-cis-RA on the inducible expression of MCP-1, we pretreated cells with RA for 2 h and stimulated them with human recombinant IL-1β (10 ng/ml; Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan) for 6 and 24 h. To examine roles of RAR in the regulation of MCP-1 by t-RA, we treated mesangial cells with t-RA (2.5 μM) together with or without a selective pan-RAR antagonist, AGN193109 (5 μM; Allergan, Irvine, CA) (30) for 6 h.

The effect of t-RA on the stability of MCP-1 transcript was examined as follows. First, the effect of the RNA synthesis inhibition on the constitutive MCP-1 mRNA level was examined by exposing the serum-deprived cells to actinomycin D (500 ng/ml; Serva, Heidelberg, Germany) for 0 to 8 h. Next, serum-deprived cells were exposed to actinomycin D for 6 h in the presence or absence of t-RA during the last 1.5 to 6 h.

To examine the role of AP-1 in the expression of MCP-1, we treated serum-deprived cells with the c-Jun/AP-1 inhibitor curcumin (Sigma; 20 μM) (29) for 8 h. To examine the effect of curcumin on the inducible expression of MCP-1, we pretreated cells with curcumin for 2 h and stimulated them with IL-1β for 24 h.

Northern Blot Analysis

Total RNA was extracted by a single-step method (31) and subjected to Northern blot analysis, as described previously (13). In brief, RNA samples were electrophoresed on 1.2% agarose gels containing 10% formaldehyde and transferred onto nitrocellulose membranes. As probes, a mouse *JE*/MCP-1 cDNA (32) and a rat glyceraldehyde-3-phosphate dehydrogenase cDNA were labeled with ^{32}P-dCTP using the random priming method. The membranes were hybridized with probes at 65°C overnight in a solution containing 4× SSC (600 mM sodium chloride, 60 mM sodium citrate), 5× Denhardt's solution, 10% dextran sulfate, 50 μg/ml herring sperm DNA, and 50 μg/ml poly(A), washed at 50°C and exposed to x-ray films at −80°C.

Assessment of Cell Viability

Serum-deprived, confluent mesangial cells were treated with t-RA (5 μM) for 6 h and 24 h, and incidence of necrosis and apoptosis was examined quantitatively by trypan blue exclusion and Hoechst staining, respectively. For the latter, cells were fixed with 4% formaldehyde in phosphate-buffered saline (PBS) for 10 min and stained by Hoechst 33258 (10 μg/ml; Sigma) for 1 h. Apoptosis was identified by fluorescence microscopy using morphologic criteria including nuclear condensation and/or fragmentation. Both assays were performed in quadruplicate.

Transient Transfection

AP-1 binds to the particular *cis* element TRE and triggers transcription of target genes. To evaluate the activity of AP-1 in mesangial cells, we used a transient transfection assay (17,22,25,29). In brief, using the calcium phosphate coprecipitation method, mesangial cells that were cultured in 24-well plates (1.0×10^5/well) were transfected with a reporter plasmid pTRE-LacZ (a gift from Dr. A. Alberts, ICRF, UK) (33) or a control plasmid pCI-βgal (a gift from Promega, Madison, WI) at 0.3 to 0.6 μg/well. pTRE-LacZ introduces a β-galactosidase (βgal) gene (*lacZ*) under the control of TRE. pCI-βgal introduces *lacZ* under the control of the immediate-early enhancer/promoter of human cytomegalovirus. After incubation for 48 h in 0.5% FCS with or without t-RA (5 μM), cells were subjected to 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-gal) assay, as described below. Activity of AP-1 was evaluated by counting X-gal–positive cells in each well. That is, the number of X-gal–positive cells transfected with pTRE-LacZ was normalized by the number of positive cells transfected with the control plasmid pCI-βgal. Assays were performed in quadruplicate.

Activity of NF-κB was similarly assessed by the transient transfection, as described previously (17,25,29). Mesangial cells were transfected with pCI-βgal, a κB reporter plasmid pHIVLTRβ-gal or its control construct pmuHIVLTRβ-gal (34) (gifts from Dr. A. Rattner, The Weizmann Institute of Science, Rehovot, Israel). pHIVLTRβ-gal introduces *lacZ* under the control of the HIV promoter that contains two κB motifs. The control plasmid pmuHIVLTRβ-gal contains a κB-mutated HIV promoter. NF-κB activity was evaluated by the number of X-gal–positive cells in each group, which was normalized by the number of positive cells transfected with the control plasmid pCI-βgal. Each normalized value of the pHIVLTRβ-gal transfection was then subtracted by the normalized value of the pmuHIVLTRβ-gal transfection, and the resultant value was used as an indicator of NF-κB activity. Assays were performed in quadruplicate. The transfection efficiency achieved in these studies was approximately 0.1 to 0.4%.

X-gal Assay

X-gal assay was performed, as described previously (35). In brief, cells were fixed in 0.5% glutaraldehyde, 2 mM $MgCl_2$, and 1.25 mM ethyleneglycol-bis(β-aminoethyl ether)-*N*,*N*'-tetraacetic acid in PBS at room temperature for 10 min and then incubated at 37°C for 2 to 4 h in a substrate solution containing 1 mg/ml X-gal, 20 mM $K_3Fe(CN)_6$, 20 mM $K_4Fe(CN)_6 3H_2O$, 2 mM $MgCl_2$, 0.01% sodium desoxycholate, and 0.02% NP-40 in PBS.

Statistical Analyses

Data were expressed as means ± SEM. Statistical analysis was performed using the nonparametric Mann-Whitney *U* test to compare data in different groups. $P < 0.05$ was used to indicate a statistically significant difference.

Results

Suppression of Constitutive but Not IL-1β–Inducible Expression of MCP-1 by t-RA

Cultured mesangial cells constitutively express low levels of MCP-1 mRNA in the absence of stimulation. We first examined the effect of t-RA on the constitutive expression of MCP-1 in mesangial cells. SM43 mesangial cells were serum deprived and treated with t-RA (5 μM) for 6 and 24 h. Northern blot analysis showed that the level of constitutive MCP-1 expression was reduced by the treatment with t-RA, modestly after 6 h and markedly after 24 h (Figure 1A).

The effect of t-RA on the level of MCP-1 mRNA was examined further using various concentrations. Mesangial cells were treated with t-RA at 0.5 to 5 μM for 24 h, and Northern blot analysis was performed. As shown in Figure 1B, substantial suppression of the steady-state level of MCP-1 was observed even at low concentrations, *e.g.*, 0.5 μM. The maximum effect was observed at concentrations higher than 2.5 μM.

Retinoic acids are known to induce apoptosis in various cell types, including tumor cells and embryonic cells. To exclude a possibility that the downregulation of MCP-1 was due to decrease in cell viability, we examined the incidence of apoptosis and necrosis in mesangial cells exposed to the highest concentration of t-RA (5 μM) for 6 and 24 h. As summarized in Table 1, no obvious induction of either apoptosis or necrosis was observed in the t-RA–treated cells.

IL-1β is known to be a prominent inducer of MCP-1 in mesangial cells (17). We next examined the effect of t-RA on the cytokine-inducible expression of MCP-1. Serum-deprived cells were pretreated with t-RA for 2 h and stimulated by IL-1β (10 ng/ml) for 24 h. Dramatic induction of MCP-1 was observed in IL-1β–stimulated cells, and the induction was not affected by the treatment with t-RA (Figure 1C).

Suppression of Constitutive but Not IL-1β–Inducible Expression of MCP-1 by 9-cis-RA

To examine whether the suppressive effect on MCP-1 was also observed by other retinoic acids, we examined the effect of 9-cis-RA on the constitutive expression of MCP-1 in mesangial cells. Serum-deprived cells were treated with 9-cis-RA (5 μM; nontoxic concentration) for 6 and 24 h and subjected to Northern blot analysis. Consistent with the effect of t-RA, the level of MCP-1 mRNA was dramatically reduced by the treatment with 9-cis-RA for 24 h (Figure 2A).

We examined further the effect of 9-cis-RA on the cytokine-inducible expression of MCP-1. Serum-deprived cells were pretreated with 9-cis-RA (5 μM) for 2 h and stimulated by IL-1β for 24 h. Marked induction of MCP-1 was observed in IL-1β–stimulated cells, and the induction was not affected by the treatment with 9-cis-RA (Figure 2B), which was consistent with the effect of t-RA.

Requirement of RAR for the Anti–MCP-1 Effect of t-RA

Both t-RA and 9-cis-RA induce target gene expression via RAR. To examine roles of RAR in the regulation of MCP-1 by RA, we treated mesangial cells with t-RA (2.5 μM) together with or without a selective pan-RAR antagonist, AGN193109 (5 μM), for 6 h, and Northern blot analysis was performed. As shown in Figure 3, AGN193109 completely abolished the suppressive effect of t-RA on MCP-1. AGN193109 itself did not obviously affect the basal level of MCP-1 (data not shown).

Effect of t-RA on the Stability of MCP-1 mRNA

Downregulation of the constitutive MCP-1 mRNA level by t-RA may be caused by transcriptional suppression or decreased stability of the transcript. To test the latter, we exam-

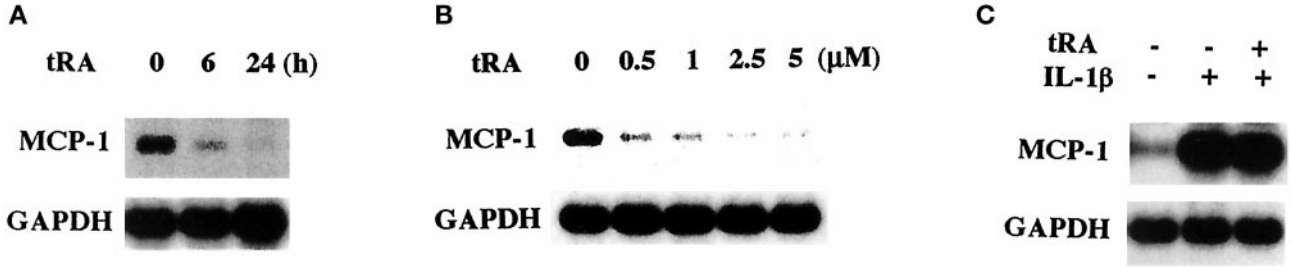

Figure 1. Suppression of constitutive but not interleukin-1β (IL-1β)-inducible expression of monocyte chemoattractant protein 1 (MCP-1) by all-trans-retinoic acid (t-RA). (A) Rat mesangial cells (SM43) were serum deprived (0.5% fetal calf serum [FCS] for 24 h), treated with t-RA (5 μM) for 6 and 24 h, and subjected to Northern blot analysis of MCP-1. Expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is shown as a loading control. (B) Mesangial cells were treated with various concentrations of t-RA (0.5 to 5 μM) for 24 h, and Northern blot analysis was performed. (C) Mesangial cells were pretreated with (+) or without (−) t-RA for 2 h and stimulated with IL-1β (10 ng/ml) for 24 h, and Northern blot analysis was performed.

Table 1. Viability of mesangial cells treated with 5 μM t-RA (%)[a]

	t-RA Exposure Time (h)		
	0	6	24
Hoechst staining	98.0 ± 0.4	98.0 ± 0.4	98.9 ± 0.4
Trypan blue exclusion	97.9 ± 0.4	97.6 ± 0.3	97.9 ± 0.7

[a] t-RA, all-trans-retinoic acid. Serum-deprived, confluent mesangial cells were treated with t-RA (5 μM) for 6 h and 24 h, and incidence of apoptosis and necrosis was examined by Hoechst staining and trypan blue exclusion, respectively. Both assays were performed in quadruplicate. Data are means ± SEM.

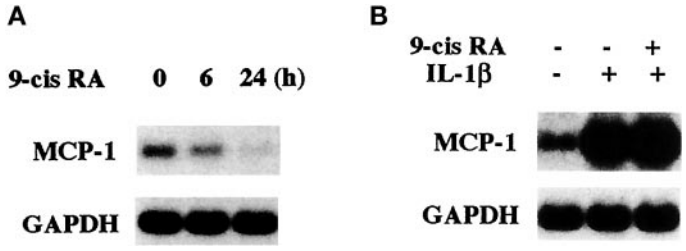

Figure 2. Suppression of constitutive but not IL-1β–inducible expression of MCP-1 by 9-cis-RA. (A) Mesangial cells were serum deprived, treated with 9-cis-RA (5 μM) for 6 and 24 h, and subjected to Northern blot analysis of MCP-1. (B) Mesangial cells were pretreated with (+) or without (−) 9-cis-RA for 2 h and stimulated with IL-1β (10 ng/ml) for 24 h, and Northern blot analysis was performed.

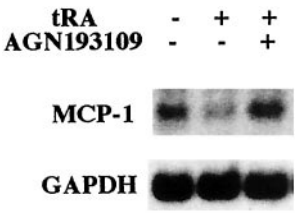

Figure 3. Requirement of retinoic acid receptors (RAR) for the anti–MCP-1 effect of t-RA. Mesangial cells were treated with t-RA (2.5 μM) together with or without a selective pan-RAR antagonist, AGN193109 (5 μM), for 6 h, and Northern blot analysis was performed.

ined whether t-RA affects the stability of MCP-1 mRNA. Serum-deprived mesangial cells were treated with actinomycin D (500 ng/ml) for 2 to 8 h, and the level of MCP-1 transcript was examined. As shown in Figure 4A, treatment with actinomycin D effectively reduced the level of MCP-1 mRNA after 6 h. Using this condition, we examined the effect of t-RA. Mesangial cells were exposed to actinomycin D for 6 h in the presence or absence of t-RA during the last 1.5 to 6 h. Northern blot analysis showed that treatment of t-RA did not affect the stability of MCP-1 mRNA at any time points (Figure 4B).

Roles of NF-κB and AP-1 in the Constitutive and IL-1β–Inducible Expression of MCP-1

The 5'-flanking region of the MCP-1 gene contains TRE and NF-κB binding sites. The roles of NF-κB and AP-1 in the constitutive and IL-1β–inducible expression of MCP-1 were examined using mutant mesangial cells in which the function of NF-κB or AP-1 is selectively attenuated. SM/IκBαM cells stably express a super-repressor mutant of IκBα and exhibit blunted activation of NF-κB when stimulated by IL-1β and TNF-α (28). SM/JUNDN cells stably express a dominant-interfering form of c-Jun and show attenuated activity of AP-1 under both unstimulated and stimulated conditions (25). Mock-transfected SM/Neo cells, SM/IκBαM cells, and SM/JUNDN cells were treated with or without IL-1β, and levels of MCP-1 mRNA were evaluated by Northern blot analysis. Under the IL-1β–stimulated condition, SM/IκBαM cells exhibited blunted expression of MCP-1, when compared with SM43 and SM/Neo cells (Figure 5A, left). SM/JUNDN cells showed the same level of MCP-1 mRNA as that observed in control mesangial cells. However, under the unstimulated condition, the expression of MCP-1 was attenuated in both SM/IκBαM cells and SM/JUNDN cells (Figure 5A, right). These data suggested that AP-1 was involved in the constitutive expression but not in the inducible expression of MCP-1 in mesangial cells. This was confirmed further by using the pharmacologic inhibitor of c-Jun/AP-1, curcumin. Mesangial cells were pretreated with curcumin (20 μM) and stimulated with or without IL-1β. As shown in Figure 5B, the IL-1β–inducible expression of MCP-1 was not affected by curcumin. In contrast, the constitutive expression of MCP-1 was attenuated substantially by the treatment with curcumin.

Effects of t-RA on the Activity of AP-1 and NF-κB

As described above, the IL-1β–inducible expression of MCP-1 was dependent only on NF-κB, but its constitutive expression was dependent on both AP-1 and NF-κB. t-RA inhibited the constitutive MCP-1 expression without affecting the inducible expression. These data suggested a possibility that t-RA inhibited the constitutive expression of MCP-1 via selective intervention in the AP-1 pathway. To explore this possibility, we examined the effect of t-RA on the constitutive activity of AP-1 and NF-κB. Mesangial cells were transfected with reporter plasmids, serum-deprived in the presence or absence of t-RA for 48 h, and activity of AP-1 and NF-κB was examined. As reported previously (17,25), serum-depleted mesangial cells exhibited substantial levels of AP-1 and NF-κB activity. Treatment with t-RA significantly inhibited the constitutive activity of AP-1 (Figure 6A). In contrast, t-RA did not have any effects on the constitutive activity of NF-κB (Figure 6B).

Discussion

MCP-1 is induced in mammalian cells in response to pathologic stimuli, including cytokines/growth factors, bacterial components, and mechanical stress. Under various pathologic situations, the inducible expression of MCP-1 contributes to accumulation of monocytes/macrophages at inflammatory sites. Conversely, constitutive expression of MCP-1 is observed in certain normal tissues, including bronchial epithelium and renal glomeruli (36,37). Because resident macrophages are present in these tissues (38), the low levels of

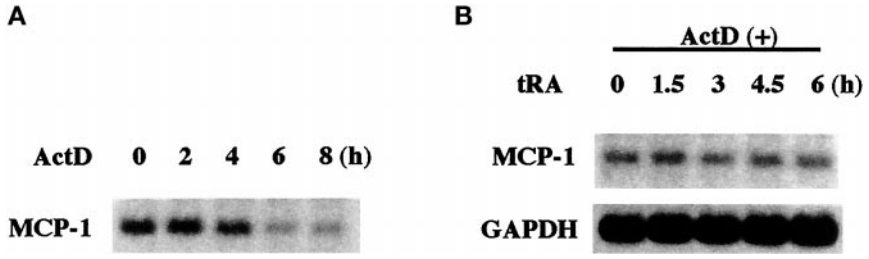

Figure 4. Effect of t-RA on the stability of MCP-1 mRNA. (A) Serum-deprived mesangial cells were treated with actinomycin D (ActD; 500 ng/ml) for 2 to 8 h, and the level of MCP-1 transcript was examined by Northern analysis. (B) Serum-deprived mesangial cells were exposed to actinomycin D for 6 h in the presence or absence of t-RA during the last 1.5 to 6 h and subjected to Northern blot analysis.

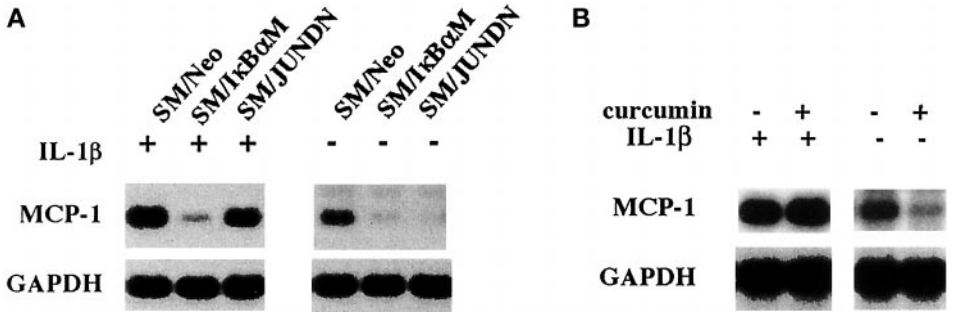

Figure 5. Roles of nuclear factor-κB (NF-κB) and activator protein 1 (AP-1) in the constitutive and cytokine-inducible expression of MCP-1. (A) Transfectants stably expressing a neomycin phosphotransferase gene (*neo*) alone (SM/Neo), *neo* and a dominant-negative mutant of c-*jun* (SM/JUNDN), and *neo* and a super-repressor mutant of IκBα (SM/IκBαM) were treated with (left) or without (right) IL-1β. Northern blot analysis was performed on the expression of MCP-1. (B) Mesangial cells were pretreated with (+) or without (−) the c-Jun/AP-1 inhibitor curcumin (20 μM) and treated with (left) or without (right) IL-1β for 8 h. Northern blot analysis was performed.

constitutive expression may contribute to continuous attraction of monocytes into these sites. Currently, little information is available regarding how the constitutive expression of MCP-1 is regulated in certain cell types. In the present study, we demonstrated that the constitutive and cytokine-inducible expression of MCP-1 was regulated differently in mesangial cells, *i.e.*, the constitutive expression was dependent on but the cytokine-inducible expression was independent of AP-1.

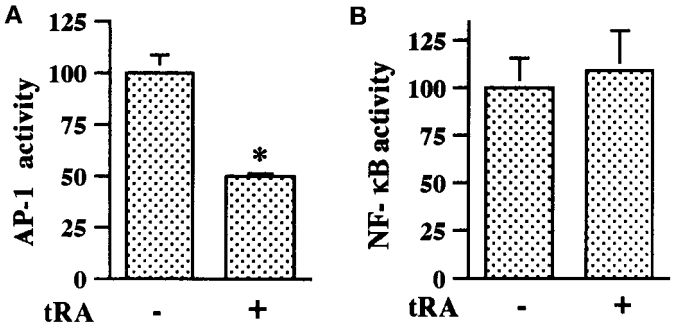

Figure 6. Effects of t-RA on the constitutive activity of AP-1 and NF-κB. Mesangial cells cultured in 24-well plates were transfected transiently with an AP-1 reporter plasmid pTRE-LacZ (A) or an NF-κB reporter plasmid pHIVLTRβ-gal (B). After the transfection, cells were incubated in 0.5% FCS in the presence (+) or absence (−) of t-RA (5 μM) for 48 h and subjected to 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-gal) assay. The activity of AP-1 and NF-κB was evaluated as described in the Materials and Methods section. Assays were performed in quadruplicate. Data are shown as means ± SEM. *, statistically significant difference ($P < 0.05$).

RA generally has been regarded as an inhibitor of AP-1 (20). However, previous studies indicated that the anti–AP-1 activity of RA is somewhat controversial. For example, RA does not inhibit c-*jun* and c-*fos* expression and activity of AP-1 in activated myofibroblasts and monocytes (39,40). RA rather may upregulate expression of c-*fos*/c-*jun* and activity of AP-1 in tumor cells and embryonic stem cells (41–44). The effect of RA on the AP-1 pathway supposedly is different from cell type to cell type. In this investigation, we tested the effect of t-RA, an anti-inflammatory agent for glomerulonephritis (45), on the expression of MCP-1 in mesangial cells. Our results showed that t-RA inhibited the constitutive expression but not IL-1β–inducible expression. The suppressive effect of t-RA was via the inhibition of AP-1 because (*1*) the constitutive expression but not the cytokine-inducible expression was dependent on AP-1, (*2*) t-RA inhibited constitutive activity of AP-1 but not NF-κB, and (*3*) genetic and pharmacologic inhibitors of AP-1 suppressed only the constitutive expression of MCP-1.

The mechanisms involved in the suppressive effect of t-RA on the basal AP-1 activity are unknown. We previously showed that t-RA inhibited induction of c-*fos* and c-*jun* in H_2O_2-exposed mesangial cells. However, under the serum-deprived, unstimulated condition, t-RA did not suppress the constitutive expression of c-*fos* and c-*jun* but rather upregu-

lated both mRNA (Lucio-Cazana J, Kitamura M, unpublished data), which is consistent with previous reports (41–44). The anti–AP-1 effect of t-RA should, therefore, be ascribed to other mechanisms.

Biological actions of RA are mediated by RAR and RXR. The RAR family is known to be activated by t-RA and by 9-cis-RA, but the RXR family is activated only by 9-cis-RA (46). That both t-RA and 9-cis-RA inhibited MCP-1 expression suggested the importance of RAR in the transcriptional suppression by RA. Indeed, the experiments using the pan-RAR antagonist revealed that RAR were essential. A previous report showed that RA inhibited activation of AP-1 via physical interaction of RAR-RXR complexes with c-Jun (47). Sequestration of AP-1 proteins by RAR-RXR heterodimers (48) may be involved in the anti–AP-1 effect of t-RA. Another recent report also showed that disruption of homodimerization or herterodimerization of AP-1 components is a mechanism through which ligand-activated RAR suppress the AP-1 activity (49). Of note, RARα, RARβ, RARγ, and RXRα mRNA are constitutively expressed in serum-deprived mesangial cells (Xu Q, Kitamura M, unpublished observation).

In summary, these data elucidated the different mechanisms involved in the constitutive and cytokine-inducible expression of MCP-1. t-RA, a potential anti-inflammatory drug, has the ability to inhibit selectively the constitutive expression of MCP-1 via intervention in the AP-1 pathway.

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