Bioflavonoid Quercetin Inhibits Interleukin-1-Induced Transcriptional Expression of Monocyte Chemoattractant Protein-1 in Glomerular Cells via Suppression of Nuclear Factor-κB

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Abstract. Flavonoids are semiessential food components that possess anti-inflammatory properties. This report describes a novel potential of bioflavonoid quercetin as an inhibitor of monocyte chemoattractant protein-1 (MCP-1) in glomerular cells. Cultured mesangial cells as well as isolated glomeruli expressed MCP-1 mRNA in response to interleukin-1β (IL-1β). Quercetin dramatically inhibited the cytokine-triggered MCP-1 expression. To explore the mechanisms involved, effects of quercetin on the putative transcriptional activators of MCP-1, nuclear factor-κB (NF-κB) and activator protein-1 (AP-1), were examined. Exposure of the cells to IL-1β caused activation of NF-κB without significant upregulation of AP-1 activity. NF-κB inhibitor MG132 diminished the IL-1-induced expression of MCP-1 in mesangial cells and isolated glomeruli, whereas c-Jun/AP-1 inhibitor curcumin did not affect this process. Consistently, NF-κB-inactive mesangial cells expressing a super-repressor mutant of IκBα showed blunted expression of MCP-1 by IL-1β. In contrast, AP-1-inactive mesangial cells expressing a dominant-negative mutant of c-Jun exhibited the same level of MCP-1 mRNA as that in control cells. These results suggest that: (*1*) quercetin has the ability to attenuate activation of NF-κB; and (*2*) it inhibits IL-1-triggered MCP-1 expression via suppression of NF-κB, but not AP-1, in glomerular cells.

Monocyte chemoattractant protein-1 (MCP-1) is a member of the chemokine family and specifically attracts monocytes (1). MCP-1, initially purified from mononuclear leukocytes and a myelomonocytic cell line, is ubiquitously produced 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 cultured mesangial cells stimulated by proinflammatory cytokines produce MCP-1 (2,3). MCP-1 synthesized by activated mesangial cells plays a role in macrophage attraction during glomerular inflammation (8). A possible therapeutic approach to glomerulonephritis, therefore, is to inhibit local expression of MCP-1 by pharmacologic agents. To achieve this goal, we sought chemical inhibitors of MCP-1 and found that flavonoid quercetin strongly inhibits cytokine-triggered induction of MCP-1 in cultured mesangial cells and isolated glomeruli.

Flavonoids are semi-essential food components that are ubiquitously present in nature. These constituents of the diet are important in the maintenance of the body. Clinically relevant functions ascribed to flavonoids include antihypertensive activity, anti-inflammatory properties, hypocholesterolemic activity, and platelet stabilization (9). Quercetin is one of the most widely distributed flavonoids in the plant kingdom. Previous reports have shown that quercetin may affect various signaling pathways mediated by protein tyrosine kinases, cAMP-dependent kinase, protein kinase C, and calmodulin-dependent kinase (9).

Several signaling pathways have been proposed for the transcriptional regulation of MCP-1. The 5′ flanking region of the MCP-1 gene contains multiple 12-*o*-tetradecanoylphorbol-13-acetate response elements (TRE) and κB sites (10–12), suggesting potential roles of activator protein-1 (AP-1) and nuclear factor-κB (NF-κB). 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 (13–15). 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 (10,16,17). In endothelial cells, NF-κB and AP-1 cooperatively upregulate expression of MCP-1 in response to IL-1β (18). To explore molecular mechanisms involved in the suppressive effect of quercetin on

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MCP-1, the present study investigated: (*1*) whether NF-κB and AP-1 are required for the induction of MCP-1 by IL-1β; and (*2*) how quercetin modulates the activity of these transcription factors in glomerular cells.

Materials and Methods

Mesangial Cells and Isolated Glomeruli

Mesangial cells (SM43) were established from isolated glomeruli of a male Sprague Dawley rat and identified as being of mesangial cell phenotype as described before (19). Cells were maintained in DMEM/Ham's F-12 medium (Life Technologies, 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 1% FCS were generally used for experiments. Normal glomeruli were isolated on ice from adult male Sprague Dawley rats (250 to 300 g body wt, four rats) by the conventional sieving method (20) and used for experiments.

Stable Transfectants

SM/JUNDN1 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 (21). TAM-67 is a deletion mutant that is lacking amino acids 3 to 122 of c-Jun (22). 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 manner (22). SM/JUNDN1 cells exhibit depressed activity of AP-1 under both basal and stimulated conditions (21).

SM/IκBαM cells in which NF-κB is selectively inactivated were established as follows. pLIκBαMSN (23), which introduces a superrepressor mutant of IκBα (IκBαM) and a neomycin phosphotransferase gene (*neo*), was transfected into a helper-free ecotropic packaging line, ΩE (19). Stable transfectants were selected in the presence of neomycin analogue G418 (500 μg/ml). Conditioned media of the transfectants were used as sources of the IκBαM retrovirus. In the presence of 10 μg/ml polybrene, SM43 mesangial cells were exposed to diluted retrovirus, as described before (19). Stable infectants were selected in the presence of G418 (750 μg/ml), and SM/IκBαM cells were established. SM/IκBαM cells exhibit blunted activation of NF-κB when stimulated by proinflammatory cytokines IL-1β and TNF-α (24).

As a control, mock-transfected mesangial cells SM/Neo that express *neo* alone were created, as described previously (25).

Pharmacologic Manipulations

Confluent mesangial cells cultured in the presence of 1% FCS for 24 to 48 h were treated by recombinant human IL-1β (10 ng/ml; Otsuka Pharmaceutical Co., Tokushima, Japan) for 8 to 24 h. To examine effects of pharmacologic agents, mesangial cells were pretreated with quercetin (1 to 100 μM; Sigma Immunochemicals, St. Louis, MO), c-Jun/AP-1 inhibitor curcumin (10 to 20 μM; Sigma) (25), or NF-κB inhibitors *N*-acetylcysteine (5 to 10 mM; Sigma) (26) and MG132 (25 to 50 μM; Peptide Institute, Osaka, Japan) (27) for 1.5 h and stimulated by IL-1β for 8 to 24 h.

Isolated glomeruli were suspended in 1% FCS, stimulated by IL-1β (10 ng/ml) for 6 h in the presence or absence of quercetin (50 μM) or MG132 (50 μM), and subjected to Northern blot analysis.

Northern Blot Analysis

Total RNA was extracted by a single-step method (28) and subjected to Northern blot analysis, as described before (14). 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 (29) and a rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA (30) 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 Kodak XAR films at −80°C.

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, a transient transfection assay was used (31). In brief, using the calcium phosphate coprecipitation method, mesangial cells cultured in 24-well plates (1.0 to 1.2 × 10^5/well) were transfected with a reporter plasmid pTRE-LacZ (a gift from Dr. A. Alberts, Imperial Cancer Research Fund, United Kingdom) (32) or a control plasmid pCI-βgal (a gift from Promega, Madison, WI) at 0.3 to 0.5 μg per well. pTRE-LacZ introduces a β-galactosidase 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 10% FCS with or without quercetin (50 μM), cells were subjected to 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-gal) assay, as described below. To examine the effect of quercetin on the activity of TRE in IL-1-stimulated cells, transfected cells were incubated in the presence of 1% FCS for 48 h, pretreated with or without quercetin for 1.5 h, and stimulated by IL-1β (10 ng/ml) for 24 h. Activity of AP-1 was evaluated by counting X-gal-positive cells in each well (25). 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 assay. As described above, mesangial cells were transfected with pCI-βgal, a κB reporter plasmid pHIVLTRβ-gal, or its control construct pmuHIVLTRβ-gal (33) (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 (25). 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 before (34). In brief, cells were fixed in 0.5% glutaraldehyde, 2 mM $MgCl_2$, and 1.25 mM ethyleneglycol-bis(β-aminoethyl ether)-*N*,*N*′-tetra-acetic acid in phosphate-buffered saline 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 $K_4Fe(CN)_6 \cdot 3\ H_2O$, 2 mM

$MgCl_2$, 0.01% sodium desoxycholate and 0.02% NP-40 in phosphate-buffered saline.

Electrophoretic Mobility Shift Assay

Confluent mesangial cells cultured in 1% FCS for 24 h were pretreated with or without quercetin for 1.5 h and stimulated by IL-1β for 4 and 24 h. After the treatment, cells were harvested on ice, lysed in lysis buffer (10 mM Hepes, pH 7.9, containing 1.5 mM $MgCl_2$, 10 mM KCl, 50 μM dithiothreitol, 100 μM phenanthroline, 1 μg/ml pepstatin, 100 μM 1-trans-epoxysuccinyl-leucylamide(4-guanidino)butane, 100 μM 3,4-dichloroisocoumarin, 10 mM NaF, 100 μM sodium orthovanadate, 25 mM β-glycerophosphate, and 0.2% [vol/vol] NP-40) for 10 min on ice, and centrifuged. The pellets were resuspended in extraction buffer (20 mM Hepes, pH 7.9, containing 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM ethylenediaminetetra-acetic acid, 25% [vol/vol] glycerol, and 100 μM dichloroisocoumarin), incubated for 15 min at 4°C, and centrifuged. The supernatants containing nuclear protein were used for electrophoretic mobility shift assay to evaluate NF-κB activity (35).

As probes, double-stranded wild-type NF-κB (5′-AGT TGA GGG GAC TTT CCC AGG C-3′) and mutant NF-κB (5′-AGT TGA GGC GAC TTT CCC AGG C-3′) oligonucleotides were purchased from Promega. These oligonucleotides were radiolabeled with [γ-^{32}P]ATP by T4 polynucleotide kinase. DNA-binding reactions were performed in binding reaction buffer (10 mM Tris/HCl, pH 7.5, 100 mM NaCl, 1 mM ethylenediaminetetra-acetic acid, 4% [vol/vol] glycerol, 5 mM dithiothreitol, 100 μg/ml nuclease-free bovine serum albumin) containing the nuclear extract (15 μg), ^{32}P-labeled oligonucleotide probe (0.14 pmol), and poly(dI-dC) (6 μg). After incubation for 15 min at room temperature, samples were electrophoresed on native 5% acrylamide gels. After the electrophoresis, gels were dried and exposed to x-ray film. To confirm the specificity of the reaction, competition assays were performed with 100-fold excess of unlabeled wild-type oligonucleotides that were added to the reaction mixtures 5 min before the addition of the labeled NF-κB probe. A negative control experiment without nuclear extract was also performed in parallel.

Statistical Analyses

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

Results

Inhibition of Cytokine-Triggered MCP-1 Expression by Quercetin

Mesangial cells were pretreated with quercetin (50 μM) for 1.5 h and stimulated by IL-1β (10 ng/ml). Under the basal culture condition (1% FCS), mesangial cells exhibited only slight expression of MCP-1 mRNA (0.8 kb). When stimulated by IL-1β, expression of MCP-1 was markedly induced. Pretreatment with quercetin inhibited both its basal and inducible expression (Figure 1A). The suppressive effect of quercetin was dose-dependent. Modest repression of MCP-1 was observed at concentrations of 5 to 10 μM. Substantial suppression was observed at concentrations higher than 25 μM (Figure 1B).

The effect of quercetin on the expression of MCP-1 was further examined using isolated glomeruli. Isolated glomeruli incubated in 1% FCS exhibited modest MCP-1 expression.

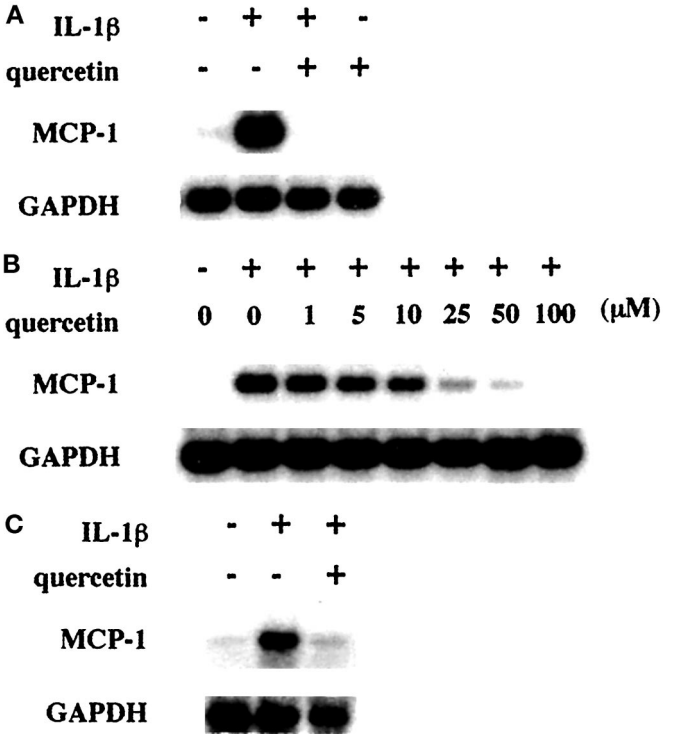

Figure 1. Inhibition of cytokine-triggered expression of monocyte chemoattractant protein-1 (MCP-1) by quercetin in cultured mesangial cells and isolated glomeruli. (A) Rat mesangial cells (SM43) were pretreated with (+) or without (−) quercetin (50 μM) for 1.5 h and stimulated by interleukin-1β (IL-1β; 10 ng/ml) for 8 h. Northern blot analysis was performed on the expression of MCP-1. Expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is shown as a loading control. (B) Mesangial cells were pretreated with serial concentrations of quercetin (1 to 100 μM) and stimulated by IL-1β. Expression of MCP-1 was examined by Northern blot analysis. (C) Isolated normal rat glomeruli were suspended in 1% fetal calf serum (FCS), stimulated by IL-1β (10 ng/ml) for 6 h in the presence (+) or absence (−) of quercetin (50 μM), and subjected to Northern blot analysis.

Stimulation of glomeruli by IL-1β markedly enhanced the induction of MCP-1 mRNA. Consistent with the result in cultured mesangial cells, quercetin abrogated the IL-1-induced expression of MCP-1 in isolated glomeruli (Figure 1C).

Effect of Quercetin on the Activity of AP-1 and NF-κB

Several signaling molecules including NF-κB and AP-1 have been proposed as transcriptional regulators for MCP-1. To examine the possibility that quercetin inhibits MCP-1 expression via suppression of these transcription factors, reporter assays were performed. Serum-stimulated mesangial cells were transiently transfected with either a κB reporter plasmid or a TRE reporter plasmid, treated with or without quercetin, and activity of NF-κB and AP-1 was evaluated. Under serum-stimulated culture conditions, mesangial cells exhibit constitutive activity of NF-κB and AP-1 (21,25). Treatment with quercetin significantly inhibited the activity of both NF-κB (33 ± 6% *versus* untreated control [100%], mean ± SEM, $P <$

0.05) and AP-1 (49 ± 4% *versus* untreated control [100%]) (Figure 2A).

We next examined whether IL-1β induces activation of NF-κB and AP-1, and if so, how quercetin modulates the activation of these transcription factors. Mesangial cells transfected with reporter plasmids were cultured in 1% FCS, pretreated with quercetin, and stimulated by IL-1β. X-gal assay showed that IL-1β significantly induced activation of NF-κB (431 ± 41% *versus* untreated control [100%]) (Figure 2B, left) but did not upregulate the activity of AP-1 (121 ± 11% *versus* untreated control [100%]) (Figure 2B, right). Treatment with quercetin dramatically diminished the activation of NF-κB in IL-1-stimulated mesangial cells from 431 ± 41% (IL-1β alone) to 85 ± 15% (quercetin + IL-1β), when compared with untreated control (100%) (Figure 2B, left).

The suppressive effect of quercetin on the NF-κB activation was further confirmed by electrophoretic mobility shift assay. Mesangial cells were pretreated with or without quercetin for 1.5 h and stimulated by IL-1β for 4 and 24 h. As shown in Figure 2C, the DNA binding activity of NF-κB induced by IL-1β was significantly inhibited by quercetin. The mutant probe and cold competition demonstrated that the band observed is NF-κB-specific. Four hours after the stimulation, the inhibitory effect of quercetin was partial. However, after 24 h, the DNA binding activity observed in IL-1-stimulated cells was completely abrogated in the cells treated with quercetin.

Roles of NF-κB and AP-1 in the IL-1-Triggered MCP-1 Expression

The roles of NF-κB and AP-1 in the expression of MCP-1 were examined using pharmacologic inhibitors. Mesangial cells were pretreated with an inhibitor of c-Jun/AP-1, curcumin (25), or an inhibitor of NF-κB, MG132 (27), and stimulated by IL-1β. As shown in Figure 3A, induction of MCP-1 by IL-1β was substantially diminished by MG132 (25 to 50 μM). Similar suppression was also observed by another NF-κB inhibitor, *N*-acetylcysteine (10 mM) (data not shown). In contrast, the induction of MCP-1 was not obviously affected by the treatment with the AP-1 inhibitor curcumin (10 to 20 μM) (Figure 3A), which effectively inhibits expression of the AP-1-dependent genes gelatinase B and stromelysin in mesangial cells (25,36).

The crucial role of NF-κB in the cytokine induction of MCP-1 was further examined using isolated glomeruli. Normal glomeruli were stimulated by IL-1β for 6 h in the presence or absence of MG132 and subjected to Northern blot analysis. Consistent with the result in mesangial cells, MG132 abolished the induction of MCP-1 in isolated glomeruli (Figure 3B).

The roles of NF-κB and AP-1 were further investigated using mesangial cells that stably express a super-repressor mutant of IκBα and a dominant-interfering form of c-Jun. As reported previously, SM/IκBαM cells exhibit blunted activation of NF-κB when stimulated by IL-1β and TNF-α (24). SM/JUNDN cells show attenuated activity of AP-1 under both basal and stimulated conditions (21). Untransfected SM43 mesangial cells, mock-transfected SM/Neo cells, SM/IκBαM cells, and SM/JUNDN cells were treated with or without

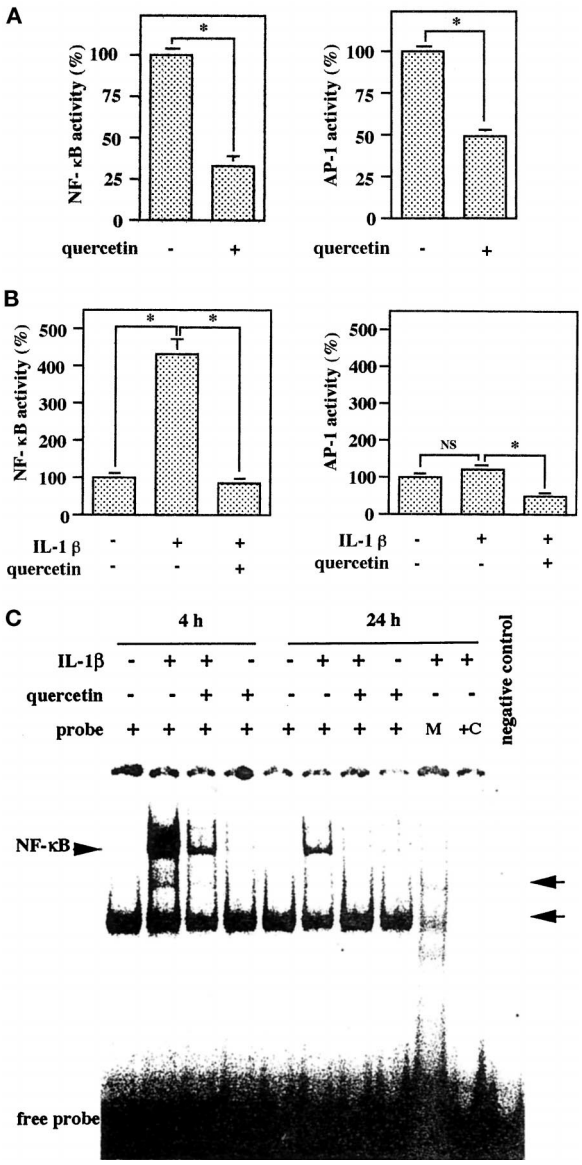

Figure 2. Effect of quercetin on the activity of activator protein-1 (AP-1) and nuclear factor-κB (NF-κB). (A) Mesangial cells cultured in 24-well plates were transiently transfected with an NF-κB reporter plasmid pHIVLTRβ-gal or an AP-1 reporter plasmid pTRE-LacZ. After the transfection, cells were incubated in 10% FCS in the presence (+) or absence (−) of quercetin (50 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 Materials and Methods. Assays were performed in quadruplicate. Data are shown as means ± SEM. *$P < 0.05$. (B) Mesangial cells transfected with reporter plasmids were incubated in the presence of 1% FCS for 48 h, pretreated with or without quercetin for 1.5 h, and stimulated by IL-1β (10 ng/ml) for 24 h. Activity of AP-1 and NF-κB was evaluated by X-gal assay. Assays were performed in quadruplicate. *$P < 0.05$. (C) Mesangial cells were pretreated with (+) or without (−) quercetin for 1.5 h and stimulated by IL-1β for 4 and 24 h. After the treatment, cells were subjected to electrophoretic mobility shift assay. Probes: +, wild-type NF-κB oligonucleotide; M, mutant NF-κB oligonucleotide; +C, wild-type NF-κB oligonucleotide + unlabeled oligonucleotide (100-fold excess). Negative control is a sample without nuclear extract. Nonspecific bindings detected by the mutant probe are indicated on the right by arrows.

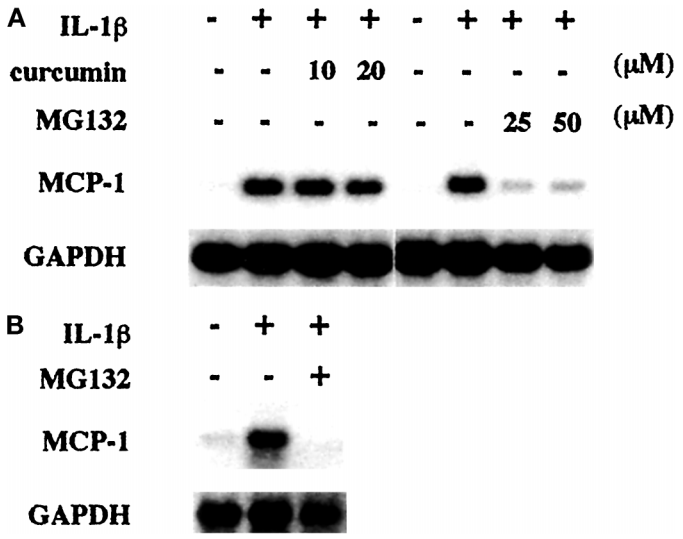

Figure 3. Effects of pharmacologic inhibition of NF-κB and AP-1 on the cytokine-induced expression of MCP-1. (A) Mesangial cells were pretreated with the c-Jun/AP-1 inhibitor curcumin (10 to 20 μM) or the NF-κB inhibitor MG132 (25 to 50 μM) for 1.5 h and stimulated by IL-1β (10 ng/ml) for 24 h. Expression of MCP-1 was examined by Northern blot analysis. (B) Isolated glomeruli were suspended in 1% FCS, stimulated by IL-1β (10 ng/ml) for 6 h in the presence (+) or absence (−) of MG132 (50 μM), and subjected to Northern blot analysis.

IL-1β, and expression of MCP-1 was evaluated by Northern blot analysis. Compared with SM43 and SM/Neo cells, SM/IκBαM cells exhibited blunted expression of MCP-1 in response to IL-1β (Figure 4). In contrast, SM/JUNDN cells showed the same level of MCP-1 mRNA as that observed in control mesangial cells.

Discussion

In this report, we describe a novel potential of quercetin as an inhibitor of MCP-1 in glomerular cells. We found that the IL-1-triggered induction of MCP-1 was dramatically suppressed by quercetin. This inhibitory effect was through suppression of NF-κB, but not AP-1, in mesangial cells.

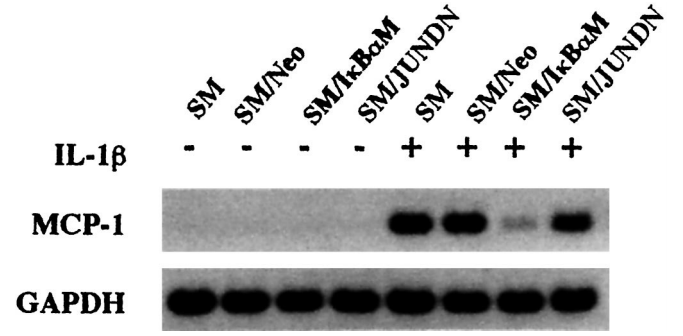

Figure 4. Effects of genetic inactivation of NF-κB and AP-1 on the cytokine-induced expression of MCP-1. Untransfected SM43 mesangial cells (SM) and transfectants stably expressing a neomycin phosphotransferase gene (*neo*) alone (SM/Neo), *neo* and a dominant-negative mutant of c-*jun* (SM/JUNDN), and *neo* and a super-repressor mutant of IκBα (SM/IκBαM) were treated with (+) or without (−) IL-1β (10 ng/ml) for 8 h. Northern blot analysis was performed on the expression of MCP-1.

The molecular mechanisms involved in the suppressive effect of quercetin on NF-κB are currently unknown, but several possibilities may be postulated. Quercetin is known to have the ability to modulate intracellular redox states (9). The first possibility is that quercetin may inhibit NF-κB by functioning as an antioxidant (37). It is known that NF-κB is a redox-sensitive transcription factor and activated by oxidant stress (38). A previous study showed that IL-1-triggered MCP-1 expression was inhibited by antioxidants (39). The inhibitory effect of quercetin, therefore, may be ascribed to its ability to scavenge reactive oxygen intermediates generated in IL-1-stimulated cells.

The second possibility is that quercetin may inhibit NF-κB via suppression of certain protein kinase(s). As has been reported, quercetin is a potential inhibitor of tyrosine kinases (9). A previous study showed that in human mesangial cells, IL-1-triggered MCP-1 expression is mediated by protein tyrosine kinases (40). We showed that in rat mesangial cells, activation of NF-κB in response to IL-1β is mediated by tyrosine kinases (25). These data suggest that tyrosine kinase may be a proximal target of quercetin for its inhibitory action on NF-κB.

Quercetin may affect not only protein tyrosine kinases, but also activity of other kinases including cAMP-dependent kinase, protein kinase C, and calmodulin-dependent kinase (9). Activation of the family of mitogen-activated protein (MAP) kinases, including extracellular signal-regulated kinases and p38 MAP kinases, is also attenuated by quercetin (Y. Ishikawa and M. Kitamura, submitted for publication). On the basis of its multipotent, inhibitory action on protein kinases, it might be speculated that quercetin inhibits NF-κB via suppression of certain kinases directly linked to NF-κB activation. For example, recent investigations disclosed the crucial roles of NF-κB-inducing kinase, MAP kinase kinase kinase1, and IκB kinases in the activation of NF-κB by IL-1β (41–43). Further investigation will be required to determine the effect of quercetin on the NF-κB-activating kinases.

Induction of MCP-1 by cytokines and growth factors plays an important role in various pathologic situations. During glomerular injury, infiltration of monocytes/macrophages is a common pathologic feature (4). High levels of MCP-1 expression are observed in the mesangium of inflamed glomeruli (5–7). In the early phase of glomerulonephritis, the cells responsible for the MCP-1 production are resident mesangial cells (8). Using blocking antibodies, several reports have shown that *in vivo* neutralization of local MCP-1 attenuated crescentic glomerulonephritis in mice and rats (8,44,45). MCP-1 produced by mesangial cells supposedly contributes to accumulation of macrophages and generation of glomerular injury. From this viewpoint, administration of quercetin could be useful for therapeutic intervention in macrophage-mediated glomerulonephritis. Of note, it has been shown that quercetin attenuated ischemia-reperfusion injury with concomitant suppression of MCP-1 in the kidney (46).

In this report, we highlighted the effect of quercetin on the

induction of MCP-1, but expression of other cytokine-inducible genes may also be attenuated by quercetin. The fact that quercetin significantly inhibited activity of both NF-κB and AP-1 (47) (present data) suggests that it can suppress induction of various genes controlled by NF-κB and AP-1. Under pathologic circumstances, activation of these transcription factors is triggered by inflammatory stimuli, including cytokines/growth factors, microbial pathogens, and oxidant stress. A wide array of genes is subsequently induced and contributes to the initiation and progression of diseases (48,49). Based on this, pharmacologic inhibitors of NF-κB and AP-1, including quercetin, should serve as useful agents for therapeutic intervention.

Quercetin and other flavonoids have been considered as therapeutic agents for various pathologic conditions, including cancer, viral infection, inflammation/allergy, hypertension, and atherosclerosis. These agents possess carcinostatic and antiviral activities, suppress cell proliferation, modify eicosanoid synthesis, protect LDL from oxidation, prevent platelet aggregation, stabilize immune cells, and promote relaxation of cardiovascular smooth muscle (9). Our data suggest that some of these therapeutically relevant actions of quercetin can be ascribed to its inhibitory effects on NF-κB and AP-1.

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