Interleukin-6 (IL-6) is a pleiotropic cytokine, whose plasma levels are elevated in inflammatory diseases such as atherosclerosis. We have previously reported that peroxisome proliferator-activated receptor α (PPARα) ligands (fibrates) lower elevated plasma concentrations of IL-6 in patients with atherosclerosis and inhibit IL-1-stimulated IL-6 secretion by human aortic smooth muscle cells (SMC). Here, we show that aortic explants isolated from PPARα-null mice display an exacerbated response to inflammatory stimuli, such as lipopolysaccharide (LPS), as demonstrated by increased IL-6 secretion. Furthermore, fibrate treatment represses IL-6 mRNA levels in LPS-stimulated aortas of PPARα wild-type, but not of PPARα-null mice, demonstrating a role for PPARα in this fibrate action. In human aortic SMC, fibrates inhibit IL-1-induced IL-6 gene expression. Furthermore, activation of PPARα represses both c-Jun- and p65-induced transcription of the human IL-6 promoter. Transcriptional interference between PPARα and both c-Jun and p65 occurs reciprocally, since c-Jun and p65 also inhibit PPARα-mediated activation of a PPAR response element-driven promoter. This transcriptional interference occurs independent of the promoter context as demonstrated by cotransfection experiments using PPARα, p65, and c-Jun Gal4 chimeras. Overexpression of the transcriptional coactivator cAMP-responsive element-binding protein-binding protein (CBP) does not relieve PPARα-mediated transcriptional repression of p65 and c-Jun. Finally, glutathione S-transferase pull-down experiments demonstrate that PPARα physically interacts with c-Jun, p65, and CBP. Altogether these data indicate that fibrates inhibit the vascular inflammatory response via PPARα by interfering with the NF-κB and AP-1 transactivation capacity involving direct protein-protein interaction with p65 and c-Jun.

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Atherosclerosis is a complex vascular disease characterized by endothelial injury, monocyte infiltration in the subendothelial space, followed by differentiation into macrophages followed by cholesterol deposition. This further results in foam cell formation, smooth muscle cell (SMC) proliferation, and migration from the media to the intima (1). The presence of macrophages, T lymphocytes, as well as numerous cytokines in the atherosclerotic lesion suggests an important immunological component in the pathogenesis of atherosclerosis (1, 2). Interleukin-6 (IL-6), a cytokine, which has been detected in human and rabbit atherosclerotic lesions (3, 4), is secreted by endothelial cells, monocytes/macrophages, and SMC (2). IL-6 controls the growth and T cell activation, SMC proliferation, and migration and is a major regulator of the acute phase response (5). Even though IL-6 might also possess anti-inflammatory properties (6, 7), this cytokine is considered as a good marker of vascular inflammation.

Peroxisome proliferator-activated receptors (PPARs) belong to the superfamily of nuclear receptors which are ligand-activated transcription factors (8). PPARs regulate gene expression by binding with their heterodimeric partner retinoid X receptor to specific PPAR-response elements (PPREs) (9). Three different PPAR subtypes have been identified: PPARα, PPARβ (NUC-1 or PPARδ), and PPARγ. Fatty acid derivatives and eicosanoids were identified as natural ligands for PPARs (10–14). Furthermore, fibrates are synthetic ligands for PPARα (10), which mediates the lipid-lowering activity of these drugs (15). Several indirect observations suggest that fibrates may also exert a direct anti-atherogenic activity at the level of the vascular wall, which occurs independently of their lipid-lowering activity. First, treatment of cholesterol-fed rabbits with the PPARα ligand fenofibrate decreases atherosclerotic plaque formation in the thoracic aorta, in the absence of any lowering of plasma lipid levels (16). Second, in a number of intervention trials, such as BECAIT and LOCAT, fibrate treatment slows the progression of coronary atherosclerosis without significantly affecting plasma atherogenic lipoprotein concentrations (17, 18). Finally, Devchand et al. (11) showed that absence of

* This work was supported by grants of the Institut Pasteur de Lille, INSERM, Comité Français de Coopération des Recherches sur l’Athérosclérose et le cholesterol, Rhône-Poulenc Rorer, Laboratoires Fournier, and the Région Nord-Pas-de-Calais/FEDER. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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$ Supported by a grant from the Région Nord-Pas-de-Calais.

|| Holds a fellowship of the IWT.

§§ Research director with the FWO-Vlaanderen.
PPARα expression in mice prolonged the inflammatory response. We and others (19, 20) reported that fibrates decrease plasma concentrations of inflammatory cytokines, such as IL-6 and tumor necrosis factor α, in human patients with angiographically established atherosclerosis and prevent the induction of IL-6 production by IL-1β in SMC.

Although much is known about gene activation by PPARs acting via PPREs, less information exists about the mechanisms of negative gene modulation by PPARs. Recently, PPARs have been suggested to exert anti-inflammatory activities by antagonizing the AP-1, NF-κB, and STAT pathways in macrophages and SMC (19, 21, 22). To address the physiological role of PPARα in the regulation of the inflammatory response at the level of the vascular wall, studies were performed using PPARα-null mice as a model. Our results demonstrate, using IL-6 secretion as an inflammatory marker, that aortas from PPARα-null mice display an exacerbated inflammatory response to LPS. We next carried out experiments to characterize the molecular mechanisms implicated in the down-modulation of IL-6 gene promoter activity by PPARα activators.

MATERIALS AND METHODS

Cell Culture and Chemical Reagents—Human aortic SMC (Cascade Biologies, Portland, OR) were cultured in SMC basal medium containing 5% FCS and 5% CO2 in a 5% CO2 humidified atmosphere at 37 °C. Human umbilical vein endothelial cells (ECV-304, Cambrex, Walkersville, MD) were cultured in ECV-304 basal medium containing 5% FCS and 5% CO2.

RESULTS

Aortas from PPARα-Null Mice Display an Exacerbated Inflammatory Response to LPS Stimulation and Are Refractory to Fenofibrate Treatment—In order to provide genetic evidence for a role of PPARα in the vascular inflammatory response, basal and LPS-stimulated IL-6 production by aortic segments from PPARα−/− and +/+ mice were compared. In the absence of LPS stimulation, basal IL-6 secretion was similar in aortas of both groups of mice (Fig. 1A). LPS stimulation resulted in a significant increase of IL-6 production (approximately 3-fold) in wild-type mice aortas, in agreement with previous observations (26). However, this increase was much greater in aortas isolated from the PPARα−/− mice (12-fold, p < 0.03). These observations indicate that PPARα deficiency results in an increased vascular inflammatory response, as assessed by enhanced IL-6 secretion. Next, the influence of treatment with the PPARα-agonist fenofibrate on the inflammatory response was analyzed in aortas of PPARα−/− and +/+ mice. In the absence of LPS stimulation, basal IL-6 mRNA levels were very low (data not shown) and became only detectable after LPS injection. In LPS-injected PPARα−/− mice, treatment with fenofibrate decreased significantly IL-6 mRNA levels in aortas, whereas fenofibrate did not have any effect in PPARα+/+ mice (Fig. 1B). These data indicate that the anti-inflammatory properties of fenofibrate are PPARα-dependent and that PPARα controls the vascular inflammatory response at the gene expression level in vivo.

PPARα Activators Inhibit IL-1-Induced IL-6 Gene Expression in Human Aortic SMC—Next, it was determined whether PPARα activation inhibits the induction of IL-6 mRNA levels by inflammatory cytokines, such as IL-1β in human aortic SMC. As expected, stimulation of SMC with IL-1β resulted in a severalfold increase of IL-6 mRNA (Fig. 2). This induction was,
however, inhibited in the presence of Wy-14643. These data indicate that PPARα activation inhibits IL-6 gene induction by inflammatory cytokines in vitro.

**PPARα Inhibits IL-6 Gene Transcription by Interfering with the Promoter Transactivation by c-Jun and p65**—Next, it was studied whether PPARα interferes with IL-6 gene expression at the transcriptional level. Several regulatory elements such as an AP-1, a C/EBP, an NF-κB, and a multiple response element, have been identified in the human IL-6 promoter (34), of which the AP-1 and NF-κB response elements have been shown to mediate the IL-6 response to inflammatory stimuli such as IL-1β (35). To test whether PPARα interferes with the transcriptional activation of the IL-6 promoter by the transcription factors AP-1 and/or NF-κB, transient cotransfection experiments were performed. Because of the inability to transfect primary cultured human aortic SMC, COS-1 cells were used for these transient transfection experiments. Cotransfection of the p65 NF-κB subunit resulted in a strong activation of wild-type IL-6 promoter activity (11-fold) (Fig. 3A). Cotransfection of human PPARα alone did not influence basal IL-6 promoter activity. However, the activation of the IL-6 promoter by p65 was significantly (p = 0.007) decreased in the presence of PPARα activated by Wy-14643. As expected, p65 cotransfection did not activate a promoter construct mutated in the NF-κB site, nor a construct containing only the IL-6 promoter TATA box region in front of the luciferase gene (Fig. 3A). Interestingly, p65 induction of the IL-6 promoter mutated in the AP-1 site was less pronounced as compared with the wild-type promoter, suggesting functional interaction between the NF-κB and AP-1 sites. However, similarly as the wild-type, cotransfection of PPARα in the presence of its ligand was able to repress p65-mediated induction (p = 0.001) of the IL-6 promoter mutated in the AP-1 site (Fig. 3A).

Cotransfection of c-Jun and c-Fos also strongly activated the wild-type human IL-6 promoter (Fig. 3B). This induction was reduced by PPARα cotransfection (p = 0.042) in the presence of Wy-14643. As expected, the IL-6 promoter mutated on the AP-1 site as well as the minimal IL-6 promoter were not activated by c-Jun/c-Fos. Similarly as for p65 activation on the AP-1 site mutated promoter, the NF-κB site-mutated promoter was less inducible by c-Jun/c-Fos than the wild-type promoter, but PPARα was able to repress the induction by c-Jun/c-Fos (p = 0.039). However, PPARα cotransfection did not result in a significant inhibition of the basal activity of the NF-κB site mutated promoter. Taken together, these data indicate that PPARα represses IL-6 promoter activation by interfering negatively with the AP-1 and NF-κB transcriptional activities.

**PPARα Represses AP-1 and NF-κB Activities Independently of the Promoter Context**—Next, it was investigated whether PPARα could interfere with AP-1 and NF-κB transactivation independently of the promoter context. Therefore, we analyzed the effect of PPARα on the transcriptional activation of a Gal4-dependent reporter, activated by the p65 or c-Jun chimeras (Fig. 4). As a control, PPARα did not influence transcriptional activity of the Gal4 DBD alone. Transfection of the chimera containing the NF-κB p65 subunit led to a strong transcriptional activation (almost 20-fold) of the reporter construct. This induction was significantly reduced (~60%) by PPARα cotransfection in the presence of Wy-14643. Cotransfection of the c-Jun chimera resulted in a less pronounced induction of promoter activity (6-fold), but an almost complete repression (~74%) of c-Jun-mediated transactivation was observed in the presence of cotransfected PPARα in the presence of its ligand. These results indicate that PPARα interferes negatively with the c-Jun as well as with the NF-κB transactivation capacities in a manner independent of the promoter context.

**p65 and c-Jun Reciprocally Repress PPARα Transactivation**

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**Fig. 1.** Aortas from PPARα-null mice display an exacerbated inflammatory response to LPS stimulation and are refractory to fenofibrate treatment. A, aortas from male PPARα−/− and +/+ mice (n = 8/group) were isolated, cut into two segments, and exposed to LPS (10 μg/ml) or vehicle for 24 h. Medium was collected and IL-6 concentration measured, as described under "Materials and Methods." Values were normalized to DNA content and expressed as mean ± S.E. Statistical analysis was performed using a two-way analysis of variance (p < 0.05). Statistical significant differences are indicated by different letters. B, male PPARα−/− and +/+ mice (n = 6/group) were fed with rodent chow or fenod chow diet supplemented with 0.2% fenofibrate for 2 weeks. At the end of the treatment period, half of the mice of each group received an intraperitoneal injection of LPS (1 mg/kg). The other half received a vehicle (water) injection. After 3 h, aortas from individual mice were isolated and subjected to RNA analysis. IL-6 mRNA levels were measured by Northern blot analysis and normalized to 36B4 mRNA levels. Values (mean ± S.E.) are expressed as a percentage of the untreated control animals. Since IL-6 mRNA levels in the vehicle injected animals are below the detection limit, only results from LPS-injected animals are depicted. Statistical significant differences from controls are indicated by an asterisk (*, p < 0.05).
Wy-14643 (10 μM) was refed with DMEM supplemented with 0.2% FCS in the presence of human PPARα, led to a dose-dependent inhibition of PPARα-mediated repression of NF-κB by PPARα involves mainly the C-terminal domains of the receptor, whereas transrepression of c-Jun implicates the N terminus.

**PPARα Interacts Physically with p65, c-Jun, and CBP**—To determine whether PPARα interacts physically with p65, c-Jun, and CBP, GST pull-down experiments were performed (Fig. 8). Interaction of PPARα protein with the p65 Rel homology domain (aa 12–317) could be detected, whereas the C-terminal transactivation domain of p65 (aa 286–551) did not bind to PPARα (Fig. 8A). Furthermore, PPARα also interacted with the JNK-responsive part of c-Jun (aa 1–79) (Fig. 8B). This interaction occurs via the N-terminal DBD containing part of PPARα, since the C-terminal deletion mutant of PPARα also binds to c-Jun (Fig. 8C), in agreement with the results from the transfection experiments (Fig. 6D). Finally, in line with a previous report (39), PPARα was found to associate with the N-terminal aa 1–213 of CBP. Interestingly, the LBD-lacking PPARα variant also interacted strongly with CBP (aa 1–170). The C-terminal deletion mutant of PPARα (aa 1–170) could be detected, whereas the C-terminal amino acids (LBD) fused to p65 (Fig. 6C). PPARα cotransfection in the presence of Wy-14643 resulted in a significant reduction of both p65 and c-Jun transactivation (Fig. 8). Interaction of PPARα with the p65 Rel homology domain (aa 12–317) could be detected, whereas the C-terminal transactivation domain of p65 (aa 286–551) did not bind to PPARα (Fig. 8A). Furthermore, PPARα also interacted with the JNK-responsive part of c-Jun (aa 1–79) (Fig. 8B). This interaction occurs via the N-terminal DBD containing part of PPARα, since the C-terminal deletion mutant of PPARα also binds to c-Jun (Fig. 8C), in agreement with the results from the transfection experiments (Fig. 6D). Finally, in line with a previous report (39), PPARα was found to associate with the N-terminal aa 1–213 of CBP. Interestingly, the LBD-lacking PPARα variant also interacted strongly with CBP (aa 1–213) (Fig. 8C). Altogether these results indicate that PPARα interacts with the N terminus of c-Jun and the Rel homology domain of p65 and both the C- and N-terminal halves of PPARα bind to CBP.

**PPARα Negatively Regulates the Vascular Inflammatory Response**—In order to delineate which domains of PPARα are involved in the transcriptional cross-talk with AP-1 and NF-κB, transfection experiments were performed using a chimera containing the PPARα C-terminal amino acids (LBD) fused to the Gal4 DBD (amino acids 1–147) in the presence or absence of c-Jun or p65 (Fig. 6, A and B). Neither treatment with Wy-14643 nor cotransfection of p65 and c-Jun exerted a major effect on the reporter activity in the absence of cotransfected Gal4-LBD (Fig. 6, A and B). However, in the presence of the latter, Wy-14643 strongly activated (10-fold) the Gal4-responsive promoter. This induction was significantly repressed by p65 (Fig. 6A), whereas cotransfection of c-Jun had almost no effect (Fig. 6B). When the influence of a recently identified, natural truncated form of PPARα, which lacks the entire LBD and only contains amino acids 1–170 of the wild-type PPARα, was tested on Gal4-p65 driven transactivation (Fig. 6C), a significant, but less pronounced, repression (−20%) of p65 transactivation was observed when compared with the wild-type form (−60%). By contrast, the truncated form of PPARα was able to repress c-Jun transactivation to a similar extent as the wild-type PPARα (−60%) (Fig. 6D). Taken together these data indicate that transrepression of NF-κB by PPARα involves mainly the C-terminal domains of the receptor, whereas transrepression of c-Jun implicates the N terminus.

**pp65 and c-Jun Functionally Interfere with Different PPARα Domains**—In order to delineate which domains of PPARα are involved in the transcriptional cross-talk with AP-1 and NF-κB, transfection experiments were performed using a chimera containing the PPARα C-terminal amino acids (LBD) fused to the Gal4 DBD (amino acids 1–147) in the presence or absence of c-Jun or p65 (Fig. 6, A and B). Neither treatment with Wy-14643 nor cotransfection of p65 and c-Jun exerted a major effect on the reporter activity in the absence of cotransfected Gal4-LBD (Fig. 6, A and B). However, in the presence of the latter, Wy-14643 strongly activated (10-fold) the Gal4-responsive promoter. This induction was significantly repressed by p65 (Fig. 6A), whereas cotransfection of c-Jun had almost no effect (Fig. 6B). When the influence of a recently identified, natural truncated form of PPARα, which lacks the entire LBD and only contains amino acids 1–170 of the wild-type PPARα, was tested on Gal4-p65 driven transactivation (Fig. 6C), a significant, but less pronounced, repression (−20%) of p65 transactivation was observed when compared with the wild-type form (−60%). By contrast, the truncated form of PPARα was able to repress c-Jun transactivation to a similar extent as the wild-type PPARα (−60%) (Fig. 6D). Taken together these data indicate that transrepression of NF-κB by PPARα involves mainly the C-terminal domains of the receptor, whereas transrepression of c-Jun implicates the N terminus.

**FIG. 3.** PPARα inhibits IL-6 gene transcription by interfering with the promoter transactivation by c-Jun and p65. COS cells were transfected with the indicated IL-6 promoter constructs (1 μg) in the presence of IPPARα (1 μg), p65 (1 μg) (A), c-Jun and c-Fos (1 μg) (B), or empty expression plasmids. After 5 h, cells were washed and refed with DMEM supplemented with 0.2% FCS in the presence of Wy-14643 (10 μM) when PPARα was cotransfected. Statistical analysis was assessed by analysis of variance (p < 0.05). Statistical significant differences between groups were then evaluated by the Student’s t test. Statistical significance was assigned when p < 0.05 and is indicated in the text.

**FIG. 4.** PPARα represses c-Jun and p65 transcriptional activity in a promoter-independent manner. COS cells were transfected respectively with the pGal4,50hu IL-6p-Luc+ (140 ng) reporter plasmid in the presence of the indicated Gal4 chimeras (80 ng) and the human PPARα (80 ng) expression plasmids or its corresponding empty vector. Cells were subsequently incubated with Wy-14643 (10 μM). Statistical significant differences (p < 0.05) were evaluated by the Student’s t test. Statistical significant effects of PPARα cotransfection are indicated by an asterisk.

**of a PPRE-driven Promoter**—In order to determine whether the transcriptional cross-talk between PPARα, NF-κB, and AP-1 activities occurs in a reciprocal manner, transfection assays were performed to test the effect of p65 and c-Jun on a PPARα-dependent PPRE-driven promoter. In the absence of cotransfected PPARα, the PPRE-driven reporter was slightly activated by addition of Wy-14643 (Fig. 5). As expected, cotransfection of PPARα significantly induced the reporter activity in the presence of Wy-14643 (3.5-fold). Cotransfection of increasing amounts of p65 (Fig. 5A) or c-Jun (Fig. 5B) expression vectors led to a dose-dependent inhibition of PPARα-induced reporter activity without affecting basal promoter activity. This result indicates the existence of a bidirectional antagonism between PPARα, c-Jun, and NF-κB activities.

**PPARα Interacts Physically with p65, c-Jun, and CBP**—To determine whether PPARα interacts physically with p65, c-Jun, and CBP, GST pull-down experiments were performed (Fig. 8). Interaction of PPARα protein with the p65 Rel homology domain (aa 12–317) could be detected, whereas the C-terminal transactivation domain of p65 (aa 286–551) did not bind to PPARα (Fig. 8A). Furthermore, PPARα also interacted with the JNK-responsive part of c-Jun (aa 1–79) (Fig. 8B). This interaction occurs via the N-terminal DBD containing part of PPARα, since the C-terminal deletion mutant of PPARα also binds to c-Jun (Fig. 8C), in agreement with the results from the transfection experiments (Fig. 6D). Finally, in line with a previous report (39), PPARα was found to associate with the N-terminal aa 1–213 of CBP. Interestingly, the LBD-lacking PPARα variant also interacted strongly with CBP (aa 1–213) (Fig. 8C). Altogether these results indicate that PPARα interacts with the N terminus of c-Jun and the Rel homology domain of p65 and both the C- and N-terminal halves of PPARα bind to CBP.

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**of a PPRE-driven Promoter**—In order to determine whether the transcriptional cross-talk between PPARα, NF-κB, and AP-1 activities occurs in a reciprocal manner, transfection assays were performed to test the effect of p65 and c-Jun on a PPARα-dependent PPRE-driven promoter. In the absence of cotransfected PPARα, the PPRE-driven reporter was slightly activated by addition of Wy-14643 (Fig. 5). As expected, cotransfection of PPARα significantly induced the reporter activity in the presence of Wy-14643 (3.5-fold). Cotransfection of increasing amounts of p65 (Fig. 5A) or c-Jun (Fig. 5B) expression vectors led to a dose-dependent inhibition of PPARα-induced reporter activity without affecting basal promoter activity. This result indicates the existence of a bidirectional antagonism between PPARα, c-Jun, and NF-κB activities.
PPARα Negatively Regulates the Vascular Inflammatory Response

have demonstrated that PPARα plays a role in acute inflammation control. Here, we show that PPARα has anti-inflammatory properties at the level of the vascular wall, since aortas from PPARα-null mice display an exacerbated inflammatory response to LPS stimulation, as measured by IL-6 production. Furthermore, PPARα mediates the anti-inflammatory actions of fibrates, such as fenofibrate, at the level of the vascular wall. This result extends previous reports showing that PPARα ligands repress cytokine-induced IL-6 production in SMC (19), inducible nitric-oxide synthase activity in murine macrophages (42), and VCAM-1 expression in endothelial cells (43). The physiological relevance of these observations is further corroborated by the demonstration that fibrates lower plasma levels of inflammatory cytokines such as IL-6, tumor necrosis factor α, and interferon γ in patients with atherosclerosis (19, 20).

Interestingly, not only PPARα, but also PPARγ (22, 44, 45), ligands have been reported to inhibit production of inflammatory cytokines by monocytes/macrophages in vitro. All these studies underline a potential modulatory role of PPAR ligands in the pathogenesis of atherosclerosis. Furthermore, IL-6 production is also inhibited by estrogen receptor (46) and glucocorticoid receptor agonists (24), suggesting that PPARs share anti-inflammatory properties with a number of other nuclear receptors.

PPARα ligands exert their activity by negatively regulating IL-1-induced IL-6 gene expression in SMC. Results from mutation analysis demonstrate that PPARα represses IL-6 promoter activation by negatively interfering with c-Jun and NF-κB transactivation. Similarly, COX-2 repression in SMC by PPARα, as well as repression of inducible nitric-oxide synthase gelatinase B, scavenger receptor-A (22), and tumor necrosis factor α expression (44) in murine and human macrophages by PPARγ have been suggested to be effected by antagonizing the AP-1, STAT, and NF-κB pathways (19, 21, 22).

Several molecular mechanisms can be invoked to explain transcriptional negative cross-talk between PPARα and other transcription factors such as c-Jun or p65. PPARα may compete for binding to identical or overlapping response elements. However, our results show that PPAR activation does not activate basal IL-6 promoter activity, indicating the absence of a functional PPRE. Furthermore, the interference between PPAR and c-Jun or p65 occurs in a promoter-independent manner, since it is observed using Gal4 fusion proteins. Therefore, competition for binding site recognition can be excluded.

In this study, we found that PPARα represses p65 as well as c-Jun transactivation of the human IL-6 promoter. Furthermore, our transfection results demonstrate that this interference is reciprocal as described previously for other nuclear receptors.

FIG. 5. p65 and c-Jun repress PPARα transactivation of a PPRE-driven promoter. COS cells were transfected with a reporter construct driven by six copies of the apo AII PPRE (J6-TK-Luc) (10 ng) in the presence of hPPARα (30 ng) and increasing amounts (1, 10, 100 ng) of p65 (A) or c-Jun (B) expression vectors. After 5 h, cells were washed and refed with DMEM supplemented with 0.2% FCS in the presence of Wy-14643 (10 μM) or solvent (Me2SO 0.1%). Statistical significant differences were evaluated by the Student's t test and are indicated by asterisks (*, p < 0.05; **, p < 0.01).

FIG. 6. p65 and c-Jun functionally interfere with different PPARα domains. A and B, COS cells were transfected with the Gal4-TK-pGL3 reporter plasmid containing five Gal4 response elements, the PPARα chimera Gal4-hPPARα (0.1 μg), and p65 (A) or c-Jun (B) expression vectors. After 5 h, cells were washed and refed with DMEM supplemented with 0.2% FCS in the presence of Wy-14643 (10 μM) or solvent (Me2SO 0.1%). C and D, COS cells were transfected with the p(Gal4)50hu IL-6P-Luc+ (340 ng) reporter plasmid in the presence of the Gal4-p65 chimera (80 ng) (C) or the Gal4-c-Jun chimera (80 ng) (D) and the wild-type or LBD-lacking human PPARα expression plasmids or its corresponding empty vector (80 ng). After 5 h, cells were washed and refed with DMEM supplemented with 0.2% FCS in the presence of Wy-14643 (10 μM). Statistical significant differences were evaluated by the Student's t test and are indicated by asterisks (*, p < 0.05; **, p < 0.01).

**DISCUSSION**

Chronic inflammation is a hallmark of atherosclerosis (1, 2, 41). It has therefore been postulated that negatively interfering with the inflammatory response at the level of the vascular wall might lead to a selective inhibition of the atherogenic process. The PPARα signaling pathway constitutes a potentially interesting target for anti-inflammatory drug development. Indeed, using PPARα-deficient mice, Devchand et al. (11)
presses c-Jun transactivation mainly via its N terminus, the truncated variant was less efficient in NF-κB repression. In vitro transfection experiments suggest that the cross-talk between PPARα and c-Jun or p65, in which PPARα represses c-Jun transactivation in a LBD-independent manner. This result is in line with previous works showing that the receptor DBD is required for the interaction between AP-1 and nuclear receptors such as glucocorticoid receptor (47–51), the retinoic acid receptor (52), the progesterone receptor (52), and the androgen receptor (53). To assess the hypothesis of a physical interaction between PPARα and c-Jun or p65, we performed GST pull-down experiments. Our results indicate that PPARα associates with aa 1–79 of c-Jun protein via the N-terminal part of the receptor, since the PPARα natural occurring splicing variant lacking the LBD was still able to interact with c-Jun. This result was corroborated by the transfection experiments using the Gal4 fusion proteins showing that PPARα represses c-Jun transactivation in a LBD-independent manner. This result is in line with previous works showing that the receptor DBD is required for the interaction between AP-1 and nuclear receptors such as glucocorticoid receptor (47–51), androgen receptor (53), and retinoic acid receptor (52). Our data extend a previous study, confirmed that PPARα interacts weakly with p65 and that this interaction occurs through aa 12–317 of p65. This region contains the Rel homology domain which mediates DNA binding, dimerization, and interaction with IκB. Through this domain, p65 was previously reported to interact with other nuclear receptors such as the glucocorticoid receptor (51), Palvimo et al. (55) also found a weak interaction between p65 and androgen receptor. In addition to the GST pull-down experiments, results from transfection experiments suggest that the cross-talk between PPARα and p65 occurs mainly via the LBD of PPARα, since the truncated variant was less efficient in NF-κB repression. In view of our data, we propose a model of transcriptional cross-talk between PPARα and c-Jun or p65, in which PPARα represses c-Jun transactivation mainly via its N terminus, whereas p65 transrepression occurs in a LBD-dependent manner.

Since it has been suggested that inhibition of transcriptional activation by nuclear receptors can be effected by competing for limiting amounts of co-activators (36–38), we investigated how CBP might interfere with NF-κB and AP-1 activities and their repression by PPARα. Cotransfection assays showed that low amounts of CBP are indeed sufficient to increase the activated state of c-Jun or p65, whereas the relative repression by PPARα remains unaffected. Furthermore, the three key players involved in PPARα-dependent transrepression on CBP-stimulated NF-κB and AP-1-dependent reporters are able to interact with each other in vitro. GST pull-down assays confirmed that PPARα interacts with the N-terminal part of CBP (aa 1–213), i.e. the nuclear receptor-associating domain, as described previously (39). Furthermore, an additional interacting domain of PPARα with CBP was mapped to its N-terminal part. To our knowledge, this is the first demonstration that PPARα interacts with CBP via its N-terminal domain. Although so far most coactivators have been shown to interact with the nuclear receptor LBD, the N-terminal part of the thyroid receptor has also been shown to mediate coactivator interaction (56). Finally, and as already stated above, PPARα is also able to interact with the DNA-binding domain of p65, as well as with the JNK-responsive part of c-Jun, whereas both proteins have already been described to associate with CBP (40, 57, 58). Hence, the various mutual interactions between the different transcription factors involved and/or CBP as well as their relative abundance may therefore be the critical parameters to determine the actual state of activation and/or repression.

Finally, recent reports (59, 60) demonstrate that nuclear...
receptors and AP-1 or NF-κB can functionally interact by interfering with signaling pathways (such as protein phosphorylation), and this modulates transcription factor activation. Caeles et al. (59) demonstrated that various nuclear receptors block AP-1 activation by interfering with the JNK cascade activation. Since PPAR interacts with the JNK phosphorylation-responsive part of c-Jun, our results do not allow us to exclude this aspect in the mechanism of PPAR-mediated gene repression.

Apart from being a marker for vascular inflammation, down-regulation of IL-6 may have important (patho)physiological consequences, since this cytokine may be involved in the pathogenesis of atherosclerosis (2). Biswas et al. (61) reported that IL-6 induces monocyte chemotactic protein-1 expression in peripheral blood mononuclear cells and U937 macrophages. Thus, suppression of IL-6 secretion by PPAR ligands may indirectly inhibit the production of potent chemokines involved in monocyte recruitment into the subendothelial space, resulting in less foam cell formation. In conclusion, the results from this study show that, in addition to their lipid-lowering properties, PPARα activators may also have beneficial effects in atherosclerosis by inhibiting vascular inflammation.

Acknowledgments—We acknowledge the technical contribution of O. Vidal, B. Derudas, P. Poulin, and K. Van Wesemael.

REFERENCES

1. Ross, R. (1999) Nature 399, 114–124
2. Ross, R. (1993) N. Engl. J. Med. 329, 1575–1580
3. Rus, H. G., Vlaicu, R., and Niculescu, F. (1996) Folia Pharmacol. Jpn. 41, 229–233
4. Tillo, H., Dinarello, C. A., and Mier, J. W. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 2927–2932
5. Madej, A., Okopien, B., Kowalski, J., Zielinski, M., Wysocki, J., Szygula, B., and Tedgui, A. (1995) J. Biol. Chem. 270, 26757–26762

Table of references begins here.