Antiinflammatory Roles of Peroxisome Proliferator–activated Receptor γ in Human Alveolar Macrophages

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Peroxisome proliferator–activated receptor γ (PPARγ) is a ligand-activated transcriptional factor belonging to the nuclear receptor superfamily. PPARγ, which is predominantly expressed in adipose tissue, plays a major regulatory role in glucose metabolism and adipogenesis. Interestingly, recent studies have demonstrated PPARγ expression in monocytes/macrophages and its antiinflammatory activities. However, it is unclear whether alveolar macrophages (AMs) express functional PPARγ. The present study was conducted to investigate the expression of PPARγ by AMs and to elucidate its functional role. Using reverse transcription–polymerase chain reaction and Western blotting, we demonstrated the strong expression of PPARs messenger RNA and protein in freshly isolated human AMs. Ligands of PPARγ, 15-deoxy-$\Delta^{12,14}$prostaglandin J_2, and troglitazone significantly decreased LPS-induced tumor necrosis factor-α production by AMs. These ligands markedly upregulated the expression of CD36, a scavenger receptor that mediates the phagocytosis of apoptotic neutrophils. Indeed, ligand-treated AMs ingested a significantly higher number of apoptotic neutrophils than untreated AMs. These data indicate that PPARγ expressed by AMs play an antiinflammatory role through inhibiting cytokine production and increasing their CD36 expression together with the enhanced phagocytosis of apoptotic neutrophils, which is an essential process for the resolution of inflammation. This suggests the potential therapeutic application of PPARγ ligands in inflammatory disorders of the lung.

Keywords: peroxisome proliferator–activated receptor γ; alveolar macrophage; tumor necrosis factor-α; CD36; phagocytosis

Alveolar macrophages (AMs) are highly specialized mononuclear phagocytic cells located in the alveolar space. They are the first immune-competent cells to encounter inhaled antigens and therefore play an important role in regulating immune responses of the lung (1). Indeed, AMs have been shown to contribute to a variety of disease processes in the lung, including infection, allergic disorders, and fibrosis, by their trophic and cytotoxic activities. Under such pathologic conditions, proinflammatory stimuli activate AMs to produce a variety of inflammatory mediators, such as cytokines, chemokines, and reactive oxygen intermediates, and to enhance their bactericidal/cytotoxic capacity, which all leads to the augmentation of local inflammation (2). In contrast, recent studies have also highlighted a novel property of AMs in resolving lung inflammation through their capacity to phagocytose infiltrating cells into the lung (3, 4). Collectively, AMs regulate lung inflammation in different ways depending on the phase of the lung inflammation, thus tightly elaborating the processes of this inflammation. Based on these observations, AMs could be one of the major therapeutic targets for inflammatory disorders of the lung. Peroxisome proliferator–activated receptors (PPARs) are members of the nuclear hormone receptor superfamily that heterodimerize with the retinoid X receptor (5, 6). The three known PPAR subtypes, α, γ, and δ (β), which are associated with selective ligands, show distinct tissue distributions (7, 8). PPARγ is predominantly expressed in adipose tissue, and its target genes encode enzymes involved in fatty acid synthesis (9). It is thus thought that PPARγ plays an important role in glucose metabolism and adipogenesis. PPARγ is activated by diverse synthetic and naturally occurring ligands, including antidiabetic thiazolidinediones (10, 11), polyunsaturated fatty acids (12, 13), 15-deoxy-$\Delta^{12,14}$prostaglandin J_2 (15d-PGJ_2) (14, 15), and components of oxidized low-density lipoproteins (16). Unexpectedly, recent studies have indicated the expression of PPARγ in monocytes (Mo) and macrophages (17–20). Low levels of PPARγ were detected in murine bone marrow macrophages, whereas activated peritoneal macrophages showed much higher levels of its expression (18). Although the function of PPARγ in Mo and macrophages is not fully determined, several reports have suggested an antiinflammatory activity of PPARγ in these cells. Indeed, PPARγ ligands inhibited inducible nitric oxide synthase expression in murine peritoneal macrophages (18) as well as the production of the inflammatory cytokines, such as tumor necrosis factor α (TNF-α), interleukin-1β, and interleukin-6, from human Mo (19). It is suggested that these inhibitory effects of PPARγ are mediated by negatively regulating the expression of proinflammatory genes and antagonizing the activities of transcription factors (18, 21, 22). Taken together, these cumulative data provide a new insight into the function of PPARγ as an antiinflammatory mediator in Mo and macrophages. However, the expression of PPARγ and its functional role in AMs are currently unknown. Interestingly, recent data demonstrated that CD36, a scavenger receptor, is upregulated by PPARγ on Mo and macrophages (16, 23). PPARγ modulates CD36 gene expression through direct interaction with the proximal promoter via a specific response element. The primary function of CD36 has been shown to be associated with clearance of apoptotic cells arising during development, normal homeostasis, and inflammation (24, 25). At the inflammatory sites, CD36 functions as a receptor for apoptotic neutrophils, which mediates their phagocytosis by macrophages (24, 26, 27). During early inflammatory processes, neutrophils are recruited from the circulation in response to a series of coordinated signals (28). In the resolving phase, the accumulated neutrophils undergo apoptosis and are subsequently phagocytosed by macrophages (29). This removal of apoptotic neutrophils by macrophage phagocytosis is a critical process for the resolution of inflammation. In the lung, it is thus hypothesized that the activation of PPARγ enhances CD36 expression by AMs, which in turn augments their phagocytic capacity for apoptotic neutrophils. However, few data are avail-

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able about CD36 expression by AMs (30). The aim of the present study was to investigate the expression of PPARγ by AMs. To address this, we first determined the expression of PPARγ in human AMs by reverse transcription polymerase chain reaction and Western blotting. Next, we examined the effect of the PPARγ ligand on the production of TNF-α and CD36 expression by the AMs. Finally, we clarified whether the ligand dependent stimulation of PPARγ in the AMs augments their capacity to phagocytose apoptotic neutrophils. Some of the results of this study have been previously reported in the form of abstracts (31, 32).

METHODS

Preparation of Human Alveolar Macrophages

Bronchoalveolar lavage was performed on normal healthy donors and AMs were purified as previously described (33) (*see* online supplement for details). This study was approved by the ethics committee of Hamamatsu University School of Medicine, and informed consent was obtained.

Preparation of Human Peripheral Blood Monocytes

Peripheral blood mononuclear cells were isolated from heparinized whole blood of normal, healthy donors by density gradient centrifugation with Lymphoprep (Nycomed, Oslo, Norway). $CD14^+$ cells were separated by magnetic sorting with a MACS (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions (*see* online supplement for details).

Neutrophil Isolation and Induction of Apoptosis

Neutrophils were isolated from heparinized whole blood of normal, healthy donors by a combination of dextran sedimentation and centrifugation with Polymorphprep (Nycomed). The purity of neutrophils was greater than 98%, as determined by their morphology. The neutrophils were aged by culturing to undergo apoptosis (28) (*see* online supplement for details).

RNA Isolation and Complimentary DNA Synthesis

Total RNA was isolated from AMs and Mo by the acid guanidinium thiocyanate-phenol-chloroform extraction method (34) (*see* online supplement for details).

Polymerase Chain Reaction

We designed the primers for PPARγ and β-actin to span an intron of each genome. For the other receptors, we used the primers described in previous a previous report (35). The primer sequences were as follows: PPARα: 5′-TCATCAAGAAGACGGAGTCG-3′ and 5′-CGGTTACCTACAGCTCAGAC-3′, amplifying a 213-bp product; PPARδ: 5′-TCCCTCTTTCTCAGTTCCTC-3′ and 5′-CAGGAGACAGAAGTGAGGAC-3′, amplifying a 289-bp product; PPARγ: 5′-CAGCATTTCTACTCCACATT-3′ and 5′-ATCTCCACAGACACGACATT-3′, amplifying a 222-bp product; and β-actin: 5′-GGGCATGGGTCAGAAGGATT-3′ and 5′-GAGGCGTACAGGGATAGCAC-3′, amplifying a 302-bp product. Polymerase chain reaction amplification was performed in the DNA Thermal Cycler (Perkin-Elmer Cetus, Norwalk, CT) and the expressions of this messenger RNA (mRNA) were evaluated by densitometry (*see* online supplement for details).

Preparation of Cell Extracts and Western Blot Analysis

For Western blotting, a positive control was generated by transiently transfecting a PPARγ expression plasmid (7) into CV-1 cells using a calcium-phosphate precipitation method. Protein extracts of AMs, Mo, and trasfected CV-1 cells were prepared and the expression of PPARγ protein was assessed by Western blotting (*see* online supplement for details).

Measurement of TNF-α Production

AMs were seeded at a density of 1.5×10^5 cells/well in RPMI 1640 with 5% fetal calf serum into 24-well tissue culture plates. The cells were treated with 15d-PGJ_2 (Cayman, Ann Arbor, MI) or troglitazone, kindly provided by Sankyo (Tokyo, Japan), for 1 hour before the addition of 0.1 ng/ml LPS (Sigma, St. Louis, MO). After 24 hours incubation at 37°C in 5% CO_2, the supernatants were harvested. The concentrations of TNF-α were measured by enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN).

Measurement of CD36 Expression by Flow Cytometric Analysis

AMs were seeded at a density of 1×10^6 cells/ml in RPMI 1640 with 10% fetal calf serum into 12-well tissue culture plates. After a 6-day-incubation with 15d-PGJ_2 or troglitazone, the cells were harvested. They were incubated with fluorescein isothiocyanate–conjugated anti-CD36 (clone FA6.152, mouse IgG1; Immunotech, Marseille, France) monoclonal antibodies for 30 minutes on ice. After washing in PBS, the cells were analyzed with EPICS Profile-II (Beckman Coulter, Fullerton, CA). The expression of CD36 was evaluated as the percentage positive cells and the mean fluorescence intensity (MFI).

Phagocytosis Assay

Phagocytosis of apoptotic neutrophils by AMs was assessed using previously described methods (24, 36, 37) (*see* online supplement for details). AMs cultured in the presence of 15d-PGJ_2 for 6 days were pretreated with or without 50 μg/mL of anti-CD36 monoclonal antibody (Immunotech) for 30 minutes.

Statistical Analysis

Unpaired *t* test or one-way analysis of variance was used for comparisons of groups. p Values of less than 0.05 were considered significant. All data are expressed as mean ± SEM.

RESULTS

Expression of PPARγ mRNA and Protein in Human Ams

Using reverse transcription polymerase chain reaction, mRNA of each PPAR subtype (PPARα, PPARγ, and PPARδ) was detected in freshly isolated human AMs (Figure 1A). Compared with peripheral blood Mo, the relative intensity of PPARγ mRNA was 10-fold higher in AMs ($p < 0.01$), whereas no significant difference was found in the expression of PPARα or PPARδ between them (Figure 1B). Western blot analysis showed strong expression of PPARγ protein in AMs, whereas a weak signal was detected in Mo (Figure 1C).

PPARγ Ligands Inhibit LPS-induced TNF-α Production by Ams

To define the functional role of PPARγ in AMs, we first examined the effect of 15d-PGJ_2, a natural ligand of PPARγ, and troglitazone, antidiabetic thiazolidinedione, on cytokine production by AMs. We measured the TNF-α concentrations in culture supernatants of LPS-stimulated AMs after treatment with graded doses of 15d-PGJ_2 or troglitazone. LPS (0.1 ng/ml) elicited considerable amounts of TNF-α production by AMs. When adding these ligands to the cultures, the LPS-induced TNF-α production by AMs was significantly inhibited in a dose-dependent manner (Figure 2), indicating that the PPARγ activation reduced the production of TNF-α by AMs. In contrast, the same ligands failed to decrease LPS-induced TNF-α production by blood Mo (data not shown).

PPARγ Ligands Upregulate CD36 Expression by Ams

We tested the effect of 15d-PGJ_2 and troglitazone on the expression of CD36, which mediates phagocytosis of apoptotic neutrophils by AMs. Although freshly isolated AMs did not express CD36, the treatment with 15d-PGJ_2 or troglitazone readily induced CD36 expression, which increased in a time-dependent manner (Figure 3A). Six days after the treatment, more than 50% of cultured AMs became positive for CD36, while the control cultures failed to induce its expression (Figure 3B). In blood Mo, 15d-PGJ_2 or troglitazone did not significantly increase CD 36 expression (data not shown).

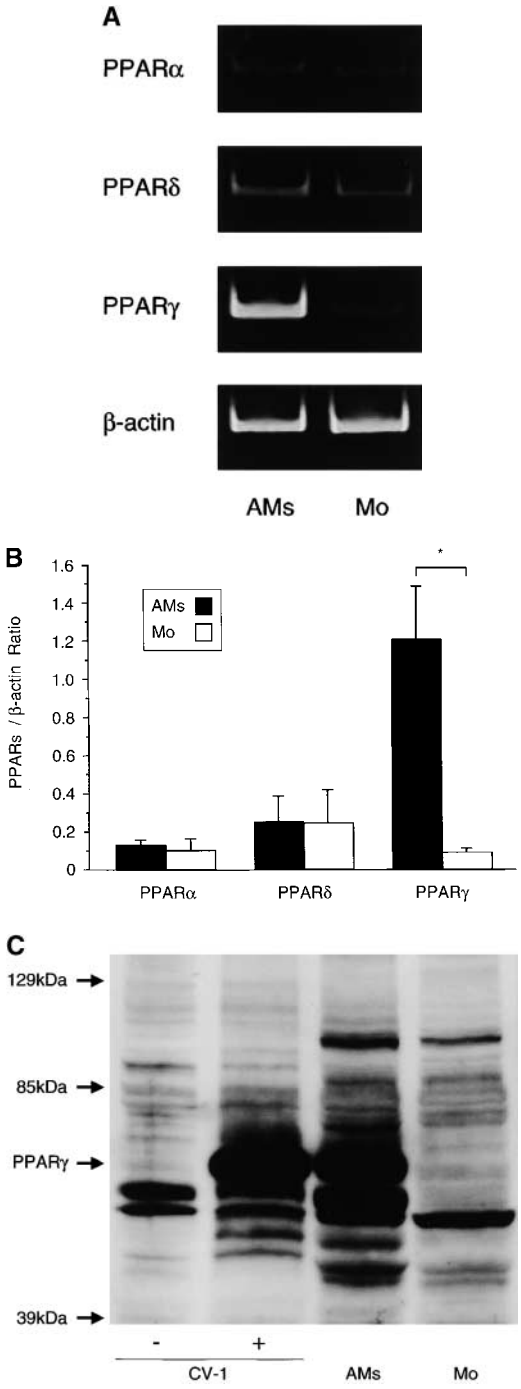

Figure 1. PPARγ messenger RNA (mRNA) and protein expression in human alveolar macrophages (AMs). (*A*) Reverse transcription polymerase chain reaction (RT-PCR) was performed on mRNA isolated from AMs and human peripheral blood monocytes (Mo). (*B*) PPARs mRNA expression was determined by semiquantitative RT-PCR. Results are shown as mean ± SEM of three independent experiments. *, $p < 0.01$. (*C*) Western blot analysis of PPARγ protein expression in AMs and Mo reveals a band of the appropriate size. *CV-1 (+)* and *CV-1 (−)* represent cell extracts from CV-1 cells transfected with PPARγ-expressing vector and control vector, respectively. The identity of this band is confirmed by comigration with a band seen in PPARγ-transfected CV-1 cells (+).

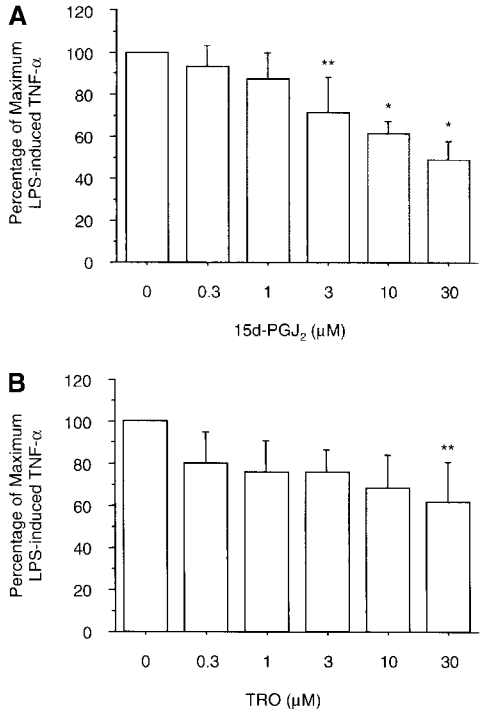

Figure 2. Effects of 15d-PGJ_2 or troglitazone (TRO) on LPS-induced tumor necrosis factor (TNF)-α production from AMs. AMs (1.5×10^5 cells/well) were plated in a 24-well culture plate. 15d-PGJ_2 or TRO was added to the wells, and after 60-minute incubation, LPS (0.1 ng/mL) was added. Cell media were harvested after 24 hours incubation. TNF-α levels were determined by ELISA. The data indicate the proportion of the TNF-α levels in cultures with vehicle. Results are shown as mean ± SEM of four independent experiments. *$p < 0.01$ and **$p < 0.05$ as compared with vehicle.

15d-PGJ_2 Enhanced Capacity of AMs to Phagocytose Apoptotic Neutrophils

To determine whether ligand activation of PPARγ augments phagocytic capacity of AMs, we performed an *in vitro* phagocytosis assay using apoptotic neutrophils. After culturing for 6 days in the presence of 15d-PGJ_2, AMs that had been induced to express CD36 were used for the phagocytosis assay. As shown in Figure 4A, we easily detected ingested neutrophils in AMs and assessed their phagocytic capacity by counting the numbers of ingested neutrophils. 15d-PGJ_2–treated AMs ingested a significantly higher number of apoptotic neutrophils than did untreated AMs ($p < 0.01$) (Figure 4B). Treatment of anti-CD36 antibody inhibited phagocytosis of 15d-PGJ_2–treated AMs ($p < 0.01$) (Figure 4B), suggesting that enhanced phagocytic capacity of these AMs by 15d-PGJ_2 is largely associated with the increase in their CD36 expression. Because phagocytosis of apoptotic neutrophils at inflammatory sites is a critical determinant for resolving inflammation, AMs stimulated by ligand for PPARγ may be actively involved in this process of the lung.

DISCUSSION

In the present study, we examined the expression of PPARγ by human AMs and its functional role in lung inflammation. We demonstrated for the first time that freshly isolated human AMs strongly expressed PPARγ mRNA and protein. Further, ligands of PPARγ reduced the LPS-induced TNF-α production and enhanced the CD36 expression by AMs together with an increase

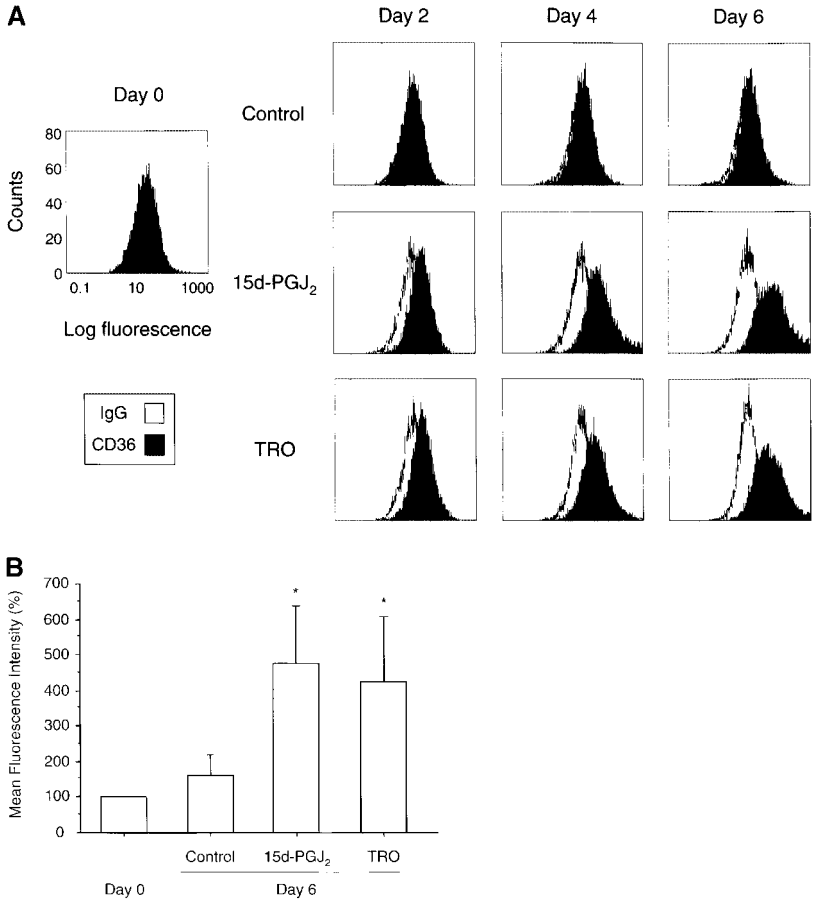

Figure 3. Effects of 15d-PGJ_2 or TRO on CD36 expression by AMs. AMs were incubated in the presence of 15d-PGJ_2 (10 μM) or TRO (10 μM) for 6 days. Expression of CD36 was determined by flow cytometry using an anti–human CD36 antibody. (*A*) Fluorescence distribution of the counted cell population is presented from a representative experiment out of four. The numbers indicate the percentage of CD36-positive cells. (*B*) Mean fluorescence intensity (MFI) of the counted cell population is presented. The data indicate the proportion of the MFI at freshly isolated AMs. Results are shown as mean $\pm$ SEM of three or four independent experiments. *$p < 0.05$ as compared with control.

in their capacity to phagocytose apoptotic neutrophils. These data suggest that PPARγ in AMs has an antiinflammatory role.

Evidence has been accumulated indicating that PPARγ plays a role in regulating inflammation. In Mo, PPARγ ligands suppress the expression of TNF-α, interleukin-6, and interleukin-1β (19), whereas in macrophages they inhibit the expression of inducible nitric oxide synthase, matrix metalloprotease-9, and scavenger receptor-A (18). Several studies have demonstrated that an administration of PPARγ ligands *in vivo* inhibited adjuvant-induced arthritis (38), colitis (39), and atherosclerosis (40) in animal models. These findings have raised the possibility that ligands for PPARγ might be useful for the treatment of inflammatory disorders. However, PPARγ expression and its function in resident tissue macrophages, particularly human AMs, still remain unclear. In the present study, we first determined PPARγ expression by human AMs. Using reverse transcription polymerase chain reaction and Western blotting, we demonstrated the expression of PPARs mRNA and protein in freshly isolated AMs. mRNA expression of PPARγ, but not of PPARα or δ, by AMs was much higher than that by peripheral blood Mo. Moreover, the expression of PPARγ protein was much stronger in AMs than Mo. Although the mechanisms of the strong expression of PPARγ in AMs are not clear, several studies have shown that this expression increased during the differentiation in monocyte/macrophage lineage cells (20). For example, PPARγ expression was shown to be upregulated during the differentiation of bone marrow progenitor cells in response to macrophage colony-stimulating factor and granulocyte/macrophage colony stimulating factor (41). Because AMs are highly differentiated macrophages that are specifically adapted to the particular environment of the lung, this may be attributable to their enhanced expression of PPARγ.

To elucidate the functional role of PPARγ in human AMs, we next examined the effect of its ligand on cytokine production by AMs. We found that 15d-PGJ_2 and troglitazone, the specific ligands of PPARγ, markedly decreased the LPS-induced TNF-α production by AMs in a dose-dependent manner. Although the precise mechanisms by which PPARγ ligands exert antiinflammatory effects are poorly understood, recent studies have revealed that PPARγ inhibits the expression of inflammatory genes by the interference with nuclear factor κB (NF-κB), activator protein-1, and signal transducers and activators of transcription-1 (18, 22, 42, 43). For example, PPARγ was shown to suppress NF-κB–mediated activation in interleukin-12 p40 promoter through both inhibition of the NF-κB–DNA binding and physical interactions between NF-κB and PPARγ (21). Because TNF-α promoter activity occurs by stimulation of the NF-κB signaling pathway (44), the inhibition of TNF-α production by the ligands of PPARγ is likely due to antagonizing NF-κB activity in AMs. However, further studies will be required to clarify the precise molecular mechanisms of PPARγ-mediated inhibition of cytokine production by AMs. A variety of inhaled microbes and particles can potentially induce inflammation in the lung, resulting in tissue damage. To limit this inflammation, AMs have been proposed to play an important role in maintaining immunologic homeostasis in the lung via their immunosuppressive activity (45, 46). In this context, it seems to be rational that AMs express high levels of functional PPARγ protein.

Finally, we determined whether PPARγ activation increased CD36 expression by human AMs, and consequently augmented their phagocytic capacity for apoptotic neutrophils. Although freshly isolated AMs did not express CD36, treatment with the PPARγ ligands readily induced its expression, which increased

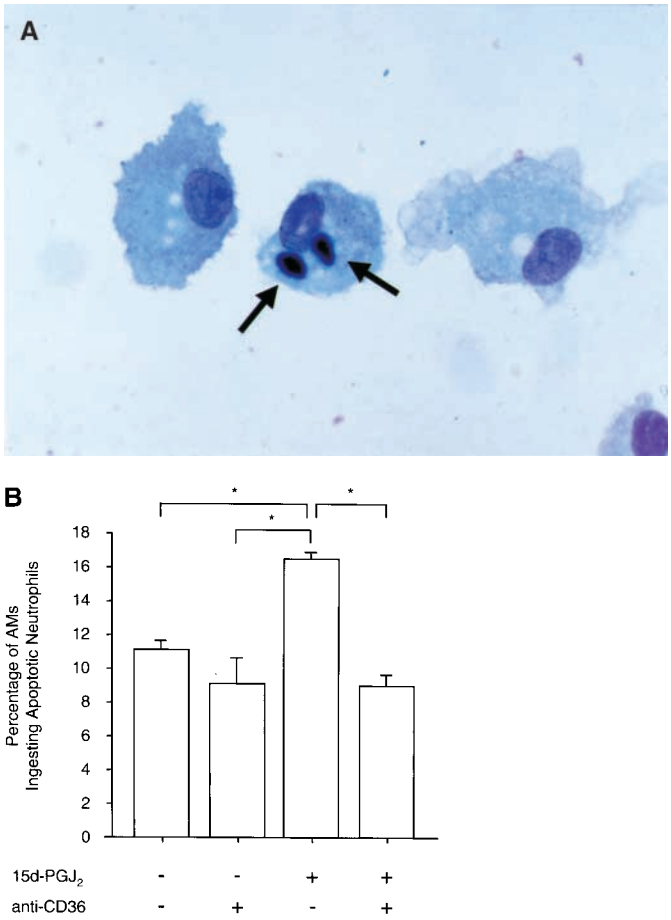

Figure 4. Phagocytosis of apoptotic neutrophils by AMs. AMs were incubated in the presence of 15d-PGJ_2 (10 μM) for 6 days, washed, and cocultured with a suspension of aged neutrophils for 60 minutes. (*A*)(original magnification: ×1000) is a representative micrograph from one experiment that shows phagocytosis of apoptotic neutrophil by AMs. (*A*) Phagocytosed apoptotic neutrophils (*arrow*) are found in the cytoplasm of AMs. Phagocytic assay was done as described in METHODS. (*B*) 15d-PGJ_2 increases the percentage of AMs ingesting apoptotic neutrophils. This increase is blocked by the treatment of anti-CD36 antibody. AMs were pretreated with or without 50 μg/ml of anti-CD36 antibody for 30 minutes. Results are shown as mean ± SEM of three or five independent experiments.

in a time-dependent manner. The human CD36 gene contains a PPARγ-responsive element, which was shown to be functional in Mo/macrophages (23, 47). Thus, the PPARγ ligand likely activated the transcription of the CD36 gene in AMs. Recently, it has been reported that CD36 also functions as a receptor for apoptotic neutrophils as well as *Plasmodium falciparum*–parasitized erythrocytes (48, 49), thereby mediating their phagocytosis. Indeed, CD36 was shown to be required for phagocytosis of apoptotic neutrophils by human blood monocyte–derived macrophages that cooperate with either a phosphatidylserine, vitronectin receptor ($\alpha_v\beta_3$), or thrombospondin (24, 26, 27). In inflammation, removal of infiltrating cells that undergo apoptosis is thought to be an essential process for its resolution (4, 29). Of the cells involved in this process, macrophages are the most prominent. In the present study, we found that human AMs that had been induced to express CD36 after treatment with 15d-PGJ_2 showed enhanced phagocytosis of apoptotic neutrophils. The enhancement of phagocytic capacity is likely to be mediated by increased CD36 expression because anti-CD36 antibody abrogated this enhancement. Collectively, our data suggest that PPARγ activation in AMs promotes the resolution of lung inflammation by their enhanced phagocytic capacity for apoptotic neutrophils.

15d-PGJ_2 is a naturally occurring prostaglandin D_2 metabolite that has been identified as a ligand for PPARγ (14, 15). Interestingly, a recent study by Gilroy and colleagues demonstrated an increased local production of 15d-PGJ_2 in acute lung inflammation (50). They measured the exudate levels of prostaglandins, such as PGE_2, PGD_2 (precursor of prostaglandin J series), and 15d-PGJ_2, and showed that PGD_2 and 15d-PGJ_2 increased at both initial and, to a greater extent, late phases of lung inflammation, while PGE_2 was produced only at the initial phase. These data suggested that the profiles of prostaglandins produced in the inflammation may change from PGE_2-dominant profile to PGD_2- and 15d-PGJ_2–dominant profile during the resolution of inflammation. Based on our findings, locally generated 15d-PGJ_2 during the late phase of lung inflammation could activate PPARγ in AMs, which in turn reduces their cytokine production and increase their CD36 expression together with an enhancement of their phagocytic capacity.

In summary, we demonstrated that human AMs strongly expressed PPARγ, of which activation led to the inhibition of LPS-induced TNF-α production and the induction of CD36 expression by the AMs, thereby enhancing their phagocytosis of apoptotic neutrophils. These data indicate an antiinflammatory role for PPARγ expressed by AMs in the lung, suggesting a potential therapeutic application of its ligand in inflammatory disorders of the lung.

Conflict of Interest Statement: K.A. has no declared conflict of interest; S.S. has no declared conflict of interest; T.S. has no declared conflict of interest; K.C. has no declared conflict of interest; H.N. has no declared conflict of interest.

References

1. Parod RJ, Brain JD. Immune opsonin-independent phagocytosis by pulmonary macrophages. *J Immunol* 1986;136:2041–2047.
2. Sibille Y, Reynolds HY. Macrophages and polymorphonuclear neutrophils in lung defense and injury. *Am Rev Respir Dis* 1990;141:471–501.
3. Haslett C. Granulocyte apoptosis and its role in the resolution and control of lung inflammation. *Am J Respir Crit Care Med* 1999;160:S5–S11.
4. Cox G, Crossley J, Xing Z. Macrophage engulfment of apoptotic neutrophils contributes to the resolution of acute pulmonary inflammation *in vivo*. *Am J Respir Cell Mol Biol* 1995;12:232–237.
5. Kliewer SA, Umesono K, Noonan DJ, Heyman RA, Evans RM. Convergence of 9-cis retinoic acid and peroxisome proliferator signaling pathways through heterodimer formation of their receptors. *Nature* 1992; 358:771–774.
6. Bardot O, Aldridge TC, Latruffe N, Green S. PPAR-RXR heterodimer activates a peroxisome proliferator response element upstream of the bifunctional enzyme gene. *Biochem Biophys Res Commun* 1993;192: 37–45.
7. Kliewer SA, Forman BM, Blumberg B, Ong ES, Borgmeyer U, Mangelsdorf DJ, Umesono K, Evans RM. Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. *Proc Natl Acad Sci USA* 1994;91:7355–7359.
8. Braissant O, Foufelle F, Scotto C, Dauca M, Wahli W. Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-α, -β, and -γ in the adult rat. *Endocrinology* 1996;137:354–366.
9. Chawla A, Schwarz EJ, Dimaculangan DD, Lazar MA. Peroxisome proliferator-activated receptor (PPAR) γ: adipose-predominant expression and induction early in adipocyte differentiation. *Endocrinology* 1994;135:798–800.
10. Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPAR γ). *J Biol Chem* 1995;270:12953–12956.
11. Willson TM, Cobb JE, Cowan DJ, Wiethe RW, Correa ID, Prakash SR, Beck KD, Moore LB, Kliewer SA, Lehmann JM. The structure–activity relationship between peroxisome proliferator–activated receptor γ agonism and the antihyperglycemic activity of thiazolidinediones. *J Med Chem* 1996;39:665–668.
12. Kliewer SA, Sundseth SS, Jones SA, Brown PJ, Wisely GB, Koble CS,

Devchand P, Wahli W, Willson TM, Lenhard JM, *et al.* Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors α and γ. *Proc Natl Acad Sci USA* 1997;94:4318–4323.
13. Krey G, Braissant O, L'Horset F, Kalkhoven E, Perroud M, Parker MG, Wahli W. Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay. *Mol Endocrinol* 1997;11:779–791.
14. Kliewer SA, Lenhard JM, Willson TM, Patel I, Morris DC, Lehmann JM. A prostaglandin J_2 metabolite binds peroxisome proliferator-activated receptor γ and promotes adipocyte differentiation. *Cell* 1995;83:813–819.
15. Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM, Evans RM. 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 is a ligand for the adipocyte determination factor PPAR γ. *Cell* 1995;83:803–812.
16. Nagy L, Tontonoz P, Alvarez JGA, Chen H, Evans RM. Oxidized LDL regulates macrophage gene expression through ligand activation of PPARγ. *Cell* 1998;93:229–240.
17. Greene ME, Blumberg B, McBride OW, Yi HF, Kronquist K, Kwan K, Hsieh L, Greene G, Nimer SD. Isolation of the human peroxisome proliferator activated receptor gamma cDNA: expression in hematopoietic cells and chromosomal mapping. *Gene Expr* 1995;4:281–299.
18. Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation. *Nature* 1998;391:79–82.
19. Jiang C, Ting AT, Seed B. PPAR-γ agonists inhibit production of monocyte inflammatory cytokines. *Nature* 1998;391:82–86.
20. Chinetti G, Griglio S, Antonucci M, Torra IP, Delerive P, Majd Z, Fruchart JC, Chapman J, Najib J, Staels B. Activation of proliferator-activated receptors α and γ induces apoptosis of human monocyte-derived macrophages. *J Biol Chem* 1998;273:25573–25580.
21. Chung SW, Kang BY, Kim SH, Pak YK, Cho D, Trinchieri G, Kim TS. Oxidized low-density lipoprotein inhibits interleukin-12 production in lipopolysaccharide-activated mouse macrophages via direct interactions between peroxisome proliferator–activated receptor-γ and nuclear factor-κB. *J Biol Chem* 2000;275:32681–32687.
22. Li M, Pascual G, Glass CK. Peroxisome proliferator–activated receptor γ–dependent repression of the inducible nitric oxide synthase gene. *Mol Cell Biol* 2000;20:4699–4707.
23. Tontonoz P, Nagy L, Alvarez JGA, Thomazy VA, Evans RM. PPARγ promotes monocyte/macrophage differentiation and uptake of oxidized LDL. *Cell* 1998;93:241–252.
24. Savill J, Hogg N, Ren Y, Haslett C. Thrombospondin cooperates with CD36 and the vitronectin receptor in macrophage recognition of neutrophils undergoing apoptosis. *J Clin Invest* 1992;90:1513–1522.
25. Bird DA, Gillotte KL, Horkko S, Friedman P, Dennis EA, Witztum JL, Steinberg D. Receptors for oxidized low-density lipoprotein on elicited mouse peritoneal macrophages can recognize both the modified lipid moieties and the modified protein moieties: implications with respect to macrophage recognition of apoptotic cells. *Proc Natl Acad Sci USA* 1999;96:6347–6352.
26. Fadok VA, Warner ML, Bratton DL, Henson PM. CD36 is required for phagocytosis of apoptotic cells by human macrophages that use either a phosphatidylserine receptor or the vitronectin receptor ($\alpha_v\beta_3$). *J Immunol* 1998;161:6250–6257.
27. Godson C, Mitchell S, Harvey K, Petasis NA, Hogg N, Brady HR. Cutting edge: lipoxins rapidly stimulate nonphlogistic phagocytosis of apoptotic neutrophils by monocyte-derived macrophages. *J Immunol* 2000;164:1663–1667.
28. Doherty DE, Downey GP, Worthen GS, Haslett C, Henson PM. Monocyte retention and migration in pulmonary inflammation: requirement for neutrophils. *Lab Invest* 1988;59:200–213.
29. Savill JS, Wyllie AH, Henson JH, Walport MJ, Henson PM, Haslett C. Macrophage phagocytosis of aging neutrophils in inflammation: programmed cell death in the neutrophil leads to its recognition by macrophages. *J Clin Invest* 1989;83:865–875.
30. Taylor ML, Noble PW, White B, Wise R, Liu MC, Bochner BS. Extensive surface phenotyping of alveolar macrophages in interstitial lung disease. *Clin Immunol* 2000;94:33–41.
31. Asada S, Sasaki S, Inui N, Ide K, Todate A, Suda T, Chida K, Nakamura H. Enhanced expression of peroxisome proliferator–activated receptor gamma mRNA in alveolar macrophages [abstract]. *Am J Respir Crit Care Med* 2000;161:A770.
32. Asada K, Sasaki S, Inui N, Yokomura K, Suda T, Chida K, Nakamura H. Peroxisome proliferator–activated receptor γ agonists inhibit lipopolysaccharide-induced tumor necrosis factor α production in human alveolar macrophages [abstract]. *Am J Respir Crit Care Med* 2001; 163(Suppl.):A789.
33. Suda T, Sato A, Ida M, Gemma H, Hayakawa H, Chida K. Hypersensitivity pneumonitis associated with home ultrasonic humidifiers. *Chest* 1995; 107:711–717.
34. Ide K, Hayakawa H, Yagi T, Sato A, Koide Y, Yoshida A, Uchijima M, Suda T, Chida K, Nakamura H. Decreased expression of Th2 type cytokine mRNA contributes to the lack of allergic bronchial inflammation in aged rats. *J Immunol* 1999;163:396–402.
35. Rivier M, Safonova I, Lebrun P, Griffiths CEM, Ailhaud G, Michel S. Differential expression of peroxisome proliferator–activated receptor subtypes during the differentiation of human keratinocytes. *J Invest Dermatol* 1998;111:1116–1121.
36. Schagat TL, Wofford JA, Wright JR. Surfactant protein A enhances alveolar macrophage phagocytosis of apoptotic neutrophils. *J Immunol* 2001;166:2727–2733.
37. Morimoto K, Amano H, Sonoda F, Baba M, Senba M, Yoshimine H, Yamamoto H, Ii T, Oishi K, Nagatake T. Alveolar macrophages that phagocytose apoptotic neutrophils produce hepatocyte growth factor during bacterial pneumonia in mice. *Am J Respir Cell Mol Biol* 2001; 24:608–615.
38. Kawahito Y, Kondo M, Tsubouchi Y, Hashiramoto A, Bishop-Bailey D, Inoue K, Kohno M, Yamada R, Hla T, Sano H. 15-deoxy-$\Delta^{12,14}$-PGJ_2 induces synoviocyte apoptosis and suppresses adjuvant-induced arthritis in rats. *J Clin Invest* 2000;106:189–197.
39. Su CG, Wen X, Bailey ST, Jiang W, Rangwala SM, Keilbaugh SA, Flanigan A, Murthy S, Lazar MA, Wu GD. A novel therapy for colitis utilizing PPAR-γ ligands to inhibit the epithelial inflammatory response. *J Clin Invest* 1999;104:383–389.
40. Li AC, Brown KK, Silvestre MJ, Willson TM, Palinski W, Glass CK. Peroxisome proliferator–activated receptor γ ligands inhibit development of atherosclerosis in LDL receptor-deficient mice. *J Clin Invest* 2000;106:523–531.
41. Ricote M, Huang J, Fajas L, Li A, Welch J, Najib J, Witztum JL, Auwerx J, Palinski W, Glass CK. Expression of the peroxisome proliferator–activated receptor γ (PPARγ) in human atherosclerosis and regulation in macrophages by colony stimulating factors and oxidized low density lipoprotein. *Proc Natl Acad Sci USA* 1998;95:7614–7619.
42. Uchimura K, Nakamuta M, Enjoji M, Irie T, Sugimoto R, Muta T, Iwamoto H, Nawata H. Activation of retinoic X receptor and peroxisome proliferator–activated receptor-γ inhibits nitric oxide and tumor necrosis factor-α production in rat Kupffer cells. *Hepatology* 2001;33: 91–99.
43. Takano H, Nagai T, Asakawa M, Toyozaki T, Oka T, Komuro I, Saito T, Masuda Y. Peroxisome proliferator–activated receptor activators inhibit lipopolysaccharide-induced tumor necrosis factor-α expression in neonatal rat cardiac myocytes. *Circ Res* 2000;87:596–602.
44. Swantek JL, Christerson L, Cobb MH. Lipopolysaccharide-induced tumor necrosis factor-α promoter activity is inhibitor of nuclear factor-κB kinase-dependent. *J Biol Chem* 1999;274:11667–11671.
45. Holt PG. Alveolar macrophages: II. Inhibition of lymphocyte proliferation by purified macrophages from the rat lung. *Immunology* 1979;37: 429–436.
46. Bilyk N, Holt PG. Inhibition of the immunosuppressive activity of resident pulmonary alveolar macrophages by granulocyte/macrophage colony-stimulating factor. *J Exp Med* 1993;177:1773–1777
47. Chawla A, Barak Y, Nagy L, Liao D, Tontonoz P, Evans RM. PPAR-γ dependent and independent effects on macrophage-gene expression in lipid metabolism and inflammation. *Nat Med* 2001;7:48–52.
48. McGilvray ID, Serghides L, Kapus A, Rotstein OD, Kain KC. Nonopsonic monocyte/macrophage phagocytosis of *Plasmodium falciparum*–parasitized erythrocytes: a role for CD36 in malarial clearance. *Blood* 2000;96:3231–3240.
49. Serghides L, Kain KC. Peroxisome proliferator–activated receptor γ–retinoid X receptor agonists increase CD36-dependent phagocytosis of *Plasmodium falciparum*–parasitized erythrocytes and decrease malaria-induced TNF-α secretion by monocytes/macrophages. *J Immunol* 2001;166:6742–6748.
50. Gilroy DW, Colville-Nash PR, Willis D, Chivers J, Paul-Clark MJ, Willoughby DA. Inducible cyclooxygenase may have anti-inflammatory properties. *Nat Med* 1999;5:698–701.