# Peroxisome Proliferator-Activated Receptor-γ Regulates the Expression of Alveolar Macrophage Macrophage Colony-Stimulating Factor

**Tracey L. Bonfield,[1]*† Mary Jane Thomassen,‡ Carol F. Farver,† Susamma Abraham,† Mary T. Koloze,* Xia Zhang,§ David M. Mosser,§ and Daniel A. Culver†**

**Macrophage CSF (M-CSF) regulates monocyte differentiation, activation, and foam cell formation. We have observed that it is elevated in human pulmonary alveolar proteinosis (PAP) and in the GM-CSF knockout mouse, a murine model for PAP. A potential regulator of M-CSF, peroxisome proliferator-activated receptor-γ (PPARγ), is severely deficient in both human PAP and the GM-CSF knockout mouse. To investigate the role of PPARγ in alveolar macrophage homeostasis, we generated myeloid-specific PPARγ knockout mice using the Lys-Cre method to knock out the floxed PPARγ gene. Similar to the GM-CSF-deficient mouse, absence of alveolar macrophage PPARγ resulted in development of lung pathology resembling PAP in 16-wk-old mice, along with excess M-CSF gene expression and secretion. In ex vivo wild-type alveolar macrophages, we observed that M-CSF itself is capable of inducing foam cell formation similar to that seen in PAP. Overexpression of PPARγ prevented LPS-stimulated M-CSF production in RAW 264.7 cells, an effect that was abrogated by a specific PPARγ antagonist, GW9662. Use of proteasome inhibitor, MG-132 or a PPARγ agonist, pioglitazone, prevented LPS-mediated M-CSF induction. Using chromatin immunoprecipitation, we found that PPARγ is capable of regulating M-CSF through transrepression of NF-κB binding at the promoter. Gel-shift assay experiments confirmed that pioglitazone is capable of blocking NF-κB binding. Taken together, these data suggest that M-CSF is an important mediator of alveolar macrophage homeostasis, and that transcriptional control of M-CSF production is regulated by NF-κB and PPARγ.** *The Journal of Immunology,* **2008, 181: 235–242.**

Peroxisome proliferator-activated receptor-γ (PPARγ)[2] is a member of the ligand-activated nuclear hormone receptor family that can function as a transcription factor (1). PPARs have been reported in the regulation of genes involved in lipid and glucose metabolism as well as inflammation (2, 3). Although weakly expressed in monocytes, PPARγ is up-regulated during differentiation into macrophages and is predominantly nuclear in location (4). Our studies are the first to describe the constitutive expression of PPARγ in healthy human alveolar macrophages and the striking deficiency of PPARγ in pulmonary alveolar proteinosis (PAP).

Alveolar proteinosis is an autoimmune disease in which neutralizing autoantibodies to GM-CSF result in a deficiency of bioactive GM-CSF in the lung (5). Intraalveolar accumulation of periodic acid-Schiff (PAS)-positive lipoproteinaceous material inhibits gas exchange in the lungs of patients with PAP (6). The deficiency of PPARγ can be reversed in vitro by GM-CSF and therapeutically in disease by treating with exogenous GM-CSF (7). Alveolar macrophages obtained from the GM-CSF knockout (KO) mouse, the animal model of PAP, are also deficient in PPARγ.

Monocytes recruited into the lungs are thought to mature into alveolar macrophages in a process dependent upon GM-CSF (8, 9). GM-CSF and macrophage CSF (M-CSF) are CSFs associated with the regulation of myeloid differentiation (10–13). The lack of GM-CSF has been thought to be a trigger for increased production of M-CSF as a compensatory mechanism, although this issue remains incompletely defined (14, 15). In support of this, we and others have observed that both human PAP and the GM-CSF KO mice overproduce M-CSF (5, 16). Furthermore, therapeutic administration of biologically active GM-CSF restores PPARγ levels and decreases M-CSF in human PAP (17, 18).

GM-CSF has been implicated in the classical activation of macrophages resulting in the up-regulation of MHC class II molecules and CD86, whereas M-CSF has been associated with alternative macrophage activation (19). Alternative macrophage activation by M-CSF has been linked to the induction of IL-10 and matrix metalloproteinases (19–21), both of which are elevated in PAP (17, 22). M-CSF has also been associated with enhanced foam cell formation in vitro (23–25). These observations suggest that M-CSF may play a role in the accumulation of lipids in PAP alveolar macrophages and in the GM-CSF KO model. We hypothesized that deficient PPARγ results in the up-regulation of alveolar macrophage M-CSF, and excess M-CSF contributes ultimately to the accumulation of foam cells. We demonstrate herein that PPARγ can regulate M-CSF production through transrepression of NF-κB. Furthermore, because GM-CSF is required for alveolar macrophage

*Department of Pediatrics, Case Western Reserve University, Cleveland, OH 44109; †Departments of Pulmonary Allergy and Critical Care, Pathobiology and Anatomic Pathology Cleveland Clinic, Cleveland, OH 44195; ‡Program in Lung Cell Biology and Translational Research, Division of Pulmonary and Critical Care Medicine, East Carolina University, Greenville, NC 27858; and §Department of Cellular and Molecular Biology, University of Maryland, College Park, MD 20742

Received for publication June 12, 2007. Accepted for publication April 16, 2008.

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.

[1] Address correspondence and reprint requests to Dr. Tracey L. Bonfield, Department of Pediatrics, Case Western Reserve University, 10900 Euclid Avenue, BRB-822, Cleveland, OH 44109. E-mail address: tracey.bonfield@case.edu

[2] Abbreviations used in this paper: PPARγ, peroxisome proliferator-activated receptor-γ; BAL, bronchoalveolar lavage; ChIP, chromatin immunoprecipitation; KO, knockout; M-CSF, macrophage CSF; PAS, periodic acid-Schiff; PAP, pulmonary alveolar proteinosis; PPRE, peroxisome proliferator response element.

Copyright © 2008 by The American Association of Immunologists, Inc. 0022-1767/08/$2.00

PPARγ expression, these data link the absence of GM-CSF with the accumulation of M-CSF in alveolar proteinosis.

## Materials and Methods

### *Mice*

*Conditional PPARγ KO mice.* Homozygous floxed (+/+) PPARγ mice were generously provided by Dr. F. J. Gonzalez (26). To achieve loss of PPARγ in macrophages, homozygous floxed PPARγ mice were crossed into a transgenic mouse containing the CRE gene under the control of the murine M lysozyme promoter (27). CRE-M lysozyme (+/+) mice were obtained from the Jackson Laboratory. Thus, control mice for these experiments consist of: floxed (+/+), CRE (−/−) mice (1) and floxed (−/−), CRE (+/+) mice (2).

*GM-CSF KO mice.* This mouse strain was generated by G. Dranoff et al. (28). The mice have been backcrossed eight generations to C57BL/6. C57BL/6 wild-type mice obtained from The Jackson Laboratory were used as controls.

### *Murine cell source*

*Alveolar macrophages.* Mice were injected subcutaneously with a lethal dose of ketamine (80 mg/kg) and xylazine (10 mg/kg). Bronchoalveolar lavage (BAL) was performed by inserting a cannula through a cut in the trachea into the bronchi and infusing 10 × 1-ml aliquots of warm PBS containing 0.2% lidocaine. The BAL fluid sample is recovered by aspirating the liquid with a syringe; we recovered 80 ± 23% of instilled fluid volume. Macrophages were harvested and cultured overnight before experimentation.

*Resident peritoneal macrophages.* Mice were injected with a lethal dose of ketamine and xylazine and cells recovered by lavage. Macrophages were harvested and cultured overnight before experimentation.

*Bone marrow-derived macrophages.* BALB/c mice were used for bone marrow-derived macrophage studies. The femur and tibia were removed and wiped with a sterile alcohol tissue (29, 30). The bones were then put into RPMI containing antibiotics and, using a sterile 1 cc syringe, fresh RPMI was pushed through the bone marrow cavity. The bone marrow cells were counted with differentials and viability stains, followed by culture for 6–7 days with L929 support medium.

*Lung pathology.* Animal models and age-matched controls were euthanized; lungs were snap frozen for lipid stains.

### *Human BAL-derived macrophages*

Alveolar macrophages were derived from BAL obtained by flexible bronchoscopy as previously described (31). Differential cell counts were obtained from cytospins stained with a modified Wright's stain. All differential cell counts represented at least 200 cells. Mean viability of lavage cells was >95% by trypan blue dye exclusion.

### *Histological staining*

Alveolar, bone marrow-derived, and peritoneal macrophages were isolated from 4–6-wk-old mice. Macrophages were stained with Oil-Red-O for neutral lipids (27, 32). Alveolar macrophages from 6-wk GM-CSF KO were used as positive controls. Cells were fixed for 5 min in 1% paraformaldehyde in PBS. A stock solution of Oil-Red-O was diluted in methanol and filtered. Cells were incubated for 30 min in Oil-Red-O staining solution and then destained in 100% methanol for 5 min. The number of cells staining red in 10 high power fields (×40) was determined and expressed as percentage Oil-Red-O positive.

### *Cell culture conditions*

Cells were cultured in 6-well, 12-well, or 24-well plates, depending on the experiment, at consistent cell densities. Primary cells were allowed to adjust to culture overnight before the addition of stimuli: LPS (50–500 ng/ml), M-CSF (100 ng/ml), irreversible PPARγ antagonist GW9662 (10 μM; Sigma-Aldrich), and/or PPARγ agonist pioglitazone (10 μM, Takeda Chemical Industries). A proteasome inhibitor, 6-amino-4-(4-phenoxyphenylethylamino) quinazoline (Calbiochem), was used to block NF-κB activation. Cells were harvested for either extracts for protein-DNA interactions, activated transcription factors, or mRNA analysis. Supernatants were harvested for secreted M-CSF.

### *Transfection experiments*

PPARγ expression vector was generously supplied by Todd Leff (Wayne State University). Two micrograms of CMV-β-gal (Jahar Haque, Cleveland Clinic Foundation) or pTR100 expression vector was transfected into $2 \times 10^6$ RAW 264.7 cells in a 6-well plate using calcium phosphate precipitation method as previously described (33) Transfection efficiency was verified with cotransfection with GFP expression vector (Invitrogen).

Table I. *Primers for TaqMan of M-CSF promoter*

| Murine M-CSF Promoter | |
|---|---|
| mCSF-PPRE-F | 5′-gtgaagtgaaatggggtgct-3′ |
| mCSF-PPRE-R | 5′-gtgattacgtttagaaccactgaa-3′ |
| mCSF-NFκB-300F | 5′-tccccattcaagtcctgttc-3′ |
| mCSF-NFκB-300R | 5′-tgtgcagcctcctcaaagta-3′ |
| mCSF-NFκB-50F | 5′-gggcctctggggtgtagtat-3′ |
| mCSF-NFκB-50R | 5′-ccgaggcaaactttcacttt-3′ |

### *RNA purification and analysis*

Bone marrow, peritoneal, and alveolar macrophages from GM-CSF and PPARγ KO mice were cultured for 24 h in OptiMEM (Life Technologies/Invitrogen) before incubation with or without M-CSF stimulants (PMA, TNF), with concentration and duration being as prescribed by the initial experiment. Total RNA was extracted by RNAeasy protocol (Qiagen). Expression of mRNA was determined by real time RT-PCR as previously described (7).

### *M-CSF protein*

Murine and human ELISA assays were done according to manufacturers specifications (BioSource International).

### *Electophorectic mobility shift assay*

The assay was performed as previously described (34). In brief, RAW 264.7 cells were cultured with LPS (50 ng/ml) or pioglitazone (10 μM) for 3 h. Supershifts were performed with anti-p65 and anti-p50 (Santa Cruz Biotechnology) added before the addition of the probe. The specificity of the probe was shown by incubating the extract with a 1000-fold molar excess of cold oligonucleotide. StormImager (Molecular Dynamics) and ImageQuant software were used for quantification of autoradiographs. The sequence of the NF-κB binding site was 5′-AACTCCGGGAATTTCCCTGGCCC-3′.

### *Chromatin immunoprecipitation (ChIP) assays*

ChIP assays were performed following the instructions of the ChIP assay kit (Upstate Biotechnology) and the protocol outlined previously (29, 35–38). Briefly, using the reagents provided in the chromatin precipitation kit, we immunoprecipitated protein-DNA complex Abs specific for PPARγ (sc-7273, Santa Cruz Biotechnology), p65 (sc-8008), or p50 (sc-114)) after bone marrow-derived macrophages were cultured in the presence and absence of pioglitazone (10 μM), LPS (500 ng/ml), or GW9662 (5 μM). Input DNA was normalized by OD (39). The presence of associated PPARγ, p65, or c-Rel was measured by real-time PCR with the ABI Prism 7700 sequence detection system using SYBR Green PCR reagents (Applied Biosystems). PCR was done with nested primers to ensure specificity, and agarose gel analyses were performed after PCR amplification to ensure that a single product with expected size was amplified. The M-CSF promoter (40–44), peroxisome proliferator response element (PPRE), and NF-κB primers are shown in Table I.

### *Statistical analysis*

Analysis was performed using Prism GraphPad software version 3.0. Comparisons were performed using Student's *t* test or Mann-Whitney *U* test, as appropriate. For multiple comparisons, repeated measures ANOVA and Dunnett's test were used.

## Results

### *Alveolar macrophage phenotype is similar in PPARγ KO and GM-CSF KO mice*

To determine the impact of the PPARγ deficiency on PAP-like lung disease, we compared lung histopathology from 16-wk-old PPARγ and GM-CSF KO mice after staining with PAS (Fig. 1).

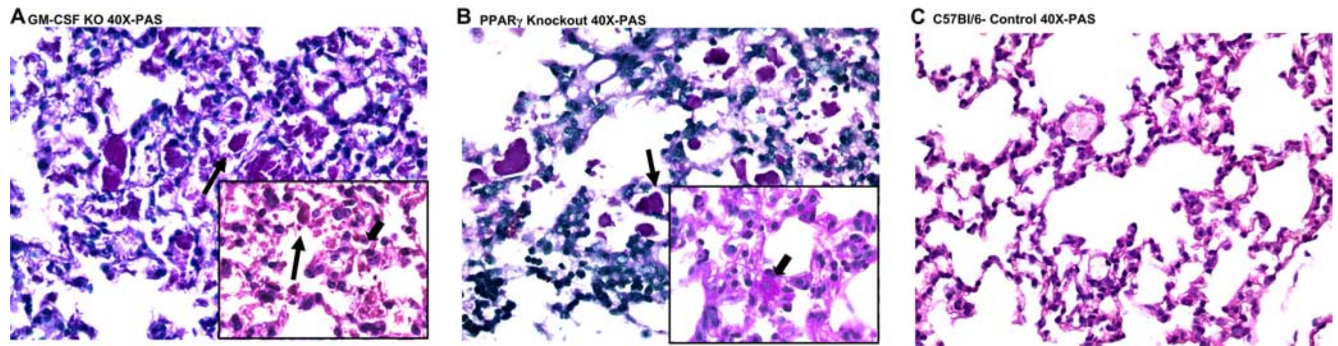


**FIGURE 1.** PPARγ knockout alveolar macrophage Pathology resembles the GM-CSF KO model. GM-CSF KO (*A*), PPARγ KO (*B*), and C57BL/6 control (*C*) mice were sacrificed, and the lungs stained with periodic-acid Schiff (×40). The GM-CSF KO model demonstrates scattered foci of intraalveolar PAS-positive material (thick arrows, *A*) and lipid-engorged alveolar macrophages (thick arrow, ×100 *inset*). All of the PPARγ KO mice (7 of 7 mice) exhibited similar PAS-positive material in alveolar macrophages (*B*, ×100 *inset*). Similar to the GM-CSF KO animals, several of these mice (2 of 7) also developed intraalveolar PAS-positive material (*B*, thin arrow).

The GM-CSF KO mice were positive for PAS as previously reported (Fig. 1*A*) (15, 28). Accumulated PAS-positive material was abundant in alveolar spaces in two out of seven PPARγ KO mice, with one exhibiting robust intraalveolar PAS staining (Fig. 1*B*). PPARγ KO mice at 6 wk did not have significant pathology (not shown). This suggests that intraalveolar accumulation occurs slowly as disease progresses. However, all PPARγ KO mice had abundant PAS staining in the alveolar macrophages (Fig. 1*B*, *inset*). The BAL total and differential cell counts are displayed in Table II ($n = 5$ per condition). These data suggest that there are significantly more cells in the GM-CSF KO mice (mean ± SD of $6.9 \pm 4.9 \times 10^4$ cells/ml) than in wild-type controls ($1.3 \pm 0.3 \times 10^4$ cells/ml, $p = 0.04$). There was a trend ($p = 0.07$) toward more cells in the PPARγ KO mice as well ($2.9 \pm 1.7 \times 10^4$ cells/ml) vs the wild-type mice. Although macrophages were the most abundant cell type in all models, there were increased numbers of lymphocytes in both the GM-CSF KO and PPARγ KO mice, and relative BAL neutrophilia as well in the PPARγ KO mice. Observations in the GM-CSF KO mice are consistent with the literature (45).

### *Exogenous M-CSF is capable of inducing PAP-like surfactant uptake in alveolar macrophages*

Because M-CSF has been associated with foam cell formation (46, 47), and we previously observed elevations of M-CSF in the setting of GM-CSF deficiency (5), we hypothesized that M-CSF overproduction contributes to the lipid-cell phenotype observed in the PPARγ and GM-CSF KO models. Alveolar macrophages from C57BL/6 mice were cultured in Lab-Tek chambers in the presence and absence of murine M-CSF (100 ng/ml) or human PAP surfactant material (200 ng/ml) (Fig. 2). The percentage of positive staining cells for each condition is depicted in Fig. 2*E*. Surfactant alone ($p = 0.001$) or M-CSF alone ($p = 0.008$) induced greater numbers of Oil-Red-O-positive alveolar macrophages than control cells. However, the combination of surfactant and M-CSF induced significantly greater uptake than either condition alone ($p = 0.03$ for the comparison of M-CSF alone or surfactant alone vs M-CSF + surfactant). Antibody-induced neutralization of M-CSF reversed the effects of exogenous M-CSF on lipid processing but did not alter the effects of surfactant alone on induction of Oil-Red-O-positive cells.

Table II. *Cell counts in BAL*

| Model | Total Cell Count (×10^4) | Alveolar Macrophages (%) | Lymphocytes (%) | Neutrophils (%) |
|---|---|---|---|---|
| Wild type | 1.34 ± 0.30 | 98 ± 9 | 2 ± 1.5 | 1 ± 1 |
| GM-CSF KO | 6.86 ± 4.93 | 70 ± 6 | 24 ± 3 | 5 ± 3 |
| PPARγ | 2.92 ± 1.67 | 72 ± 9 | 13 ± 2 | 13 ± 4 |

### *M-CSF is elevated in both the GM-CSF KO and macrophage-specific PPARγ KO mice*

We have previously reported that PPARγ is deficient in human PAP (48). We have also shown that PPARγ is deficient in the GM-CSF KO alveolar macrophages (49). To determine whether these two models also demonstrated similarities in M-CSF expression, we collected BAL cells and fluid from both KO genotypes. Gene expression was assessed using fresh whole cells, because most are alveolar macrophages (Table II). Fig. 3 demonstrates that both GM-CSF and PPARγ KO mice have increased M-CSF gene expression and protein levels compared with controls ($n = 4$–5 for all conditions, $p \leq 0.002$ vs wild type mice). The elevation of M-CSF was specific for alveolar macrophages and the lung milieu because peritoneal macrophages did not express elevated levels of M-CSF mRNA or M-CSF protein in the peritoneal fluid compared with controls, regardless of whether the cells were elicited by thioglycollate or not (data not shown).

### *PPARγ overexpression inhibits M-CSF production*

RAW 264.7 cells were transfected with 2 μg/ml PPARγ expression vector. We measured the expression of M-CSF after 24 h with and without LPS stimulation. The transfection resulted in 10,809 ± 1578% increase in PPARγ expression (Fig. 4, $p \leq 0.001$, $n = 3$). Expression of PPARγ after transfection was not affected by the addition of LPS ($p = 0.98$). Constitutive expression of M-CSF in RAW cells was decreased to 31 ± 4% of control levels (CMV β-gal vector) by transfection with a PPARγ expression vector. In the presence of LPS, M-CSF expression was induced by 20 ± 1.2-fold vs control ($n = 3$, $p = 0.02$). LPS-induced M-CSF production was inhibited by overexpression of PPARγ (10.9 ± 13.2% of LPS alone, $p = 0.017$, $n = 3$). In additional experiments, RAW cells were cultured in the presence and absence of a specific PPARγ agonist, pioglitazone, or an irreversible antagonist, GW9662. Pioglitazone repressed the expected induction of M-CSF transcription by 41 ± 6% percent (Fig. 5*A*, $p = 0.04$). Conversely, baseline RAW cells treated with GW9662 spontaneously increased their M-CSF gene expression by 532 ± 241% (Fig. 5*B*, $p = 0.02$), suggesting that basal PPARγ activity maintains repression of the M-CSF promoter, even in the absence of

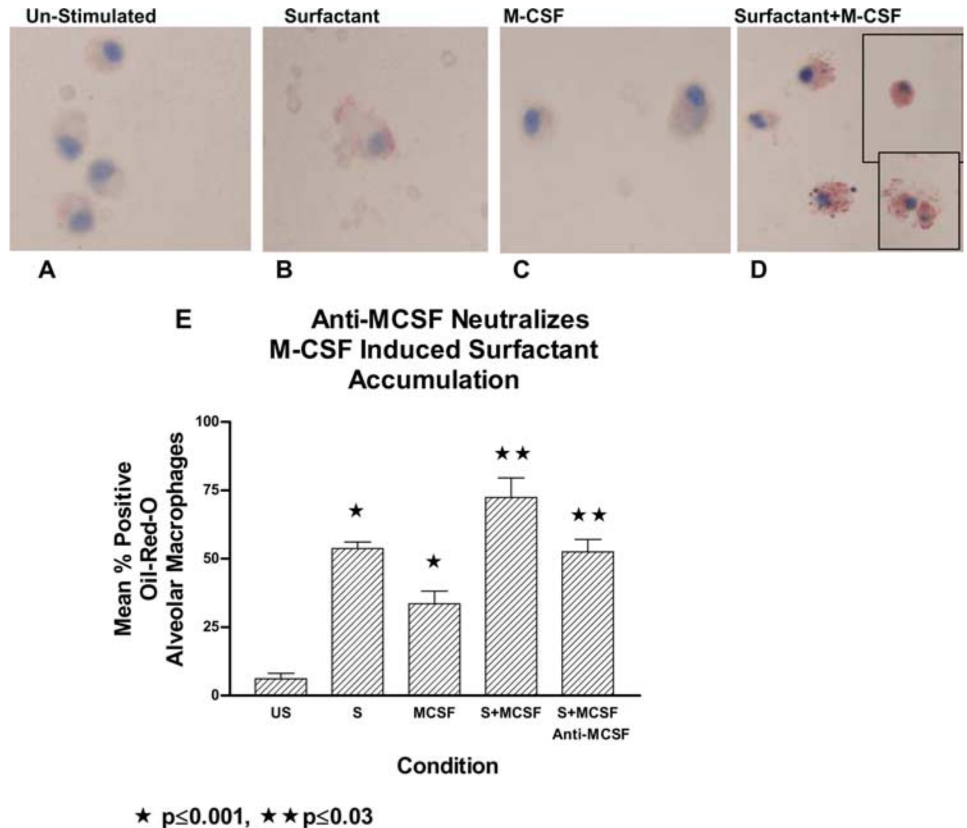


**FIGURE 2.** M-CSF induces alveolar macrophages to take up surfactant. Alveolar macrophages were obtained from C57BL/6 mice and cultured in vitro with 200 ng/ml surfactant obtained from a PAP patient in the presence and absence of human recombinant M-CSF. Figures depict ×40 images, representative of six independent experiments. Both surfactant ($p = 0.001$ vs controls) and M-CSF ($p = 0.008$) induced Oil-Red-O-positive cells. Alveolar macrophages incubated with surfactant and M-CSF had a cumulatively greater uptake of Oil-Red-O staining lipids compared with either condition alone ($p = 0.03$ vs either). Each condition was evaluated for (and counted in 6 frames of at least 200 cells) by blinded numerical designation. Data represent mean percentage of positive Oil-Red-O staining ± SEM ($n = 6$). Abs to M-CSF neutralized the effect of M-CSF on surfactant uptake to levels comparable to surfactant alone. US, Unstimulated; S, surfactant.

inflammatory stimuli. To assess the generalizability of these findings, we repeated the experiments using bone marrow-derived macrophages. LPS stimulated bone marrow-derived macrophages ($n = 4$) had a 21 ± 0.5-fold increase in M-CSF mRNA gene expression, which was decreased to 2.2 ± 0.1 ($p = 0.001$) with pioglitazone. Treatment of the bone marrow macrophages with GW9662 resulted in a 57 ± 1.7-fold increase in M-CSF mRNA expression ($p \leq 0.001$). Because previous studies have shown that alveolar macrophages from PAP patients express high levels of M-CSF (17), healthy control alveolar macrophages were cultured in the presence and absence of either pioglitazone or PPARγ agonists/antagonists to determine whether the impact of PPARγ agonists/antagonists would be the same on M-CSF production. Similar to the RAW cells and the bone marrow-derived macrophages, healthy control alveolar macrophages treated with GW9662 secreted 12 ± 2% more M-CSF than did cultured controls ($p = 0.04$). LPS-stimulated alveolar macrophage M-CSF production was decreased by 30 ± 12% with the addition of pioglitazone ($n = 3$, $p = 0.02$). The observed modest change in M-CSF protein production compared with mRNA expression may be due to the release of preformed M-CSF, enhanced M-CSF stability relative to M-CSF protein, and/or preferential production of membrane-bound M-CSF. This is the subject of ongoing studies.

*PPARγ regulates M-CSF through NF-κB*

Analysis of the M-CSF promoter, as well as prior reports, suggested that NF-κB is crucial for constitutive and inducible gene expression (50). To determine whether PPARγ binding to the NF-κB site affected M-CSF transcription, experiments were designed to measure M-CSF transcription in the presence and absence of a proteasome inhibitor, 6-amino-4-(4-phenoxyphenylethylamino) quinazoline, which prevents activation of NF-κB by blocking IκBα degradation. RAW 264.7 cells were stimulated for 6 h with LPS, resulting in 21.6 ± 0.9-fold increase in M-CSF expression (Fig. 6, $n = 4$ for each condition). LPS-induced M-CSF expression was reduced by 60 ± 26% when NF-κB activation was inhibited. Pioglitazone treatment also decreased LPS-stimulated M-CSF expression, by 68 ± 15% compared with the LPS control

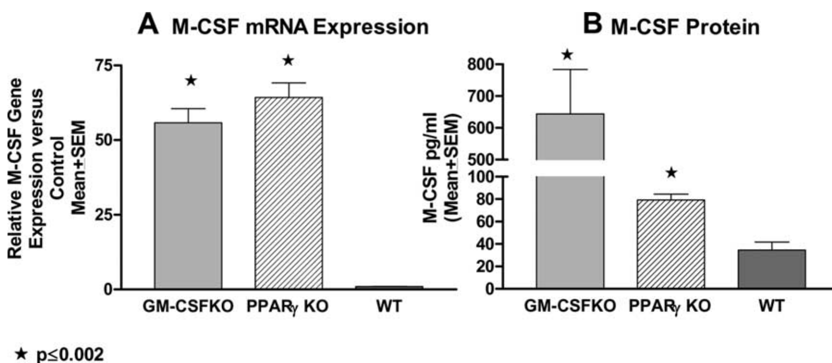


**FIGURE 3.** M-CSF gene expression and protein are elevated in BAL specimens from GM-CSF and PPARγ KO mice. Acellular BAL fluid was assessed for M-CSF protein by ELISA, and BAL cell pellets were evaluated for M-CSF mRNA. Compared with wild-type mice ($n = 5$), BAL cells from both GM-CSF KO ($n = 4$) and PPARγ KO ($n = 4$) mice demonstrated increased M-CSF gene expression ($p \leq 0.002$ for each comparison, *B*). Similarly, BAL protein levels were increased in both models when compared with wild-type mice (*B*, $p < 0.002$, $n = 4$ per condition).

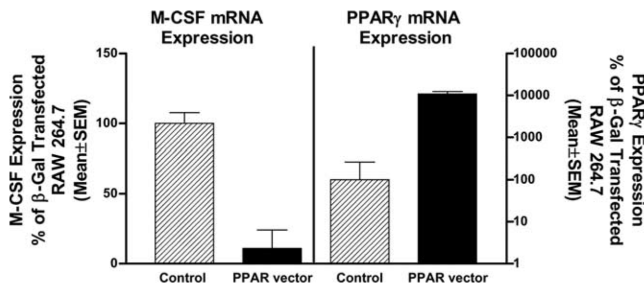


**FIGURE 4.** Transfection of PPARγ expression vector into RAW 264.7 cells attenuates LPS-mediated induction of M-CSF. RAW 264.7 cells were transfected with 1 μg PPARγ murine expression vector. We measured the expression of M-CSF after 24 h in the presence and absence of LPS. Transient transfection of the PPARγ expression vector resulted in 10,809 ± 1,578% (mean ± SEM) increased expression of PPARγ mRNA expression compared with the control (β-gal) vector (right bars, $n = 3$, $p \leq 0.001$). Compared with transfected controls (β-gal), LPS-induced M-CSF production was inhibited by 90.1 ± 13.2% with the overexpression of PPARγ expression vector (*left bars*, $p = 0.017$, $n = 3$).

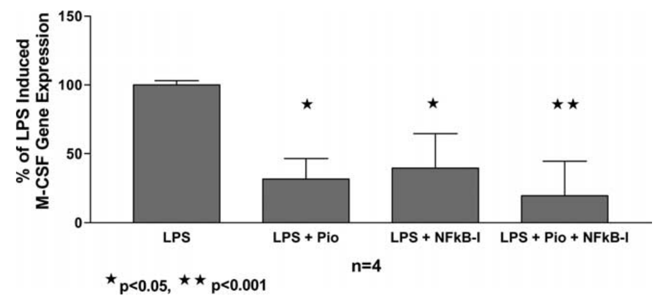


**FIGURE 6.** PPARγ regulates M-CSF through NF-κB. RAW 264.7 cells were stimulated with LPS, which resulted in a 21.6 ± 0.9-fold increase in M-CSF expression. LPS induced M-CSF expression was reduced by 60 ± 26% when the NF-κB was inhibited with the proteasomal blocker. Pioglitazone treatment also decreased LPS-stimulated M-CSF expression, by 68 ± 15% of the LPS control ($p < 0.05$ vs LPS alone). The combination of both pioglitazone and NF-κB inhibition resulted in 80 ± 25% reduction of M-CSF expression ($p < 0.001$ vs LPS). These data suggest that PPARγ can regulate M-CSF induction through repression of NF-κB.

($p < 0.05$). The combination of both pioglitazone and NF-κB inhibition resulted in a further reduction of M-CSF expression, to 19.7 ± 25% of LPS alone ($p < 0.001$ vs LPS). These data suggest that PPARγ regulates M-CSF production through repression of NF-κB. PPARγ regulation of M-CSF did not appear to involve MAPK pathways because ERK inhibitor (PD 98059) and p38 inhibitor (SB 203580) did not alter M-CSF gene expression (data not shown).

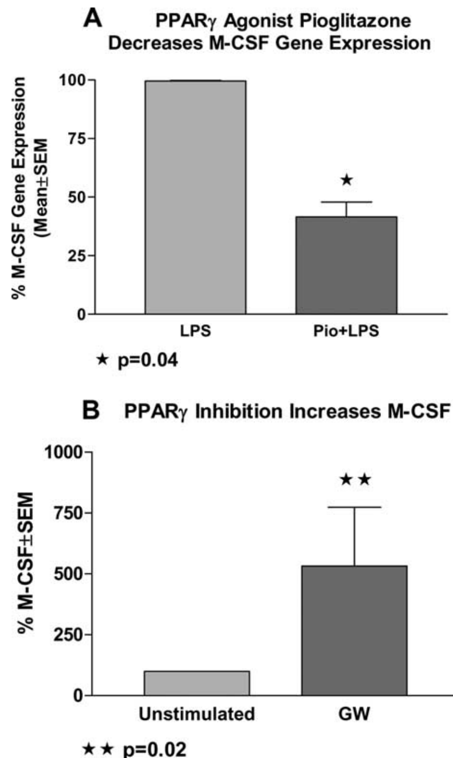


**FIGURE 5.** M-CSF secretion is enhanced by PPARγ antagonists and suppressed by PPARγ agonist. RAW cells were cultured in the presence and absence of pioglitazone or GW9662 at baseline or after stimulation with LPS. LPS-stimulated RAW cells treated with pioglitazone expressed 41 ± 6% significantly less M-CSF mRNA (*A*, $p = 0.04$, $n = 4$) and 12 ± 0.7% less M-CSF protein ($p = 0.01$) compared with the LPS cultures alone. Conversely, baseline RAW cells treated with GW9662 increased their M-CSF gene expression and protein by 532 ± 242% (*B*, $p = 0.02$, $n = 3$) and 11.2 ± 1% ($p = 0.03$), respectively.

### *Pioglitazone inhibits NF-κB activation*

Pioglitazone, a PPARγ agonist, has previously been shown to decrease activity of NF-κB-regulated gene promoters in transient transfection assays (51). To confirm that this effect is mediated by inhibition of NF-κB binding to DNA, we performed EMSAs using RAW 264.7 cells cultured for 3 h in the presence of LPS, pioglitazone, or pretreated with pioglitazone for 30 min before the addition of LPS. The extent of NF-κB DNA binding was quantified by measuring radioactivity with phosphorimaging. Constitutive NF-κB binding was low and was not significantly affected by pioglitazone alone (Fig. 7). After pretreatment with pioglitazone, LPS-induced formation of NF-κB-DNA-binding complexes was reduced by 30.6 ± 6.9% ($n = 4$, $p < 0.01$ by Tukey's test).

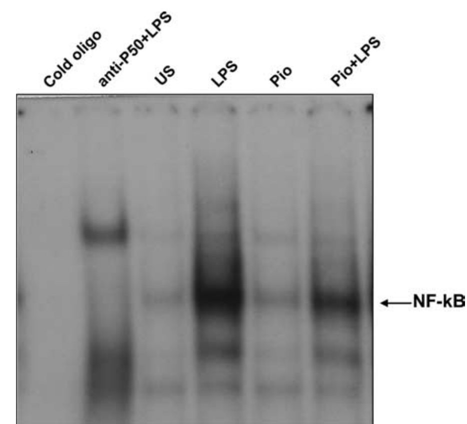


**FIGURE 7.** Pioglitazone inhibits LPS-mediated NF-κB activation. Whole-cell extracts were analyzed by EMSA using a $^{32}$P-labeled oligonucleotide containing the κB consensus sequences and analyzed on a 4% nondenaturing acrylamide gel. Specificity of the band was confirmed by supershift analysis with p50 and p65 Abs and competition with cold oligonucleotide. Representative image from four independent experiments is shown. Quantification using the StormImager with ImageQuant analysis revealed that pretreatment with pioglitazone before addition of LPS decreased NF-κB binding by 30.6 ± 6.9% (mean (SEM), $p < 0.01$ by Tukey's test). US, Unstimulated; Pio, pioglitazone.

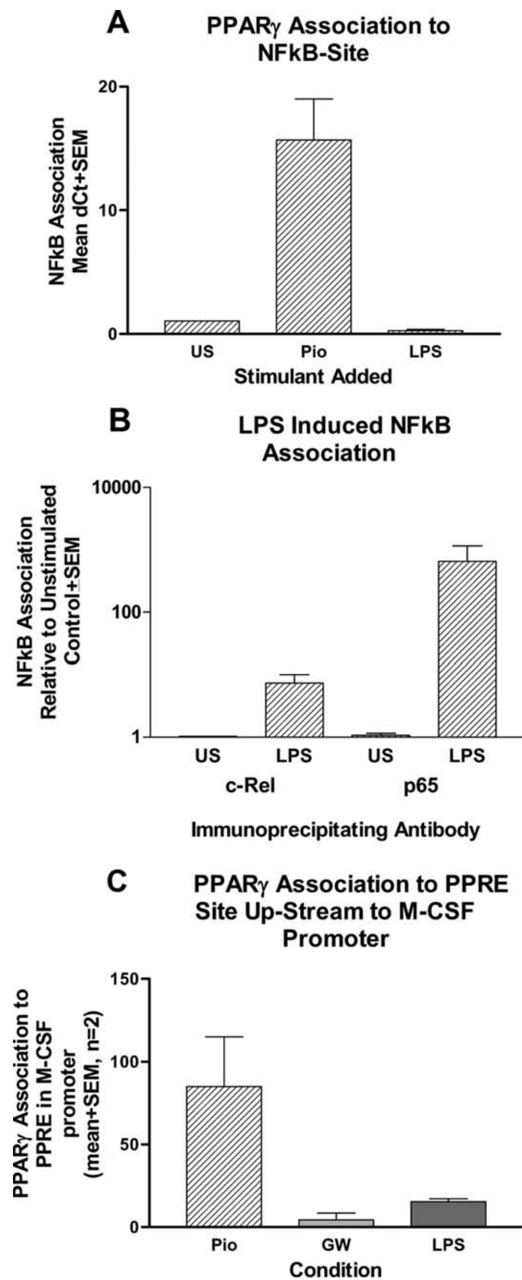


**FIGURE 8.** PPARγ inhibits LPS-induced NF-κB association with the M-CSF promoter. ChIP was performed using BALB/c bone marrow-derived macrophages. After treatment with LPS (2 μg/ml), pioglitazone (Pio, 10 μg/ml), or GW9662 (GW, 10 mM), cell lysates were subjected to immunoprecipitation with anti-PPARγ, anti-c-Rel, or anti-p65. Binding to the M-CSF promoter was determined by real-time PCR. LPS stimulation resulted in c-Rel and p65 binding to the NF-κB site upstream of the M-CSF promoter (*B*). Activation of PPARγ by pioglitazone increased PPARγ association with the NF-κB response element, which was nearly eliminated with NF-κB activation by LPS (*A*). Pioglitazone also resulted in PPARγ binding to the M-CSF PPRE promoter region (*C*).

### *PPARγ binds to NF-κB site and PPRE site upstream of the M-CSF promoter*

To assess whether PPARγ could inhibit NF-κB transactivation of the M-CSF promoter, we used bone marrow-derived macrophages for ChIP experiments. BALB/c bone marrow cells were differentiated into macrophages using L929-supplemented medium. Cells were harvested and cultured for 7 days before experiments. Bone marrow-derived macrophages were then treated with LPS (500 ng/ml) or pioglitazone (10 μM). Cell lysates were immunoprecipitated with anti-PPARγ, anti-p65, and anti c-Rel. Binding to the M-CSF promoter was determined by real-time PCR. Positive M-CSF promoter PCR reactions in the different immunoprecipitated reactions reflect transcription factor association with the M-CSF promoter. As expected, LPS resulted in p65 and c-Rel association with the NF-κB site (Fig. 8*B*). Activation of PPARγ by pioglitazone enhanced PPARγ association with the same region (Fig. 8*A*, $n = 2$), while LPS nearly abrogated PPARγ binding to the promoter. These data suggest that activation of PPARγ is capable of repressing inflammatory signaling that can activate transcription at the M-CSF promoter. The impact of PPARγ association with a PPRE site in the M-CSF promoter is unknown at this time (Fig. 8*C*).

## Discussion

GM-CSF is essential for alveolar macrophage differentiation and surfactant catabolism (7). The mechanism whereby deficiency of bioactive GM-CSF induces accumulation of surfactant material in human PAP and the GM-CSF KO model has not been delineated to date. Our data suggest that the effect of GM-CSF deficiency to enhance M-CSF expression may be an important clue about its role. The dysregulation of M-CSF appears to be due to deficiency of PPARγ and is capable in vitro of inducing lipid accumulation in alveolar macrophages. In support of this general concept, both the macrophage-specific PPARγ KO mode and the GM-CSF KO mouse have similar accumulation of PAS-positive lipid material in the intraalveolar space and alveolar macrophages. Cells harvested from the peritoneum did not demonstrate this morphology, suggesting a mechanism unique to the alveolar microenvironment.

Additionally, the data presented herein demonstrate that M-CSF production in alveolar macrophages can be regulated by PPARγ. Using chromatin immunoprecipitation, PPARγ expression vectors and inhibitors for NF-κB, ERK, and JNK, we provide evidence that PPARγ regulates M-CSF through transrepression of NF-κB activity. It is possible that the effects of pioglitazone and the NF-κB inhibition involve unique or independent mechanisms. The present report does not fully resolve this issue. We think it is likely, however, that the repressive effect of PPARγ activation is mediated by blockade of NF-κB association with the promoter. Data in support of this hypothesis include the EMSA analysis and the very robust association of PPARγ with the NF-κB response element. The magnitude of reduction of NFκB binding in the EMSA is almost identical to previously reported effects of pioglitazone on LPS induction of NFκB-luciferase in RAW cells (51). Taken together, these results suggest that the effects of pioglitazone are mediated by prevention of NFκB transactivation of the promoter. This mechanism is congruent with the model proposed for regulation of NF-κB induction of the murine iNOS promoter in RAW cells (52–55).

Normally, production of M-CSF is low, a phenomenon that is partly due to basal repression of the M-CSF promoter by PPARγ. In the presence of inflammatory signaling via NF-κB, up-regulated M-CSF promoter activity can be modulated by PPARγ. These observations are consistent with the PPARγ corepression mechanism suggested by Pascual et al. (52). From these observations, we propose the model depicted in Fig. 9, showing that in the absence of activation a corepressor complex may block M-CSF promoter activity. When PPARγ is activated it binds to and stabilizes the corepressor, preventing accumulation of coactivator proteins and NF-κB transactivation of the promoter, which requires degradation of the corepressor complex. In the absence of PPARγ, constitutively low-level NF-κB binding at the promoter allows basal M-CSF production.

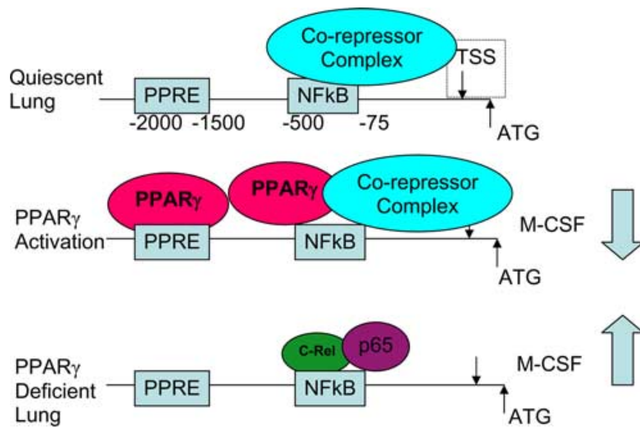


**FIGURE 9.** Model of PPARγ regulation of M-CSF transcription. Data from the ChIP and culture studies were put together to develop a model of a working hypothesis in which PPARγ regulates M-CSF through NF-κB. PPARγ inhibits association of NF-κB proteins with the promoter, an effect that is enhanced in the presence of PPARγ activation. In the presence of NF-κB activation, PPARγ binding to the promoter is down-regulated unless PPARγ is ligand-activated by pioglitazone. In the absence of PPARγ, there is a potential constitutive production of M-CSF through p65/c-Rel binding to NF-κB transcription site.

M-CSF is a CSF associated with the maturation, differentiation and survival of monocyte/macrophages (10, 41). During early myeloid differentiation, M-CSF alone cannot support the proliferation and differentiation of multipotent progenitor cells; however, it synergizes with other growth factors such as stem cell factor (also known as kit ligand) and IL-3 to produce mononuclear phagocyte progenitor cells (56). After this initial differentiation process, M-CSF itself can regulate the proliferation and differentiation of mononucelar phagocyte progenitor cells to monocytes and macrophages in addition to the survival, proliferation, and function of fully differentiated macrophages. Additionally, M-CSF synergizes with receptor activator of NF-κB ligand (RANKL) to regulate the differentiation of mononuclear phagocytes to osteoclasts. Studies have shown that M-CSF induced bone-resorbing osteoclasts can be inhibited by PPARγ ligands (57), which are congruent with our observations associating M-CSF repression with PPARγ activation.

Overabundance of M-CSF can also lead to the development of disease. M-CSF-associated enhancement of atherosclerosis is thought to be mediated by a local production of M-CSF leading to the accumulation of cholesterol-filled foam cells in atherosclerotic plaques (56). In obesity, the ratio of M-CSF-dependent macrophages to adipocytes in adipose tissue is positively correlated with both adipocyte size and body mass (23). These and other studies suggest that macrophages have an active role in morbid obesity and that macrophage-based inflammatory activity supported by locally produced M-CSF might contribute to the pathogenesis of obesity-induced insulin resistance (58). M-CSF also has a role in some inflammatory diseases. The administration of Abs to M-CSF in the collagen-induced arthritis model results in reduced severity of arthritis in mice, and M-CSF-deficient mice show a decrease in recruitment, proliferation, and activation of macrophages in models of inflammation (58, 59). Our data suggest therefore that PPARγ has the potential to alter monocyte/macrophage activation through the regulation of M-CSF, independent of GM-CSF. This mechanism explains the observation that there is constitutive hyperproduction of M-CSF in the GM-CSF KO mouse, because PPARγ is markedly deficient.

GM-CSF is essential for alveolar macrophage differentiation, surfactant catabolism, and PPARγ expression (14, 31, 48, 60). One of the implications of loss of GM-CSF-induced PPARγ expression is on the production of M-CSF. These studies are the first to directly link PPARγ to M-CSF production. M-CSF is essential in the survival, proliferation, and activation of monocyte/macrophages. The PPARγ KO expresses normal levels of GM-CSF mRNA, but also has elevated levels of M-CSF. This suggests that in any scenario in which PPARγ becomes deficient or significantly decreased such as in inflammation, a switch turns on in which macrophages may become alternatively activated potentially through the production of M-CSF. The nature and phenotype of alveolar macrophage activation ultimately defines the adaptive and innate immunity of the response to the insulting stimulus. These studies therefore are the first to directly associate PPARγ to alveolar macrophage M-CSF production and potential activation and regulation of pulmonary immunity.

## Acknowledgments

We extend our sincere gratitude to the following people for their technical expertise: Baisakhi Raychaudhuri, Anagha Malur, Nejimol John, and Steven Moeller.

## Disclosures

The authors have no financial conflicts of interest.

## References

1. Rotondo, D., and J. Davidson. 2002. Prostaglandin and PPAR control of immune cell function. *Immunology* 105: 20–22.
2. Berger, J., and D. E. Moller. 2002. The mechanisms of action of PPARs. *Annu. Rev. Med.* 53: 409–435.
3. Clark, R. B. 2002. The role of PPARs in inflammation and immunity. *J. Leukocyte Biol.* 71: 388–400.
4. Chinetti, G., S. Griglio, M. Antonucci, I. P. Torra, P. Delerive, Z. Majd, J.-C. Fruchart, J. Chapman, J. Najib, and B. Staels. 1998. Activation of proliferator-activated receptors alpha and gamma induces apoptosis of human monocyte-derived macrophages. *J. Biol. Chem.* 273: 25573–25580.
5. Bonfield, T. L., D. Russell, S. Burgess, A. Malur, M. S. Kavuru, and M. J. Thomassen. 2002. Autoantibodies against granulocyte macrophage colony-stimulating factor are diagnostic for pulmonary alveolar proteinosis. *Am. J. Respir. Cell Mol. Biol.* 27: 481–486.
6. Venkateshiah, S. B., M. J. Thomassen, and M. S. Kavuru. 2004. Pulmonary alveolar proteinosis: clinical manifestations and optimal treatment strategies. *Treat. Respir. Med.* 3: 217–227.
7. Bonfield, T. L., C. F. Farver, B. P. Barna, A. Malur, S. Abraham, B. Raychaudhuri, M. S. Kavuru, and M. J. Thomassen. 2003. Peroxisome proliferator-activated receptor-γ is deficient in alveolar macrophages from patients with alveolar proteinosis. *Am. J. Respir. Cell Mol. Biol.* 29: 677–682.
8. Foey, A. D., M. Feldmann, and F. M. Brennan. 2000. Route of monocyte differentiation determines their cytokine production profile: CD40 ligation induces interleukin 10 expression. *Cytokine* 12: 1496–1505.
9. Goyal, A., Y. Wang, M. M. Graham, A. I. Doseff, N. Y. Bhatt, and C. B. Marsh. 2002. Monocyte survival factors induce Akt activation and suppress caspase-3. *Am. J. Respir. Cell Mol. Biol.* 26: 224–230.
10. Sweet, M. J., and D. A. Hume. 2003. CSF-1 as a regulator of macrophage activation and immune responses. *Arch. Immunol. Ther. Exp.* 51: 169–177.
11. Brown, A. L., M. Peters, R. J. D'Andrea, and T. J. Gonda. 2004. Constitutive mutants of the GM-CSF receptor reveal multiple pathways leading to myeloid cell survival, proliferation, and granulocyte-macrophage differentiation. *Blood* 103: 507–516.
12. De, A. K., K. Laudanski, and C. L. Miller-Graziano. 2003. Failure of monocytes of trauma patients to convert to immature dendritic cells is related to preferential macrophage-colony-stimulating factor-driven macrophage differentiation. *J. Immunol.* 170: 6355–6362.
13. Horiguchi, J., M. K. Warren, and D. Kufe. 1987. Expression of the macrophage-specific colony-stimulating factor in human monocytes treated with granulocyte-macrophage colony-stimulating factor. *Blood* 69: 1259–1261.
14. Shibata, Y., Y. P. Berclaz, Z. C. Chroneos, M. Yoshida, J. A. Whitsett, and B. C. Trapnell. 2001. GM-CSF regulates alveolar macrophage differentiation and innate immunity in the lung through PU.1. *Immunity* 15: 557–567.
15. Trapnell, B. C., Z. Z. Zsengeller, C. Chroneos, J. A. Whitsett, and Y. P. Berclaz. 2000. Alveolar macrophages from granulocyte-monocyte colony stimulating factor (GM-CSF) deficient mice are arrested at an early differentiation stage. *Am. J. Respir. Crit. Care Med.* 161: A667.
16. Bonfield, T. L., C. M. Swaisgood, D. A. Culver, M. S. Kavuru, and M. J. Thomassen. 2004. Matrix metalloproteinase gene expression is elevated in alveolar macrophages from pulmonary alveolar proteinosis patients. *Am. J. Respir. Crit. Care Med.* 169: A56.

17. Bonfield, T. L., C. M. Swaisgood, B. P. Barna, C. F. Farver, M. S. Kavuru, and M. J. Thomassen. 2006. Elevated gelatinase activity in pulmonary alveolar proteinosis: role of macrophage-colony stimulating factor. *J. Leukocyte Biol.* 79: 133–139.
18. Shibata, Y., Z. K. Zsengeller, K. Otake, N. Palaniyar, and B. C. Trapnell. 2001. Alveolar macrophage deficiency in osteopetrotic mice deficient in macrophage colony-stimulating factor is spontaneously corrected with age and associated with matrix metalloproteinase expression and emphysema. *Blood* 98: 2845–2852.
19. Gordon, S., and D. Hughes. 1997. Macrophages and their origins, heterogeneity in relation to tissue microenvironment. In *Lung Macrophages and Dendritic Cells in Health and Disease.* M. F. Lipscomb and S. W. Russell, eds. Marcel Dekker, New York, pp. 3–31.
20. Mosser, D. M. 2003. The many faces of macrophage activation. *J. Leukocyte Biol.* 73: 209–212.
21. Nathan, C. F., T. J. Prendergast, M. E. Wiebe, E. R. Stanley, E. Platzer, H. G. Remold, K. Welte, B. Y. Rubin, and H. W. Murray. 1984. Activation of human macrophages. *J. Exp. Med.* 160: 600–605.
22. Thomassen, M. J., B. Raychaudhuri, T. L. Bonfield, A. Malur, S. Abraham, B. P. Barna, and M. S. Kavuru. 2003. Elevated IL-10 inhibits GM-CSF synthesis in pulmonary alveolar proteinosis. *Autoimmunity* 36: 285–290.
23. Hamilton, J. A., R. Byrne, W. Jessup, V. Kanagasundaram, and G. Whitty. 2001. Comparison of macrophage responses to oxidized low-density lipoprotein and macrophage colony-stimulating factor (M-CSF or CSF-1). *Biochem. J.* 354: 179–187.
24. Pang, Z. J. 2003. Effect of macrophage colony stimulating factor overexpression on oxidative injury/resistance of RAW264.7 cells. *Clin. Exp. Med.* 3: 20–26.
25. Shashkin, P., B. Dragulev, and K. Ley. 2005. Macrophage differentiation to foam cells. *Curr. Pharm. Des.* 11: 3061–3072.
26. Akiyama, T. E., S. Sakai, G. Lambert, C. J. Nicol, K. Matsusue, S. Pimprale, Y. H. Lee, M. Ricote, C. K. Glass, H. B. Brewer, and F. J. Gonzalez. 2002. Conditional disruption of the peroxime proliferator-activated receptor $\gamma$ gene in mice results in lowered expression of ABCA1, ABCGi, and apoE in macrophages and reduced cholesterol efflux. *Mol. Cell. Biol.* 22: 2607–2619.
27. Babaev, V. R., P. G. Yancey, S. V. Ryzhov, V. Kon, M. D. Breyer, M. A. Magnuson, S. Fazio, and M. F. Linton. 2005. Conditional knockout of macrophage PPAR$\gamma$ increases atherosclerosis in C57BL/6 and low-density lipoprotein receptor-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* 25: 1647–1653.
28. Dranoff, G., A. D. Crawford, M. Sadelain, B. Ream, A. Rashid, R. T. Bronson, G. R. Dickersin, C. J. Bachurski, E. L. Mark, J. A. Whitsett, and R. C. Mulligan. 1994. Involvement of granulocyte-macrophage colony-stimulating factor in pulmonary homeostasis. *Science* 264: 713–716.
29. Lucas, M., X. Zhang, V. Prasanna, and D. M. Mosser. 2005. ERK activation following macrophage Fc$\gamma$R ligation leads to chromatin modifications at the IL-10 locus. *J. Immunol.* 175: 469–477.
30. Zhang, X., J. P. Edwards, and D. M. Mosser. 2006. Dynamic and transient remodeling of the macrophage IL-10 promoter during transcription. *J. Immunol.* 177: 1282–1288.
31. Thomassen, M. J., T. Yi, B. Raychaudhuri, A. Malur, and M. S. Kavuru. 2000. Pulmonary alveolar proteinosis is a disease of decreased availability of GM-CSF rather than an intrinsic cellular defect. *Clin. Immunol.* 95: 85–92.
32. Swendsen, C. L., V. Skita, and R. S. Thrall. 1996. Alterations in surfactant neutral lipid composition during the development of bleomycin-induced pulmonary fibrosis. *Biochim. Biophys. Acta* 1301: 90–96.
33. Hogan, J. C., and J. M. Stephens. 2001. The identification and characterization of a STAT1 binding site in the PPAR$\gamma$2 promoter. *Biochem. Biophys. Res. Commun.* 287: 484–492.
34. Culver, D. A., B. P. Barna, B. Raychaudhuri, T. L. Bonfield, S. Abraham, A. Malur, C. F. Farver, M. S. Kavuru, and M. J. Thomassen. 2004. Peroxisome proliferator-activated receptor $\gamma$ activity is deficient in alveolar macrophages in pulmonary sarcoidosis. *Am. J. Respir. Cell Mol. Biol.* 30: 1–5.
35. Dunlop, T. W., S. Vaisanen, C. Frank, F. Molnar, L. Sinkkonen, and C. Carlberg. 2005. The human peroxisome proliferator-activated receptor $\delta$ gene is a primary target of 1$\alpha$,25-dihydroxyvitamin D3 and its nuclear receptor. *J. Mol. Biol.* 349: 248–260.
36. Kang, S. H., K. Vieira, and J. Bungert. 2002. Combining chromatin immunoprecipitation and DNA footprinting: a novel method to analyze protein-DNA interactions in vivo. *Nucleic Acids Res.* 30: e44.
37. Nagamine, N., Y. Kawada, and Y. Sakakibara. 2005. Identifying cooperative transcriptional regulations using protein-protein interactions. *Nucleic Acids Res.* 33: 4828–4837.
38. Lang, R., D. Patel, J. J. Morris, R. L. Rutschman, and P. J. Murray. 2002. Shaping gene expression in activated and resting primary macrophages by IL-10. *J. Immunol.* 169: 2253–2263.
39. Xu, R., V. A. Spencer, and M. J. Bissell. 2007. Extracellular matrix-regulated gene expression requires cooperation of SWI/SNF and transcription factors. *J. Biol. Chem.* 282: 14992–14999.
40. Fixe, P., and V. Praloran. 1997. Macrophage colony-stimulating-factor (M-CSF or CSF-1) and its receptor: structure-function relationships. *Eur. Cytokine Network* 8: 125–136.
41. Abboud, S. L., M. Bunegin, N. Ghosh-Choudhury, and K. Woodruff. 2003. Analysis of the mouse CSF-1 gene promoter in a transgenic mouse model. *J. Histochem. Cytochem.* 51: 941–949.
42. Sawka-Verhelle, D., L. Escoubet-Lozach, A. L. Fong, K. D. Hester, S. Herzig, P. Lebrun, and C. K. Glass. 2004. PE-1/METS, an antiproliferative Ets repressor factor, is induced by CREB-1/CREM-1 during macrophage differentiation. *J. Biol. Chem.* 279: 17772–17784.
43. Shi, C., X. Zhang, Z. Chen, K. Sulaiman, M. W. Feinberg, C. M. Ballantyne, M. K. Jain, and D. I. Simon. 2004. Integrin engagement regulates monocyte differentiation through the forkhead transcription factor Foxp1. *J. Clin. Invest.* 114: 408–418.
44. Rosenwasser, L. J., D. J. Klemm, J. K. Dresback, H. Inamura, J. J. Mascali, M. Klinnert, and L. Borish. 1995. Promoter polymorphisms in the chromosome 5 gene cluster in asthma and atopy. *Clin. Exp. Allergy* 25 (*Suppl.* 2): 74–78.
45. Paine, R., S. B. Morris, H. Jin, S. E. Wilcoxen, S. M. Phare, B. B. Moore, M. J. Coffey, and G. B. Toews. 2001. Impaired functional activity of alveolar macrophages from GM-CSF-deficient mice. *Am. J. Physiol.* 281: L1210–L1218.
46. Linton, M. F., and S. Fazio. 2003. Macrophages, inflammation, and atherosclerosis. *Int. J. Obes. Relat Metab Disord.* 27 (*Suppl.* 3): S35–S40.
47. Babaev, V. R., M. B. Patel, C. F. Semenkovich, S. Fazio, and M. F. Linton. 2000. Macrophage lipoprotein lipase promotes foam cell formation and atherosclerosis in low density lipoprotein receptor-deficient mice. *J Biol. Chem.* 275: 26293–26299.
48. Bonfield, T. L., C. F. Farver, B. P. Barna, A. Malur, S. Abraham, B. Raychaudhuri, M. S. Kavuru, and M. J. Thomassen. 2003. PPARg is deficient in alveolar macrophages from patients with alveolar proteinosis. *Am. J. Respir. Cell Mol. Biol.* 29: 677–682.
49. Thomassen, M. J., B. P. Barna, A. G. Malur, T. L. Bonfield, C. F. Farver, A. Malur, H. Dalrymple, M. S. Kavuru, and M. Febbraio. 2007. ABCG1 is deficient in alveolar macrophages of GM-CSF knockout mice and patients with pulmonary alveolar proteinosis. *J. Lipid Res.* 48: 2762–2768.
50. Rubin, J., D. Fan, A. Wade, T. C. Murphy, H. Gewant, M. S. Nanes, X. Fan, M. Moerenhout, and W. Hofstetter. 2000. Transcriptional regulation of the expression of macrophage colony stimulating factor. *Mol. Cell. Endocrinol.* 160: 193–202.
51. Nakamuta, M., M. Enjoji, K. Uchimura, S. Ohta, R. Sugimoto, K. Kotoh, M. Kato, T. Irie, T. Muta, and H. Nawata. 2002. Bisphenol $\alpha$ diglycidyl ether (BADGE) suppresses tumor necrosis factor-$\alpha$ production as a PPAR$\gamma$ agonist in the murine macrophage-like cell line, RAW 264.7. *Cell Biol. Int.* 26: 235–241.
52. Pascual, G., A. L. Fong, S. Ogawa, A. Gamliel, A. C. Li, V. Perissi, D. W. Rose, T. M. Willson, M. G. Rosenfeld, and C. K. Glass. 2005. A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-$\gamma$. *Nature* 437: 759–763.
53. Pascual, G., and C. K. Glass. 2006. Nuclear receptors versus inflammation: mechanisms of transrepression. *Trends Endocrinol. Metab.* 17: 321–327.
54. Li, M., G. Pascual, and C. K. Glass. 2000. Peroxisome proliferator-activated receptor gamma-dependent repression of the inducible nitric oxide synthase gene. *Mol. Cell. Biol.* 20: 4699–4707.
55. Ricote, M., J. T. Huang, J. S. Welch, and C. K. Glass. 1999. The peroxisome proliferator-activated receptor-$\gamma$ (PPAR$\gamma$) as a regulator of monocyte/macrophage function. *J. Leukocyte Biol.* 66: 733–739.
56. Pixley, F. J., and E. R. Stanley. 2004. CSF-1 regulation of the wandering macrophage: complexity in action. *Trends Cell Biol.* 14: 628–638.
57. Ricote, M., J. Huang, L. Fajas, A. Li, J. Welch, J. Najib, J. L. Witztum, J. Auwerx, W. Palinski, and C. K. Glass. 1998. Expression of the peroxisome proliferator-activated receptor $\gamma$ (PPAR$\gamma$) in human atherosclerosis and regulation in macrophages by colony stimulating factors and oxidixed low density lipoprotein. *Proc. Natl. Acad. Sci. USA* 95: 7614–7619.
58. Chitu, V., and E. R. Stanley. 2006. Colony-stimulating factor-1 in immunity and inflammation. *Curr. Opin. Immunol.* 18: 39–48.
59. Mottonen, M., P. Isomaki, R. Luukkainen, and O. Lassila. 2002. Regulation of CD154-induced interleukin-12 production in synovial fluid macrophages. *Arthritis Res.* 4: R9.
60. Ricote, M., A. C. Li, T. M. Willson, C. J. Kelly, and C. K. Glass. 1998. The peroxisome proliferator-activated receptor-$\gamma$ is a negative regulator of macrophage activation. *Nature* 391: 79–82.