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Sepsis-induced inhibition of neutrophil chemotaxis is mediated by activation of peroxisome proliferator-activated receptor-γ

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Neutrophils (polymorphonuclear leukocytes [PMNs]) are critical to the immune response, including clearance of infectious pathogens. Sepsis is associated with impaired PMN function, including chemotaxis. PMNs express peroxisome proliferator-activated receptor-γ (PPAR-γ), a ligand-activated nuclear transcription factor involved in immune and inflammatory regulation. The role of PPAR-γ in PMN responses, however, is not well characterized. We report that freshly isolated human PMNs constitutively express PPAR-γ, which is up-regulated by the sepsis-induced cytokines TNF-α and IL-4. PMN chemotactic responses to formylmethionyl-leucyl-phenylalanine (fMLP) and IL-8 were dose-dependently inhibited by treatment with the PPAR-γ ligands troglitazone and 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) and by transfection of PMN-like HL-60 cells with a constitutively active PPAR-γ construct. Inhibition of chemotaxis by PPAR-γ ligands correlated with decreases in extracellular signal-regulated kinase-1 and -2 activation, actin polymerization, and adherence to a fibrinogen substrate. Furthermore, PMN expression of PPAR-γ was increased in sepsis patients and mice with either of 2 models of sepsis. Finally, treatment with the PPAR-γ antagonist GW9662 significantly reversed the inhibition of PMN chemotaxis and increased peritoneal PMN recruitment in murine sepsis. This study indicates that PPAR-γ activation is involved in PMN chemotactic responses in vitro and may play a role in the migration of these cells in vivo. (Blood. 2008;112:4250-4258)

Introduction

Human peripheral polymorphonucleocytes (PMNs) represent the predominant cellular component at sites of acute inflammation. These cells serve a critical role in host defense with transendothelial migration of PMNs being a crucial component of the immune/inflammatory response.[1] In addition to their phagocytic activity, PMNs produce mediators such as reactive oxygen intermediates, cytokines/chemokines, and enzymes.[1,2] The directed migration, or chemotaxis, of PMNs occurs in response to a variety of molecules, including formylmethionyl-leucyl-phenylalanine (fMLP), a bacterial peptide, and chemokines such as IL-8, which are released from sites of inflammation or injury. fMLP and IL-8 bind to receptors on PMNs, resulting in both G-protein–dependent and –independent responses.[3] Emerging evidence indicates that sepsis, most commonly caused by bacteria, results in impaired host defenses, including a reduced ability of PMNs to migrate appropriately.[4-6] Moreover, higher mortality rates have been observed in patients with sepsis-induced immune deactivation.[7] Mechanistic pathways resulting in altered leukocyte function during sepsis, however, remain incompletely understood.

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear hormone receptor family. Three PPAR subtypes (α, β, and γ), each with a specific pattern of expression, have been identified. PPAR-γ also exists in 2 isoforms, PPAR-γ1 and PPAR-γ2, with the human PPAR-γ2 protein consisting of 28 additional amino acids compared with PPAR-γ1. Ligands for PPAR-γ include a variety of compounds, both natural and synthetic. Most of the natural ligands are fatty acids or fatty acid derivatives. Synthetic ligands for PPAR-γ include the antidiabetic thiazolidinediones such as troglitazone. PPAR-γ is highly expressed in adipose tissue and plays a crucial role in adipocyte differentiation,[8] but it is also expressed in a variety of tissue and cell types, including a majority of those in the hematopoietic system. In cells of the immune system, treatment with PPAR-γ ligands typically results in the down-regulation of inflammatory responses.[9] For example, activation of PPAR-γ in macrophages results in inhibition of cytokine, nitric oxide, and hydrogen peroxide production.[10]

Freshly isolated human peripheral PMNs were previously shown to express PPAR-γ mRNA.[11] Treatment of human PMNs with PPAR-γ ligands results in decreased adhesion-dependent H_2O_2 production[12] and blocks up-regulation of the CD11b/CD18 adhesion complex.[13] However, no previous study has examined regulation of PPAR-γ expression in human PMNs or the specific role this transcription factor may play in the migration of these cells. In this study, we assessed the expression and regulation of PPAR-γ in PMNs and investigated the effect of PPAR-γ activation on the ability of PMNs to migrate in response to chemoattractants. Furthermore, we investigated the expression of PPAR-γ in PMNs during the septic response and the effects on chemotaxis and PMN recruitment of blocking PPAR-γ activation in vivo.

Methods

Reagents

The PPAR-γ ligands troglitazone (Cayman, Ann Arbor, MI) and 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2; Cayman) and the PPAR-γ antagonist GW9662 (Cayman) were dissolved in 100% dimethyl sulfoxide (DMSO;

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Sigma-Aldrich, St Louis, MO) at 100 mM and stored in aliquots at −20°C. LTB_4 (Cayman) in ethanol was evaporated under a gentle stream of nitrogen and immediately resuspended with DMSO (purged with inert gas) at 10 mM and stored in aliquots at −20°C. Human TNF-α, IL-8, and IL-4 were purchased from R&D Systems (Minneapolis, MN). Lipopolysaccharide (LPS; *Escherichia coli* serotype 0111:B4) and fMLP was purchased from Sigma-Aldrich. Anti–PPAR-γ (H-100) antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA) and anti–glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody was purchased from Abcam (Cambridge, United Kingdom).

PMN isolation and culture conditions

Human peripheral blood PMNs were obtained from heparinized venous blood of healthy volunteers and patients in the medical intensive care unit by Ficoll-Hypaque gradient centrifugation and sedimentation in 5% dextran. This method yielded approximately 2×10^6 cells/mL, with PMNs representing more than 95% of the cells isolated. Murine PMNs were similarly isolated from blood obtained by cardiac puncture. Once isolated, PMNs were cultured in RPMI 1640 supplemented with 10% (vol/vol) fetal bovine serum (FBS; Sigma-Aldrich) and 1% (vol/vol) penicillin-streptomycin or placed in Hanks balanced salt solution (HBSS). The cells were maintained at 37°C in humidified incubators supplemented with 5% CO_2.

CLP and isolation of PMNs from blood and peritoneal fluid

Female C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME) at 6 to 8 weeks old were anesthetized by the intraperitoneal injection of a mixture of ketamine (Abbott Laboratories, Abbott Park, IL) and xylazine (Lloyd Laboratories, Shenandoah, IA), and the cecum was exposed by a 1- to 2-cm midline incision on the anterior abdomen. A 3-0 silk suture was then used to tightly ligate the cecum at its base without causing bowel obstruction. The cecum was then punctured through-and-through thrice with an 18-gauge needle. Sham-operated control animals underwent identical laparotomy but did not undergo cecal ligation and puncture (CLP). In all animals, the abdominal incision was closed, and 1 mL of saline was administered subcutaneously for fluid resuscitation. Animals were killed after surgery, and blood was collected by cardiac puncture. PMNs were isolated using the Ficoll-Hypaque method as previously described.[14] In another set of experiments, cells from the peritoneal cavity were harvested after CLP by washing the cavity with 3 mL of phosphate-buffered saline (PBS) and counted. The percentage of neutrophils in the peritoneal cavity was calculated based on their morphologic appearance in a cell smear preparation on a glass slide prepared using a cytocentrifuge (Cytospin 3; Thermo Electron, Waltham, MA) followed by staining with the Diff-Quick (Fisher Scientific, Pittsburgh, PA) method.

Treatment of mice with bacterial lipopolysaccharide

Female C57BL/6 mice (The Jackson Laboratory) at 6 to 8 weeks old were administered LPS (1 mg/kg) by intraperitoneal injection. At indicated times after LPS challenge, animals were killed, and PMNs were isolated from the blood. All experiments were approved by the University of Michigan Committee on Use and Care of Animals.

Patient selection criteria

Patients in the Medical Intensive Care Unit (MICU) at the University of Michigan Medical Center, Ann Arbor, MI, were prospectively identified as having septic shock according to definitions of the American College of Chest Physicians and Society for Critical Care Medicine Consensus Conference.[15] Patients were excluded if they were younger than 18 years old, pregnant, or had hematologic malignancies. All patients who met entry criteria were enrolled within 24 hours of the onset of sepsis. Nonseptic patients in the MICU and healthy volunteers served as controls. The Institutional Review Board at the University of Michigan Medical School approved this study, and informed consent was obtained in accordance with the Declaration of Helsinki from each subject.

PMN chemotaxis assays

PMN chemotaxis was assayed using a 12-well Boyden chemotaxis chamber (Neuroprobe, Cabin John, MD). As previously described,[16] chemotaxis was expressed as the number of PMNs per high-power (×100) field (hpf) migrating through a 3-μm polycarbonate membrane filter. Migration was induced by fMLP, IL-8, or LTB_4.

HL-60 culture and transfection

HL-60 cells (CCL-240; ATCC, Manassas, VA) were maintained at 37°C in Iscove modified Dulbecco medium supplemented with 10% (vol/vol) FBS and 1% (vol/vol) penicillin-streptomycin in a humidified atmosphere of 95% air and 5% CO_2. Differentiation of HL-60 cells into PMN-like cells was induced by incubating them in 1.25% DMSO for 6 days. Cells were transfected with either a pcDNA3-VP16-PPAR-γ construct or control vector (pcDNA3). The constitutively active VP16-PPAR-γ fusion cDNA was obtained from Dr Mitchell Lazar (University of Pennsylvania, Philadelphia) and subcloned into the mammalian expression vector pcDNA3. HL-60 cells were transfected using the Amaxa Nucleofector system (Gaithersburg, MD) according to the manufacturer's guidelines (program T-19). After transfection, cells were washed and then grown in the presence of 10% FBS. Some transfected cells were used for chemotaxis assays, performed 24 hours after completion of transfection. Other transfected cells were stimulated with fMLP or vehicle. These cells were then lysed and analyzed for phosphorylation of ERK by Bio-Plex.

Reverse transcriptase–polymerase chain reaction amplification of PMN mRNA

Total cellular RNA from PMNs was isolated in 1 mL of TRIzol reagent (Invitrogen, Carlsbad, CA) following the manufacturer's protocol. Expression of mRNA was determined semiquantitatively using a reverse transcriptase–polymerase chain reaction (RT-PCR) system kit (Access; Promega, Madison, WI) according to the manufacturer's instructions. The following primer pairs were used for specific mRNA amplification: PPAR-γ forward, 5′-TCTCTCCGTAATGGAAGACC-3′; PPAR-γ reverse, 5′-GCATTATGAGACATCCCCAC-3′; β-actin forward, 5′-GTGGGGCGCCCCAGGCACCA-3′; β-actin reverse, 5′-GCTCGGCCGTGGTGGTGAAGC-3′. After amplification, the cDNA products were separated on a 2% agarose gel containing 0.3 mg/mL ethidium bromide. Bands were visualized and photographed using UV transillumination. Real-time quantitative reverse transcriptase–polymerase chain reaction (RT-PCR, TaqMan) of PMN mRNA was also performed. Gene-specific primers and probes were designed using Primer Express software (PerkinElmer Life and Analytical Sciences, Waltham, MA; Applied Biosystems, Foster City, CA). The sequences for PPAR-γ were as follows: 5′-GGCTTCATGACAAGGGAGTTTC-3′ (forward), 5′-AACTCAAACTTGGGCTCCATAAAG-3′ (reverse), 5′-AAAGAGCCTGCGAAAGCCTTTTGGTG-3′ (probe).

Oligonucleotide primers and TaqMan probe for β-actin (internal control) were purchased from Applied Biosystems. The real-time quantitative RT-PCR was performed essentially following the manufacturer's protocol. Briefly, the reaction mixture contained 5.5 mM $MgCl_2$, 500 μM dNTP, 2.5 μM random hexamers, 200 nM FAM probe, and forward and reverse primers at 600 nM in a final volume of 25 μL and was analyzed in an ABI PRISM 7700 sequence detection system. Relative quantification of PPAR-γ mRNA levels at specific time points was plotted as fold change compared with vehicle-treated (control) PMNs, arbitrarily assigned a value of 1. β-Actin measurements were used for normalization.

Bio-Plex phosphoprotein analysis

Cells at a concentration of 1.5×10^5/mL were treated. At each time point, cells were collected, and protein lysates were prepared using the Bio-Plex Cell Lysis kit (Bio-Rad, Hercules, CA). The presence of phosphorylated p38 MAPK and ERK was detected by, respectively, Bio-Plex Phospho-p38 MAPK (Thr180/Tyr182) and Phospho-ERK singleplex assay kits (Bio-Rad); the Bio-Plex Phosphoprotein Testing Reagent kit (Bio-Rad) was used according to the manufacturer's protocol. Briefly, 50 μL of cell lysate (adjusted to a concentration of 0.9 mg protein/mL) was plated in the

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96-well filter plate coated with beads coupled to anti–phospho-p38 or anti–phospho-ERK antibody. The plate was incubated overnight at room temperature on a platform shaker at 300 rpm. After a series of washes to remove unbound proteins, biotinylated detection antibodies, each specific for a different epitope, were added to the reaction. This resulted in formation of antibody complexes assembled around the target proteins. Streptavidin-phycoerythrin was then added to bind to the biotinylated detection antibodies on the bead surface. Data were acquired with the Bio-Plex 200 system and analyzed with the Bio-Plex Manager software from Bio-Rad Laboratories. Total ERK and p38 protein were determined using the Bio-Plex singleplex assay kit (Bio-Rad).

Confocal analysis for phalloidin staining

PMNs were plated onto glass coverslips and centrifuged at 900*g* for 5 minutes. Cells were then fixed with 4% paraformaldehyde in PBS for 10 minutes at room temperature. After fixation, cells were permeabilized for 10 minutes in PBS containing 0.1% Triton X-100, washed with PBS, and incubated with tetramethylrhodamine isothiocyanate (TRITC)–labeled phalloidin (1:40; Invitrogen) for 20 minutes at room temperature. Coverslips were mounted with ProLong Gold mounting medium containing diamidino-phenylindole (Invitrogen). Images were captured using an Olympus FV-500 laser-scanning confocal microscope (Tokyo, Japan) with a 100× oil immersion objective. Images were collected, and confocal *z*-sections were acquired at 0.5-mm intervals. Images were further analyzed and processed using Adobe Photoshop software (Adobe Systems, Mountain View, CA). The fluorescence intensity of F-actin was analyzed using the Image J program (National Institutes of Health, Bethesda, MD).

Adhesion of neutrophils to fibrinogen-coated plates

Neutrophils (10^6) were seeded onto 6-well tissue culture plates that 2 hours previously had been coated with 50 μg/mL fibrinogen (Sigma-Aldrich) and incubated at 37°C in a humidified 5% CO_2 atmosphere. Cells were treated and washed with PBS to remove the nonadherent cells. Adherent cells were removed by trypsinization and counted.

Statistical analysis

Data are represented as means plus or minus SEM and were analyzed with the Prism 4.0 statistical program (GraphPad Software, San Diego, CA). Groups were compared using one-sided ANOVA or one-sided Student *t* test as applicable. Differences were considered significant if *P* was less than .05.

Results

TNF-α and IL-4 increase PPAR-γ expression in human PMNs

To assess regulation of PPAR-γ expression in human PMNs, we performed both mRNA and protein analysis. PMNs obtained from healthy volunteers showed constitutive PPAR-γ expression. Treatment of these PMNs with either the proinflammatory cytokine TNF-α (20 ng/mL) or the anti-inflammatory cytokine IL-4 (20 ng/mL) enhanced expression of PPAR-γ mRNA in a time-dependent manner, with expression increasing throughout the first 8 hours after treatment (Figure 1A,B). In contrast, treatment with LPS did not result in significant changes in PPAR-γ mRNA levels. We then assessed PPAR-γ protein expression in human PMNs by Western immunoblotting. As with the mRNA analysis, we observed constitutive PPAR-γ protein expression with enhanced expression after treatment with TNF-α or IL-4 for 16 hours (Figure 1C). Treatment with LPS did not result in a significant change in PPAR-γ protein expression.

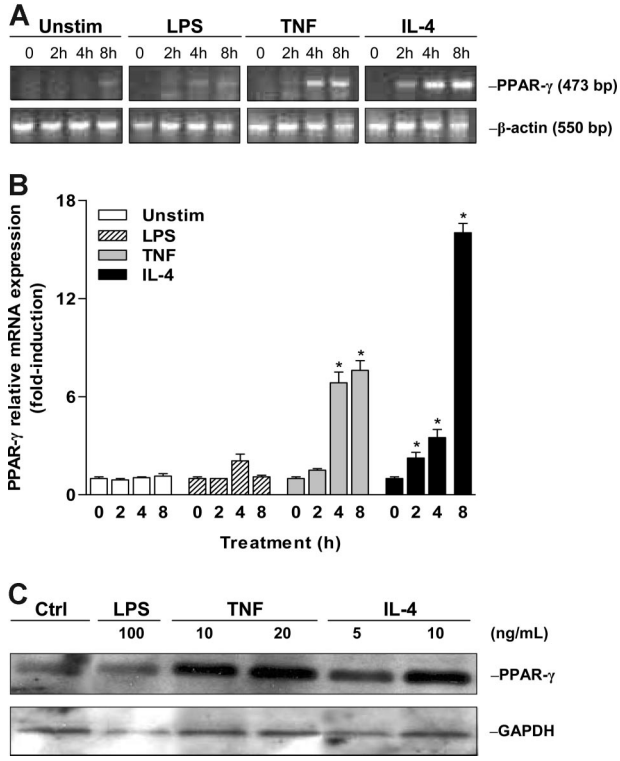

Figure 1. PPAR-γ is expressed in human PMNs. Messenger RNA expression of PPAR-γ by isolated PMNs was analyzed by (A) semiquantitative and (B) quantitative RT-PCR in PMNs after treatment (0-8 hours) with TNF-α (20 ng/mL), IL-4 (20 ng/mL), or LPS (100 ng/mL). *$P < .05$ compared with DMSO (vehicle) treated cells. Results are expressed as the average of 3 independent experiments performed in quadruplicate. Error bars represent SEM. (C) Western immunoblotting after treatment for 16 hours with TNF-α (10 and 20 ng/mL), IL-4 (5 and 10 ng/mL), or LPS (100 ng/mL). Equal loading was confirmed by stripping and probing with antibody against GAPDH. Gel and blot shown are representative of 3 separate experiments.

PPAR-γ activation inhibits PMN chemotaxis in response to fMLP or IL-8

Effective antibacterial activity by PMNs requires migration to the site of infection, representing a chemotactic response to specific chemoattractants. IL-8, a CXC chemokine produced by several cell types, is a potent chemotactic factor for PMNs.[17] Formylmethionyl peptides such as fMLP, which are generated by bacteria as a by-product of protein synthesis, likewise result in robust chemotaxis of PMNs.[18] Maximal doses of either IL-8 (100 ng/mL) or fMLP (10^{-7} M) induced significant migration of human PMNs, compared with HBSS. Migration of PMNs in response to fMLP was significantly reduced, in a dose-dependent manner, when cells were preincubated in vitro with either troglitazone (1-10 μM) or 15d-PGJ_2 (1-10 μM) for 2 hours before assessment of chemotaxis (Figure 2A). At the highest dose tested (10 μM), approximately 50% and 70% inhibition of chemotaxis was observed with troglitazone and 15d-PGJ_2, respectively. Similar results were seen when the chemokine IL-8 was used instead of fMLP as the chemoattractant (Figure 2B). The observed inhibition of chemotaxis was not due to alterations in cell viability because no significant differences in apoptosis (ssDNA method) or cell death (trypan blue staining) were seen between the PPAR-γ agonist versus vehicle (DMSO)–treated groups (data not shown). Given that results for IL-8 and fMLP were essentially identical, we used only fMLP in subsequent experiments.

Because troglitazone and 15d-PGJ_2 are known to induce effects independent of PPAR-γ activation, we transfected HL-60 cells with

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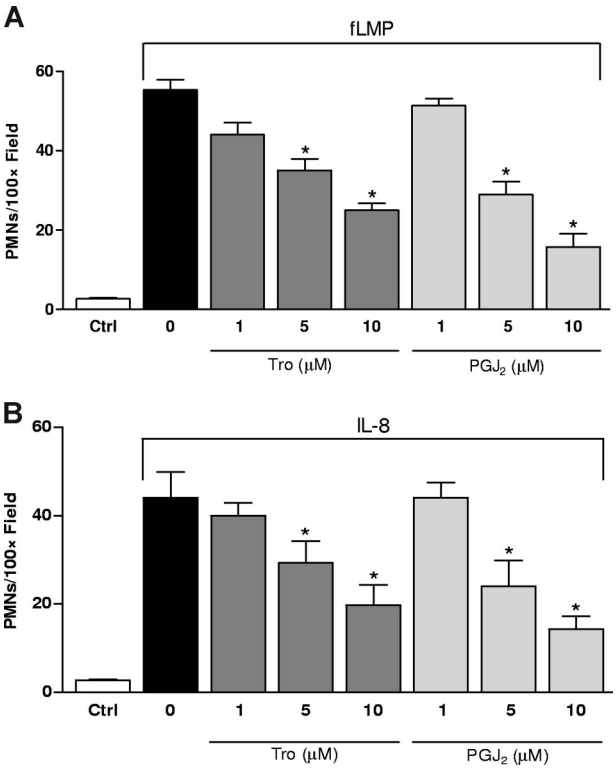

Figure 2. PPAR-γ ligands decrease human PMN chemotaxis. Cells were isolated and treated with troglitazone (Tro; 1-10 μM) or 15d-PGJ_2 (PGJ_2; 1-10 μM) for 2 hours before stimulation with (A) 10^{-7} M fMLP or (B) 100 ng/mL IL-8 for assessment of chemotaxis. All data are expressed as mean PMN count per high-powered field at 100× magnification (PMNs/hpf). $^*P < .05$ compared with DMSO (vehicle) treated cells. Results are expressed as the average of 2 independent experiments performed in triplicate. Ctrl, unstimulated cells (without fMLP or IL-8 treatment). Error bars represent SEM.

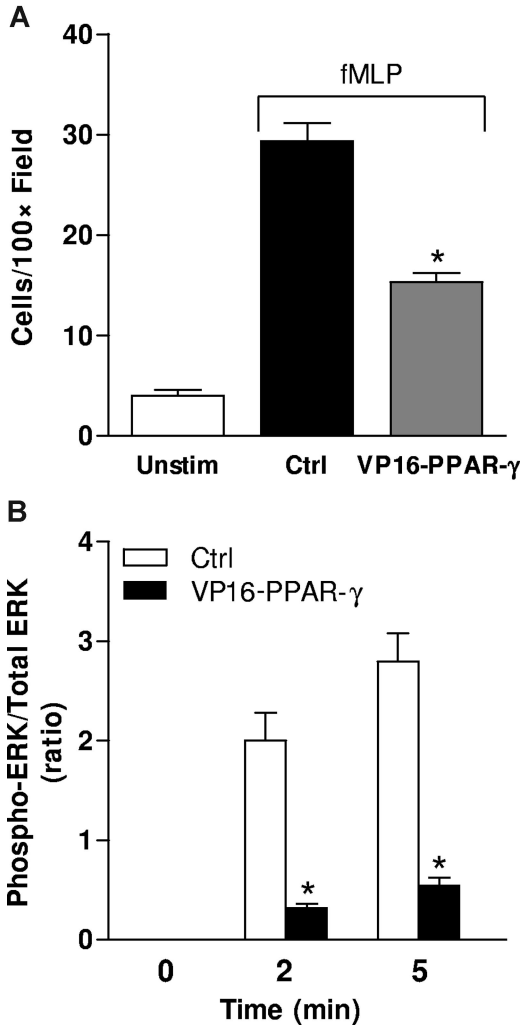

Figure 3. Constitutively active PPAR-γ decreases fMLP-induced HL-60 chemotaxis and ERK-1/2 phosphorylation. (A) DMSO-treated HL-60 cells transiently transfected with either the pcDNA3-VP16-PPAR-γ construct or pcDNA3 (control vector), were stimulated with 10^{-7} M fMLP and assessed for chemotaxis. All data are expressed as mean HL-60 count per high-powered field at 100× magnification (cells/hpf). $^*P < .05$ compared with cells transfected with control vector (Ctrl). Results are expressed as the average of 3 independent experiments performed in triplicate. Unstim, unstimulated cells without fMLP treatment. (B) Cells transfected as in panel A were lysed 0, 2, 5, and 10 minutes after treatment with 10^{-7} fLMP, and phosphorylation of ERK-1/2 was determined by Bio-Plex assay. The ratio of phosphorylated to total ERK-1/2 is displayed. No phosphorylation of ERK-1/2 was observed at 10 minutes. $^*P < .05$ compared with cells transfected with control vector (Ctrl). Results are expressed as the average of 3 independent experiments performed in triplicate. Error bars represent SEM.

a constitutively active, ligand-independent, PPAR-γ construct, pcDNA3-VP16-PPAR-γ. Before transfection, HL-60 cells were cultured in 1.25% DMSO for 6 days, an established procedure to produce PMN-like cells. Transfection of pcDNA3-VP16-PPAR-γ into DMSO-treated HL-60 cells resulted in significant attenuation of fMLP (10^{-7} M)–induced chemotaxis compared with that by cells transfected with control plasmid, pcDNA3 (Figure 3A). This result suggests a direct inhibitory role for PPAR-γ on PMN chemotactic responses.

PPAR-γ activation decreases phosphorylation of ERK-1/2 in human PMNs after exposure to fMLP

Previous work in our laboratory has shown an important role for MEK1/ERK-1/2 in human PMN chemotaxis.[19] Chemotaxis of human PMNs stimulated by either IL-8 or fMLP was found to be inhibited by U0126, a specific inhibitor of MEK1/ERK-1/2, but not by LY294002 or SB20358, which, respectively, inhibits phosphatidylinositol 3-kinase and p38 mitogen-activated protein kinase (MAPK).[19] We therefore examined the effect of PPAR-γ ligands on activation of MAPK pathways, specifically the p38 and p42/44 (ERK-1/2) MAPK cascades. Stimulation with fMLP resulted in a time-dependent increase in phosphorylated ERK-1/2 that was maximal 10 minutes after stimulation and absent at the 30-minute point. Two-hour pretreatment of PMNs with 15d-PGJ_2 (10 μM) totally abolished fMLP-induced ERK-1/2 activation at all time points (Figure 4A), whereas pretreatment with troglitazone (10 μM) markedly reduced but did not eliminate ERK phosphorylation (Figure 4A). fMLP-induced ERK phosphorylation was also reduced by transfection with the constitutively active PPAR-γ construct pcDNA3-VP16-PPAR-γ (Figure 3B). Phosphorylation of p38, however, was not reduced by pretreatment with either PPAR-γ agonist (Figure 4B). These findings suggest that PPAR-γ activation may block PMN chemotaxis by selectively inhibiting the ERK-1/2 pathway.

Troglitazone inhibits fMLP-induced actin polymerization in human PMNs

Movement of PMNs is accompanied by formation of bands with high concentrations of polymerized actin (filamentous actin, or F-actin).[20] The amount of F-actin has been shown to be increased by molecules such as fMLP,[21] as we also observed. This fMLP-stimulated increase in F-actin was significantly inhibited by treatment with troglitazone, but, interestingly, no effect was seen

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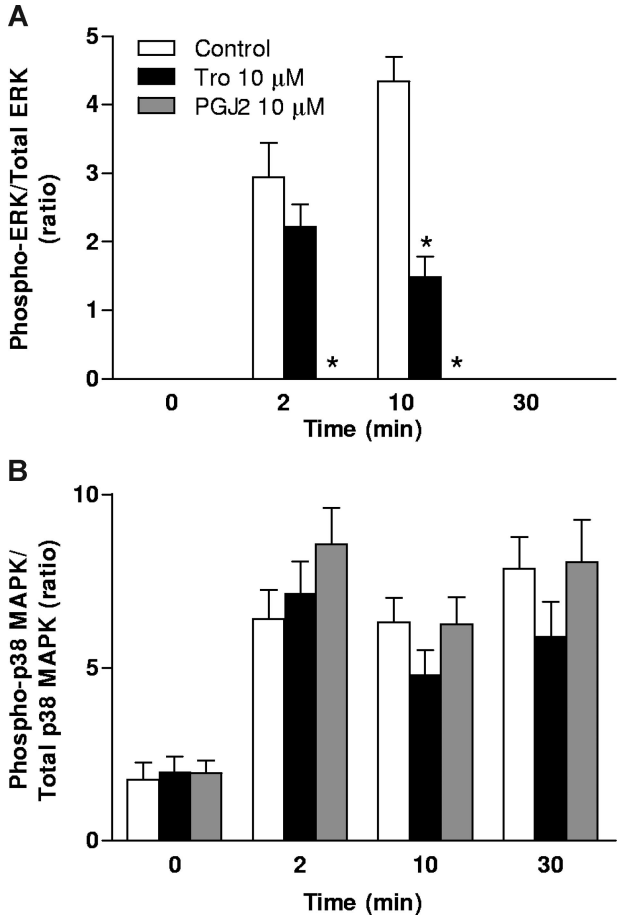

Figure 4. PPAR-γ ligands decrease fLMP-induced phosphorylation of ERK-1/2 in human PMNs. PMNs were pretreated for 2 hours with or without troglitazone (Tro; 10 μM) or 15d-PGJ_2 (PGJ2; 10 μM) before stimulation with 10^{-7} M fMLP. Cells were lysed at 0, 2, 10, and 30 minutes after fMLP treatment. Bio-Plex assay was then used to measure amounts of (A) phospho-ERK and (B) phospho-p38. Figures show the ratio of phosphorylated protein to the corresponding total protein. $^*P < .05$ compared with DMSO (Ctrl) treated cells. Results are expressed as the average of 3 independent experiments performed in triplicate. Error bars represent SEM.

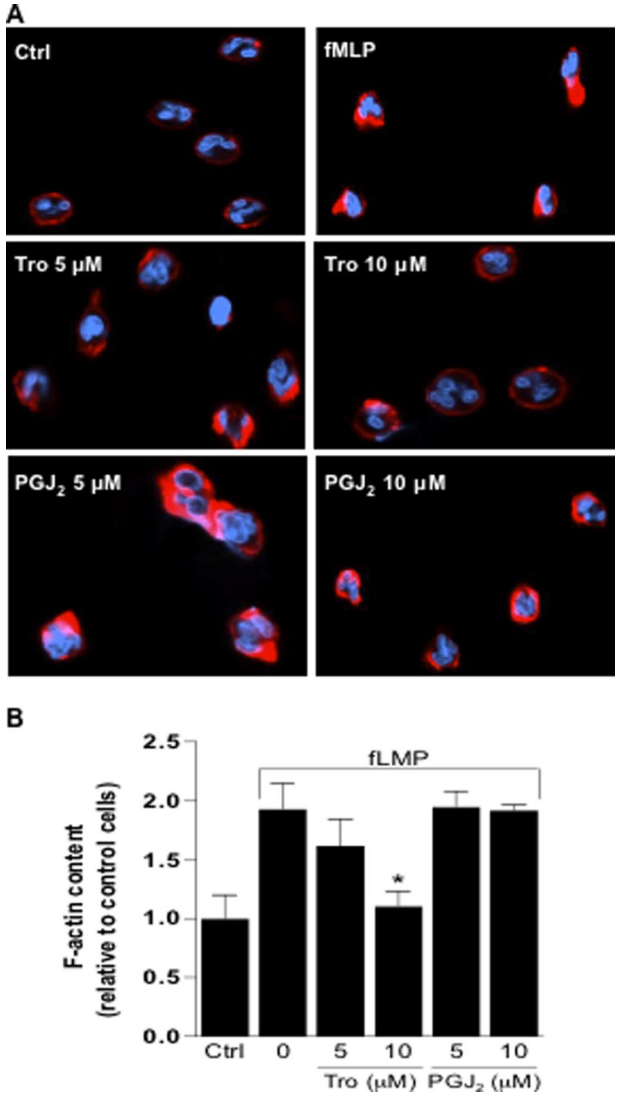

Figure 5. Troglitazone, but not 15d-PGJ_2, decreases polarization and actin polymerization in response to fMLP. PMNs were pretreated with troglitazone (5 or 10 μM) or with 15d-PGJ_2 (5 or 10 μM) for 2 hours, then stimulated with fMLP (10^{-7} M) for 10 minutes. (A) Cells were stained with TRITC-phalloidin, and confocal microscopy was used to analyze F-actin distribution and morphologic changes. Results shown are representative of 3 independent experiments. All panels depict stimulation with fMLP except control (Ctrl). (B) The change in F-actin content is expressed as a relative fold change in mean fluorescence intensity, with the fluorescence intensity of unstimulated control cells set as 1. $^*P < .05$ compared to DMSO-treated cells. Tro indicates troglitazone; PGJ_2, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2. Error bars represent SEM.

with 15d-PGJ_2 at any of the doses tested (Figure 5A). Quantitative analysis confirmed a dose-dependent reduction in actin polymerization with troglitazone but not with 15d-PGJ_2 (Figure 5B).

PPAR-γ ligands inhibit fMLP-induced adhesion of PMNs to fibrinogen

Cells migrating through a solid matrix must adhere to the substrate, such as fibrinogen, to move. fMLP was previously shown to enhance PMN adhesion to fibrinogen and thus their ability to move on this substrate. To test the ability of PPAR-γ ligands to inhibit PMN adhesion to fibrinogen in response to fMLP, we seeded PMNs onto fibrinogen-coated plates. After 20 minutes, cells were treated with troglitazone (5 or 10 μM) or 15d-PGJ_2 (5 or 10 μM) for 2 hours, followed by treatment with fMLP for 45 minutes. After washing to remove nonadherent cells, adherent cells were removed by trypsinization and counted. Compared with controls, both agonists significantly reduced the number of fibrinogen-adherent PMNs in a dose-dependent manner (Figure 6).

PMNs from septic patients or mice show increased expression of PPAR-γ

Patients with sepsis are frequently observed to have significant immunosuppression characterized by a decreased host response to infectious pathogens.[7] Inhibition of PMN migratory activity is suspected to be a clinically important event during sepsis.[5] Because of the suppressive effects of PPAR-γ activation on human PMN chemotaxis and the up-regulation of PPAR-γ that was observed with selective cytokines associated with sepsis, we examined expression of PPAR-γ in PMNs isolated ex vivo from septic patients compared with controls. PMNs were isolated from healthy controls and nonseptic and septic patients in the MICU. RNA was immediately extracted, and levels of PPAR-γ and β-actin mRNA were determined by real-time quantitative RT-PCR. PMNs isolated from the majority of sepsis patients showed significant increases in PPAR-γ mRNA expression compared with PMNs from either control group (Figure 7A left).

To further investigate the in vivo effects of sepsis on PMNs, including PPAR-γ expression and chemotaxis, we used 2 murine

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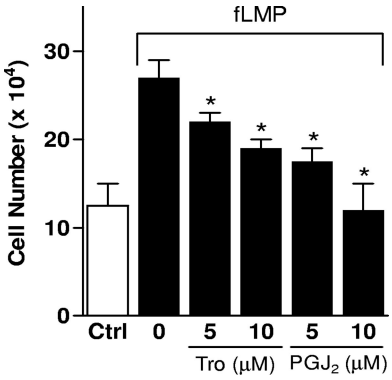

Figure 6. PPAR-γ ligands decrease fMLP-induced adhesion of PMNs to fibrinogen. Human PMNs were isolated and seeded onto fibrinogen-coated plates. After 20 minutes, PMNs were treated with troglitazone (5 or 10 μM) or 15d-PGJ_2 (5 or 10 μM) or vehicle for 2 hours followed by treatment with 10^{-7} fMLP for 45 minutes. Adherent cells were trypsinized and counted using a Coulter counter. *$P < .05$ compared with DMSO-treated cells. Results are expressed as the average of 3 independent experiments performed in quadruplicate. Tro indicates troglitazone; PGJ_2, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2; Ctrl, without fMLP treatment. Error bars represent SEM.

models of sepsis, one induced by intraperitoneal injection of LPS (1 mg/kg), the other induced by CLP. As we had found in human sepsis patients, both LPS administration and CLP produced significant time-dependent increases in blood PMN expression of PPAR-γ mRNA at 6 and 12 hours after induction of sepsis (Figure 7A center and right).

PPAR-γ inhibition reverses sepsis-induced suppression of murine PMN chemotaxis and recruitment

To test the hypothesis that suppression of PMN chemotaxis and recruitment observed during sepsis is mediated, at least in part, by PPAR-γ, we inhibited PPAR-γ activation during the septic response. Specifically, we administered GW9662, a PPAR-γ antagonist, or vehicle to mice 30 minutes after induction of sepsis by LPS administration. Six hours later, PMNs were isolated, and the chemotactic response to leukotriene B_4 was assessed. Chemotaxis by PMNs from vehicle-treated septic mice was significantly suppressed compared with that seen in PMNs from healthy mice, but treatment with GW9662 restored chemotaxis essentially to control levels (Figure 7B).

In the clinical setting, chemotaxis is directed toward the site of infection. We accordingly assessed the effect of GW9662 on infection-site recruitment using a different model of murine sepsis, CLP. Animals received GW9662 or vehicle 30 minutes after CLP or sham operation and the number of PMNs in the peritoneal cavity was assessed 6 hours after the operation. GW9662 significantly increased the number of PMNs in the peritoneal cavity compared with vehicle-treated controls (Figure 7C). GW9662 administration had no effect on PMN expression of PPAR-γ (data not shown).

Discussion

In this study, we show several significant findings. First, human PMNs constitutively express the nuclear hormone receptor PPAR-γ. Incubation with either TNF-α or IL-4, cytokines important in regulating the septic response, results in up-regulation of PPAR-γ in these cells. Second, treatment of PMNs with PPAR-γ ligands or transfection with constitutively active PPAR-γ results in inhibition of chemotaxis. Inhibition appears to occur by down-regulation of ERK phosphorylation and is accompanied by reductions in actin polymerization and PMN adhesion to a fibrinogen substrate, both required for movement of PMNs in a solid matrix. Third, PMNs

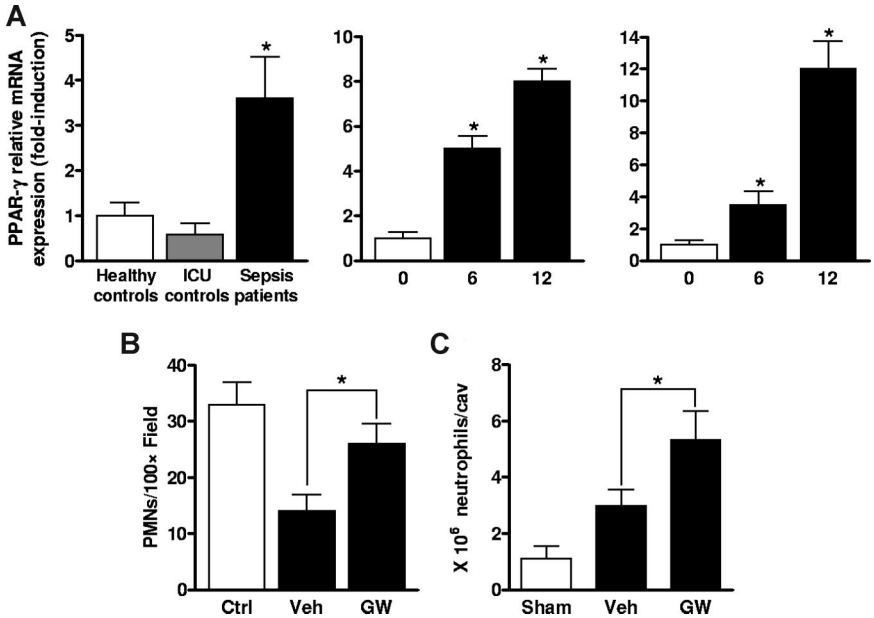

Figure 7. Sepsis-induced inhibition of PMN chemotaxis is reversed by blocking activation of PPAR-γ, expression of which is increased in sepsis. (A left) Peripheral blood PMNs were isolated from control subjects (healthy volunteers [n = 7] and nonseptic patients in the intensive care unit [n = 3]) and patients with sepsis (n = 14). Relative expression of PPAR-γ was plotted as the fold change from healthy controls. PPAR-γ mRNA levels at specific time points after induction of sepsis by (center) LPS administration (1 mg/kg) or by (right) CLP were plotted as the fold change compared with PMNs from vehicle-treated or sham-operated (control) mice, arbitrarily assigned a value of 1. In all panels, β-actin measurements were used for normalization. *$P < .05$ compared with each control group. (B) Blood PMNs were isolated from mice (n = 8 mice/group) that were rendered septic by intraperitoneal administration of LPS (1 mg/kg) 6 hours previously. Some mice also received GW9662 (1 mg/kg; GW) or vehicle (Veh) by intraperitoneal injection 30 minutes after LPS. Chemotactic responses to leukotriene B_4 (10^{-7} M) were measured and are reported as the number of cells per high-power field. *$P < .05$ compared with control (Veh). (C) Mice (n = 4/group) underwent CLP (■) or sham operation (□). Some mice also received GW9662 (1 mg/kg; GW) or vehicle (Veh) intraperitoneally 30 minutes after the operation. Six hours later animals were killed, and the number of PMNs in the peritoneal cavity was determined as described in "Methods." *$P < .05$ compared with control (Veh). Error bars represent SEM.

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isolated from septic patients or mice express higher levels of PPAR-γ compared with controls. Finally, the sepsis-induced inhibition of PMN chemotaxis and recruitment is reversed by blocking PPAR-γ activation in vivo. We suggest that these findings, taken together, provide a coherent explanation for the previously observed reduction in chemotaxis by PMNs from septic patients.[4,5]

Cytokines have been shown to regulate PPAR-γ expression and function. Treatment of macrophages with IL-4, for instance, results in enhanced expression and activity of PPAR-γ.[22] We similarly found that IL-4 increased PPAR-γ messenger RNA and protein expression in PMNs. Interestingly, we found that treatment with the proinflammatory cytokine TNF-α also increased PPAR-γ expression in PMNs. In other cell types, TNF-α was shown to decrease PPAR-γ expression.[23] We did not investigate the effects of IL-4 or TNF-α on expression of 12/15-lipoxygenase, which produces the PPAR-γ ligands 15-hydroxyeicosatetraenoic acid (15-HETE) and 13-hydroxyoctadecadienoic acid (13-HODE), although 15-lipoxygenase is known to be expressed in human neutrophils.[24] However, IL-4 up-regulates expression of 12/15-lipoxygenase in macrophages, leading to increased production of 15-HETE and 13-HODE, which would suggest that the same could be true for neutrophils.[22,25] The situation may be different for TNF-α, whereby to our knowledge no effects on 12/15-lipoxygenase have been reported. In contrast to these effects of cytokines, direct exposure to LPS in vitro did not alter PMN expression of PPAR-γ in our study. Induction of murine sepsis either by administration of LPS or by CLP, however, did increase expression of PPAR-γ by PMNs. This is in agreement with the findings of Leininger et al[26] for LPS-induced endotoxemia, although that study examined all peripheral blood leukocytes as a group. We did not investigate the mechanism through which PPAR-γ expression was up-regulated, but our in vitro results suggest that the increased PPAR-γ expression observed in vivo is likely to have been an effect of sepsis-induced cytokines.

PPAR-γ ligands exert a wide range of effects on leukocytes. Specifically, PPAR-γ ligands were previously shown to inhibit eosinophil[27] and monocyte[28] chemotaxis in a dose-dependent manner. In addition, in vivo animal studies suggest that PMN migration to sites of inflammation is impaired after PPAR-γ ligand administration.[29,30] Inhibition of PMN chemotaxis in our study was similar irrespective of the specific type of ligand used, either troglitazone or 15d-PGJ_2, and regardless of whether fMLP or IL-8 was used as the chemoattractant. Furthermore, transfection of a constitutively active PPAR-γ construct into HL-60 cells induced to differentiate into PMN-like cells inhibited chemotaxis by these cells, thus providing compelling evidence of a PPAR-γ–specific effect.

To investigate the migration-suppressing mechanism of PPAR-γ ligands, we examined their effects on MAPK activation. The ERK-1/2 MAPK pathway was shown to play an important role in the migration of several cells, including PMNs.[31-33] We found that troglitazone and 15d-PGJ_2, as well as a constitutively active PPAR-γ construct, reduced activation of the ERK-1/2 MAPK signal transduction pathway induced by fMLP. In contrast, although one study showed that chemotaxis of THP-1 cells, a human monocytic cell line, was inhibited by treatment with PPAR-γ ligands, no inhibition of MCP-1–induced MAPK activation was observed.[28] Inhibition of PMN chemotaxis in our study did not occur as a result of chemotaxin receptor down-regulation, because surface expression of PMN CXCR1, CXCR2, or FPR was not altered with either 15d-PGJ_2 or troglitazone treatment (data not shown).

Migration of PMNs also requires, in part, adhesion to fibrinogen and polymerization of actin, both of which we found to be inhibited by PPAR-γ ligands. Previous studies have shown that the rate of PMN locomotion correlates with the extent of actin polymerization[21] and that actin polymerization is increased by fMLP.[34] Interestingly, this latter study found that TNF-α inhibited actin polymerization, which parallels our findings for PPAR-γ ligands. Similar to our results, the PPAR-γ ligand rosiglitazone has been found to inhibit fMLP-induced actin polymerization in monocytes at least partially by reducing Akt phosphorylation.[35] The reasons for the disparate inhibitory effects on actin polymerization between troglitazone and 15d-PGJ_2 in this study remain unclear, but possibly reflect the complexity of interactions between 15d-PGJ_2 and actin. One study showed that 15d-PGJ_2 interacts directly with the actin molecule in neuroblastoma cells to depolymerize F-actin and block polymerization, but only modest effects were seen at a concentration of 20 μM.[36] We tested only concentrations of 5 and 10 μM because these concentrations had proved adequate to inhibit chemotaxis.

The rapid effects observed in this study occur in a shorter time span than is typical for effects mediated by nuclear transcription. This might be viewed as arguing for a PPAR-γ–independent mechanism despite similar effects shown by 2 structurally dissimilar ligands and by a constitutively active PPAR-γ construct. Even more rapid effects involving PPAR-γ ligands have been described, however. In vascular smooth muscle cells, PPAR-γ ligands were shown to activate ERK within 15 minutes by the phosphatidylinositol-3-kinase pathway.[37] Involvement of PPAR-γ was not explicitly examined, but the investigators suggest that consistent results with several PPAR-γ ligands indicate that effects are mediated by the receptor. In Chinese hamster ovary cells, treatment with tetradecanoyl phorbol acetate results in PPAR-γ exiting the nucleus within 30 minutes.[38] This effect was shown to be due to direct interactions between PPAR-γ and MEK.

Extending our in vitro data, we observed increased expression of PPAR-γ in PMNs from septic patients, compared with those from healthy controls and nonseptic MICU patients. Similarly, Soller et al[39] recently observed enhanced PPAR-γ expression in T cells isolated from septic patients compared with control subjects and showed that sera of septic patients contained elevated levels of PPAR-γ activators. Several studies have also shown alterations in PMN function during the septic response.[4,6,40-42] Two recent studies have highlighted impairment of PMN chemotactic responses during sepsis. Tavares-Murta et al[43] isolated PMNs from 20 patients with sepsis and examined their in vitro chemotaxis to fMLP and LTB_4. PMN chemotactic responses were significantly impaired in septic patients compared with controls. In addition, Chishti et al[5] observed impaired PMN chemotaxis to IL-8 but, interestingly, increased expression of the β-integrin CD11b in 15 patients with sepsis compared with 8 healthy controls.

We saw a similar increase in expression of PPAR-γ mRNA in PMNs isolated from mice in which sepsis had been induced by LPS or CLP. Because inhibition of ex vivo chemotaxis after endotoxemia was shown in animals,[44] we hypothesized that this inhibition could result from endogenous PPAR-γ ligands activating the elevated receptor levels we observed. We accordingly extended our in vitro studies by investigating effects of the PPAR-γ antagonist GW9662 on PMN chemotaxis and recruitment after LPS administration or CLP. We found that blocking PPAR-γ activation significantly reversed the suppression of chemotaxis observed after LPS

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administration and increased recruitment of PMNs to the peritoneal cavity after CLP. Analogously, in endotoxin-induced acute lung injury, the PPAR-γ ligand rosiglitazone was found to reduce neutrophil infiltration into lung tissues with this effect being reversed by GW9662.[45] Interestingly, endotoxemia is known to up-regulate inducible nitric oxide synthase and to increase NO production.[46] NO reacts nonenzymatically with endogenous unsaturated fatty acids to produce nitrated fatty acids that are potent PPAR-γ ligands.[47]

In conclusion, our results show that PPAR-γ activation results in a significant reduction in the chemotactic response of human PMNs in vitro. PMNs from patients with sepsis show enhanced PPAR-γ expression, which may be due to cytokines such as TNF-α and IL-4 that are released during the evolution of sepsis. The inhibited migration of these cells in septic patients could occur as a result of PPAR-γ activation. Our study suggests that inhibition of PPAR-γ activation may therefore result in enhanced PMN chemotaxis and recruitment and improved host immunity in diseases such as sepsis.

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Authorship

Contribution: R.C.R. designed experiments, performed research, and wrote the paper; V.R.N., V.G.K., J.E.M., and M.W.N. performed research; and T.J.S. supervised the project and edited the paper.

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References

1. Mayer-Scholl A, Averhoff P, Zychlinsky A. How do neutrophils and pathogens interact? Curr Opin Microbiol. 2004;7:62-66.
2. Kasama T, Strieter RM, Standiford TJ, Burdick MD, Kunkel SL. Expression and regulation of human neutrophil-derived macrophage inflammatory protein 1 alpha. J Exp Med. 1993;178:63-72.
3. Van Haastert PJ, Devreotes PN. Chemotaxis: signalling the way forward. Nat Rev Mol Cell Biol. 2004;5:626-634.
4. Bignold LP, Rogers SD, Siaw TM, Bahnisch J. Inhibition of chemotaxis of neutrophil leukocytes to interleukin-8 by endotoxins of various bacteria. Infect Immun. 1991;59:4255-4258.
5. Chishti AD, Shenton BK, Kirby JA, Baudouin SV. Neutrophil chemotaxis and receptor expression in clinical septic shock. Intensive Care Med. 2004; 30:605-611.
6. Frevert CW, Warner AE, Kobzik L. Defective pulmonary recruitment of neutrophils in a rat model of endotoxemia. Am J Respir Cell Mol Biol. 1994; 11:716-723.
7. Reddy RC, Chen GH, Tekchandani PK, Standiford TJ. Sepsis-induced immunosuppression: from bad to worse. Immunol Res. 2001;24: 273-287.
8. Lemberger T, Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: a nuclear receptor signaling pathway in lipid physiology. Annu Rev Cell Dev Biol. 1996;12:335-363.
9. 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.
10. Reddy RC, Keshamouni VG, Jaigirdar SH, et al. Deactivation of murine alveolar macrophages by peroxisome proliferator-activated receptor-gamma ligands. Am J Physiol Lung Cell Mol Physiol. 2004;286:L613-L619.
11. Greene ME, Blumberg B, McBride OW, et al. Isolation of the human peroxisome proliferator activated receptor gamma cDNA: expression in hematopoietic cells and chromosomal mapping. Gene Expr. 1995;4:281-299.
12. Vaidya S, Somers EP, Wright SD, Detmers PA, Bansal VS. 15-Deoxy-Delta12,1412,14-prostaglandin J2 inhibits the beta2 integrin-dependent oxidative burst: involvement of a mechanism distinct from peroxisome proliferator-activated receptor gamma ligation. J Immunol. 1999;163: 6187-6192.
13. Imamoto E, Yoshida N, Uchiyama K, et al. Inhibitory effect of pioglitazone on expression of adhesion molecules on neutrophils and endothelial cells. Biofactors. 2004;20:37-47.
14. Boyum A. Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. Scand J Clin Lab Invest Suppl. 1968;97:77-89.
15. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Crit Care Med. 1992;20:864-874.
16. Richards KL, McCullough J. A modified microchamber method for chemotaxis and chemokinesis. Immunol Commun. 1984;13:49-62.
17. Baggiolini M, Dewald B, Moser B. Interleukin-8 and related chemotactic cytokines–CXC and CC chemokines. Adv Immunol. 1994;55:97-179.
18. Schiffmann E, Corcoran BA, Wahl SM. N-formylmethionyl peptides as chemoattractants for leucocytes. Proc Natl Acad Sci U S A. 1975;72: 1059-1062.
19. Tsai WC, Rodriguez ML, Young KS, et al. Azithromycin blocks neutrophil recruitment in pseudomonas endobronchial infection. Am J Respir Crit Care Med. 2004;170:1331-1339.
20. Haston WS. F-actin distribution in polymorphonuclear leucocytes. J Cell Sci. 1987;88(Pt 4):495-501.
21. Howard TH, Meyer WH. Chemotactic peptide modulation of actin assembly and locomotion in neutrophils. J Cell Biol. 1984;98:1265-1271.
22. Huang JT, Welch JS, Ricote M, et al. Interleukin-4-dependent production of PPAR-gamma ligands in macrophages by 12/15-lipoxygenase. Nature. 1999;400:378-382.
23. Kim MS, Sweeney TR, Shigenaga JK, et al. Tumor necrosis factor and interleukin 1 decrease RXRα, PPARα, PPARγ, LXRα, and the coactivators SRC-1, PGC-1α, and PGC-1β in liver cells. Metabolism. 2007;56:267-279.
24. Kuitert LM, Newton R, Barnes NC, Adcock IM, Barnes PJ. Eicosanoid mediator expression in mononuclear and polymorphonuclear cells in normal subjects and patients with atopic asthma and cystic fibrosis. Thorax. 1996;51:1223-1228.
25. Heydeck D, Thomas L, Schnurr K, et al. Interleukin-4 and -13 induce upregulation of the murine macrophage 12/15-lipoxygenase activity: evidence for the involvement of transcription factor STAT6. Blood. 1998;92:2503-2510.
26. Leininger MT, Portocarrero CP, Houseknecht KL. Peroxisome proliferator-activated receptor gamma1 expression in porcine white blood cells: dynamic regulation with acute endotoxemia. Biochem Biophys Res Commun. 1999;263:749-753.
27. Ueki S, Matsuwaki Y, Kayaba H, et al. Peroxisome proliferator-activated receptor gamma regulates eosinophil functions: a new therapeutic target for allergic airway inflammation. Int Arch Allergy Immunol. 2004;134(suppl 1):30-36.
28. Kintscher U, Goetze S, Wakino S, et al. Peroxisome proliferator-activated receptor and retinoid X receptor ligands inhibit monocyte chemotactic protein-1-directed migration of monocytes. Eur J Pharmacol. 2000;401:259-270.
29. Naito Y, Takagi T, Uchiyama K, et al. Suppression of intestinal ischemia-reperfusion injury by a specific peroxisome proliferator-activated receptor-gamma ligand, pioglitazone, in rats. Redox Rep. 2002;7:294-299.
30. Ito K, Shimada J, Kato D, et al. Protective effects of preischemic treatment with pioglitazone, a peroxisome proliferator-activated receptor-gamma ligand, on lung ischemia-reperfusion injury in rats. Eur J Cardiothorac Surg. 2004;25:530-536.
31. Nick JA, Avdi NJ, Young SK, et al. Common and distinct intracellular signaling pathways in human neutrophils utilized by platelet activating factor and FMLP. J Clin Invest. 1997;99:975-986.
32. Hii CS, Stacey K, Moghaddami N, Murray AW, Ferrante A. Role of the extracellular signal-regulated protein kinase cascade in human neutrophil killing of Staphylococcus aureus and Candida albicans and in migration. Infect Immun. 1999;67: 1297-1302.
33. Xythalis D, Frewin MB, Gudewicz PW. Inhibition of IL-8-mediated MAPK activation in human neutrophils by beta1 integrin ligands. Inflammation. 2002;26:83-88.
34. Kutsuna H, Suzuki K, Kamata N, et al. Actin reorganization and morphological changes in human neutrophils stimulated by TNF, GM-CSF, and G-CSF: the role of MAP kinases. Am J Physiol Cell Physiol. 2004;286:C55-64.
35. Singh N, Webb R, Adams R, et al. The PPAR-γ activator, Rosiglitazone, inhibits actin polymerisation in monocytes: involvement of Akt and intracellular calcium. Biochem Biophys Res Commun. 2005;333:455-462.
36. Aldini G, Carini M, Vistoli G, et al. Identification of actin as a 15-deoxy-Delta12,14-prostaglandin J2 target in neuroblastoma cells: mass spectrometric, computational, and functional approaches to

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investigate the effect on cytoskeletal derangement. Biochemistry (Mosc). 2007;46:2707-2718.

37. Takeda K, Ichiki T, Tokunou T, Iino N, Takeshita A. 15-Deoxy-Delta^{12,14}-prostaglandin J_2 and thiazolidinediones activate the MEK/ERK pathway through phosphatidylinositol 3-kinase in vascular smooth muscle cells. J Biol Chem. 2001;276:48950-48955.
38. Burgermeister E, Chuderland D, Hanoch T, Meyer M, Liscovitch M, Seger R. Interaction with MEK causes nuclear export and downregulation of peroxisome proliferator-activated receptor gamma. Mol Cell Biol. 2007;27:803-817.
39. Soller M, Tautenhahn A, Brune B, et al. Peroxisome proliferator-activated receptor gamma contributes to T lymphocyte apoptosis during sepsis. J Leukoc Biol. 2006;79:235-243.
40. Solomkin JS, Bass RC, Bjornson HS, Tindal CJ, Babcock GF. Alterations of neutrophil responses to tumor necrosis factor alpha and interleukin-8 following human endotoxemia. Infect Immun. 1994;62:943-947.
41. Bjornson AB, Somers SD. Down-regulation of chemotaxis of polymorphonuclear leukocytes following thermal injury involves two distinct mechanisms. J Infect Dis. 1993;168:120-127.
42. Territo MC, Golde DW. Granulocyte function in experimental human endotoxemia. Blood. 1976;47:539-544.
43. Tavares-Murta BM, Zaparoli M, Ferreira RB, et al. Failure of neutrophil chemotactic function in septic patients. Crit Care Med. 2002;30:1056-1061.
44. Hartiala KT, Langlois L, Goldstein IM, Rosenbaum JT. Endotoxin-induced selective dysfunction of rabbit polymorphonuclear leukocytes in response to endogenous chemotactic factors. Infect Immun. 1985;50:527-533.
45. Liu D, Zeng BX, Zhang SH, et al. Rosiglitazone, a peroxisome proliferator-activated receptor-gamma agonist, reduces acute lung injury in endotoxemic rats. Crit Care Med. 2005;33:2309-2316.
46. Bhattacharyya J, Biswas S, Datta AG. Mode of action of endotoxin: role of free radicals and antioxidants. Curr Med Chem. 2004;11:359-368.
47. Baker PR, Lin Y, Schopfer FJ, et al. Fatty acid transduction of nitric oxide signaling: multiple nitrated unsaturated fatty acid derivatives exist in human blood and urine and serve as endogenous peroxisome proliferator-activated receptor ligands. J Biol Chem. 2005;280:42464-42475.

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