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15-Deoxy-$\Delta^{12,14}$-PGJ_2 inhibits IL-6-induced Stat3 phosphorylation in lymphocytes

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Abbreviations: 15d-PGJ_2, 15-deoxy-$\Delta^{12,14}$-PGJ_2; IL-6R, IL-6 receptor; MAPK, mitogen-activated protein kinase; MNC, mononuclear cells; PKC, protein kinase C; PPARγ, peroxisome proliferators-activated receptor γ; SOCS, suppressor of cytokine signaling

Abstract

15-deoxy-$\Delta^{12,14}$-PGJ_2 (15d-PGJ_2) is a natural ligand that activates the peroxisome proliferators-activated receptor (PPAR) γ, a member of nuclear receptor family implicated in regulation of lipid metabolism and adipocyte differentiation. Recent studies have shown that 15d-PGJ_2 is the potent anti-inflammatory agent functioning via PPARγ-dependent and -independent mechanisms. Most postulated mechanisms for anti-inflammatory action of PPARγ agonists are involved in inhibiting NF-κB signaling pathway. We examined the possibility that IL-6 signaling via the Jak-Stat pathway is modulated by 15d-PGJ_2 in lymphocytes and also examined whether the inhibition of IL-6 signaling is dependent of PPARγ. 15d-PGJ_2 blocked IL-6 induced Stat1 and Stat3 activation in primary human lymphocytes, Jurkat cells and immortalized rheumatoid arthritis B cells. Inhibition of IL-6 signaling was induced rapidly within 15 min after treatment of 15d-PGJ_2. Other PPARγ-agonists, such as troglitazone and ciglitazone, did not inhibit IL-6 signaling, indicating that 15d-PGJ_2 affect the IL-6-induced Jak-Stat signaling pathway via PPARγ-independent mechanism. Although cycloheximide reversed 15d-PGJ_2-mediated inhibition of Stat3 activation, actinomycin D had no effect on 15d-PGJ_2-mediated inhibition of IL-6 signaling, indicating that inhibition of IL-6 signaling occur independent of *de novo* gene expression. These results show that 15d-PGJ_2 specifically inhibit Jak-Stat signaling pathway in lymphocytes, and suggest that 15d-PGJ_2 may regulate inflammatory reactions through the modulation of different signaling pathway other than NF-κB in lymphocytes.

Keywords: 15-deoxy-delta(12,14)-prostaglandin J_2; Interleukin-6; inflammation mediators; lymphocytes; PPARγ; Stat1 protein; Stat3 protein

Introduction

IL-6 is a pleotropic cytokine required for immune and inflammatory responses. IL-6 has been initially considered as a pro-inflammatory cytokines, and many of its pro-inflammatory properties are mediated by driving B cell activation and autoantibody production (Muraguchi *et al.,* 1981; Hirano *et al.,* 1986; Kishimoto *et al.,* 1992), inducing the expansion and activation of T cells (Le *et al.,* 1988; Lotz *et al.,* 1988; Okada *et al.,* 1988), and inducing chemokine expression and augmentation of ICAM-1 in endothelial cells (Romano *et al.,* 1997). IL-6 is a member of the IL-6 family cytokines (IL-6, IL-11, leukemia inhibitory factor (LIF), oncostatin M (OSM), ciliary neurotrophic factor (CNTF), cardiotrophin-1 (CT-1)) and IL-6 receptor system consists of two molecules, IL-6Rα and gp130 (Taga and Kishimoto, 1997). IL-6 binds to its binding receptor (IL-6Rα) devoid of signal transducing activity, and IL-6/IL-6Rα complex induces gp130 homodimerization, and dimerization of gp130 activates the receptor-associated protein tyrosine kinases, such as Jak1, Jak2, and Tyk2. Then, tyrosine residues in the cytoplasmic portion of gp130 are phosphorylated, and latent cytoplasmic Stat (signal transducer and activator of transcription) transcription factors are recruited to a phosphotyrosine-containing sequence in the cytoplasmic portion of gp130 and are tyrosine phosphorylated and activated. Activated Stat molecules dimerize and translocate to the nucleus, where they can activate transcription after binding to a consensus sequence in gene promoters (Darnell 1997;

Taga *et al.,* 1997). In many cells, IL-6 activates predominantly Stat3, although Stat1 also can be activated. Activated Stat3 is involved in promoting T cell survival and functions (Takeda *et al.,* 1998).

15-deoxy-$\Delta^{12,14}$-PGJ_2 (15d-PGJ_2) is a natural ligand that activates the peroxisome proliferators-activated receptor γ (PPARγ), a member of nuclear receptor family and has been reported to exert anti-inflammatory activities in several kinds of cells such as macrophages and endotherial cells via PPARγ-dependent and -independent mechanism (Jiang *et al.,* 1998; Ricote *et al.,* 1998; Gilroy *et al.,* 1999; Hinz *et al.,* 2003; Imaizumi *et al.,* 2003). The precise mechanisms for anti-inflammatory effects of 15d-PGJ_2 and other PPARγ agonists are poorly understood, and most recent studies about mechanisms for anti-inflammatory action of 15d-PGJ_2 and other PPARγ agonists have been focused on inhibiting nuclear factor κB (NF-κB) signaling pathway (Li *et al.,* 2000; Rossi *et al.,* 2000; Daynes and Jones, 2002). The postulated mechanisms for suppression of inflammation are transrepression of the transcription factors such as NF-κB and activation protein 1 (AP-1) through the sequestration of essential, shared coactivators (Li *et al.,* 2000; Daynes *et al.,* 2002) and direct inhibition of IκB kinase by 15d-PGJ_2 (Rossi *et al.,* 2000). To date, limited information is available for the interaction between the anti-inflammatory effects of PPARγ and cytokine-induced Jak-STAT signaling. Two studies were recently reported that 15d-PGJ_2 exerts anti-inflammatory action via inhibition of IFNγ-induced Jak-STAT signaling pathway independent of PPARγ (Chen *et al.,* 2003; Park *et al.,* 2003). In addition, Park *et al.* (2003) reported that 15d-PGJ_2 induce the transcription of suppressor of cytokine signaling (SOCS) 1 and 3, which is the mechanism for 15d-PGJ_2 induced inhibition of IFNγ signaling. There is no report about the modulation of cytokine-induced Jak-Stat signaling by PPARγ in lymphocytes. In this study, we examined the effects of 15d-PGJ_2 and other PPARγ agonists on cytokine-induced Jak-Stat signaling pathway in primary human lymphocytes, immortalized rheumatoid arthritis B cells and Jurkat human T cell line.

Materials and Methods

Cell isolation and culture

Peripheral blood mononuclear cells (MNC) were obtained from three healthy blood donors by density gradient centrifugation using Ficoll Paque. Primary lymphocytes were purified from MNC by negative selection, using anti-CD14 magnetic beads, as recommended by the manufacturer (Miltenyi Biotech, Auburn, CA). Lymphocytes were used fresh in RPMI 1640 medium supplemented with 10% FBS. Jurkat human T cells and immortalized rheumatoid arthritis synovial B cells were also cultured at 37°C in 5% CO_2 using RPMI medium with 10% FBS. Immortalized rheumatoid arthritis synovial B cells were kindly provided by Dr. J. Sohn (Korea University, Seoul, Korea). These immortalized B cell lines were generated by Ebstein-Barr virus (EBV) transformation, a subsequent fusion with Sp2/0-Ag8 mouse myeloma cells and subcloning.

Immunoblotting

Total cellular protein was extracted from several conditioned cells using Pro-prep protein extraction solution (Intron, Seoul, Korea). Extracts corresponding to 2×10^5 cells were separated through 7.5% SDS-polyacrylamide gels, transferred to polyvinylidene difluoride membranes by semidry electrophoretic transfer, and incubated with phospho-specific (Tyr705) anti-anti-Stat3 antibody, phospho-specific (Tyr701) anti-Stat1 antibody (Cell Signaling Technology, Beverly, MA), monoclonal anti-Stat3 antibody (Transduction Laboratories, Lexington, KY), and polyclonal antibody against human PPAR-γ (Santa Cruz, CA). ECL western blotting detection reagents (Amersham Biosciences) were used for detection.

Analysis of mRNA levels

Total cellular RNA was isolated using TRIzol reagent (Gibco BRL) according to the instructions of the manufacturer. For real-time PCR, RNA was treated with RNase-free DNAse (Gibco BRL), and complementary DNA (cDNA) was prepared by reverse transcription (RT) with a random hexamer primer (Invitrogen) and M-MuLV reverse transcriptase (Gibco BRL). Oligonucleotide primers used are as follows: GAPDH, AAGGTGAAGGTCGGAGTCAACG and CCTTCTCCATGGTGGTGAAGAC; SOCS3, CACTCTTCAGCATCTCTGTCGGAAG and CATAGGAGTCCAGGTGGCCGTTGAC; SOCS1, AGGATGGTAGCACACAACCAGGT and GATCTGGAAGGGGAAGGAACTCAGGTA. We performed real time PCR using the iCycler iQ thermal cycler and detection system (Bio-Rad Laboratories). mRNA levels were normalized relative to GAPDH mRNA.

Results

15d-PGJ_2 inhibits IL-6-induced Stat activation in lymphocytes

In this study, the effects of 15d-PGJ_2 on IL-6 signaling and Stat activation were examined using primary human lymphocytes, immortalized rheumatoid arthritis synovial B cells and Jurkat human T cells. Phosphorylation of Stat3 and Stat1 was induced rapidly by IL-6 treatment. To determine the effects of 15d-PGJ_2 on IL-6-induced Stat phosphorylation, the cells were pretreated with 10 μM 15d-PGJ_2 for 2 h, followed by IL-6 stimulation for 10 min at 37°C in 5% CO_2. IL-6 activates predominantly Stat3, and activated Stat3 is mainly involved in promoting T cell survival and functions (Takeda *et al.,* 1998). So, in this study, we focused primarily the modulation of 15d-PGJ_2 on

IL-6-induced Stat3 phosphorylation. Two hours' pretreatment with 15d-PGJ$_2$ inhibited Stat3 and Stat1 phosphorylation in primary human lymphocytes (Figure 1A). Similar to IL-6, IL-10 activation of Stat3 was inhibited by 15d-PGJ$_2$ at tyrosine-phosphorylation level. However, in this study, we investigated mainly about the modulation of IL-6-induced Stat3 phosphorylation by 15d-PGJ$_2$. Also we used immortalized rheumatoid arthritis B cells and Jurkat human T cells for studying the effects of 15d-PGJ$_2$ on IL-6-induced signaling, and these results were compared with the results from primary lymphocytes. Jurkat cells were shown not to express IL-6Rα (Igaz *et al.,* 2000), so we used combination of IL-6 and soluble IL-6 receptor (sIL-6R) for examining the IL-6 signaling in Jurkat cells. 15d-PGJ$_2$ could inhibit Stat3 phosphorylation in immortalized rheumatoid arthritis B cells and Jurkat human T cells, too (Figure 1B, 1C). In unstimulated conditions, weak phosphorylation of Stat3 was seen in rheumatoid arthritis B cells, we can guess minimal production of some cytokines from rheumatoid arthritis B cells, but we did not examine this. 15d-PGJ$_2$ mediated-inhibition of IL-6 signaling was in a dose-dependent manner (Figure 1D, 1E). In contrast, phosphorylation of Stat1 by IFN-γ was not inhibited by 10 μM 15d-PGJ$_2$ (Figure 1A). This result is distinct from those reported by Park *et al.* (2003) and Chen *et al.* (2003) who showed that 10 μM of 15d-PGJ$_2$ significantly suppresses IFN-γ signaling in murine macrophages and rat astrocytes. But very high concentrations of 15d-PGJ$_2$ can inhibit IFN-γ signaling (Figure 1F). So, we demonstrated here that 15d-PGJ$_2$ also can inhibit IFN-γ signaling in a dose-dependent manner. These results indicate that IL-6-induced Stat3 phosphorylation is more easily inhibited by 15d-PGJ$_2$ as compared with IFN-γ-induced Stat1 phosphorylation and also suggest that the inhibition of cytokine signaling using the Jak-Stat pathway by 15d-PGJ$_2$ may be different in the several cell types.

Alcohol inhibits cytokine-induced Stat activation (Chen *et al.,* 1999). Since we used ethanol for dissolving 15d-PGJ$_2$, we tested the effects of ethanol on IL-6 and IFNγ signaling in lymphocytes. Low concentration of ethanol (20 μM) which we used to dissolve 15d-PGJ$_2$, as compared with the previous report (Chen *et al.,* 1999) did not inhibit IFN-γ signaling as well as IL-6 signaling (Data not shown) in lymphocytes.

To exclude the possibility that cell death may contribute to the decreased Stat phosphorylation, trypan blue staining was used to quantify the numbers of viable cells. Treatment with 15d-PGJ$_2$ for 2 h resulted in a less than 10% decrease in the number of viable

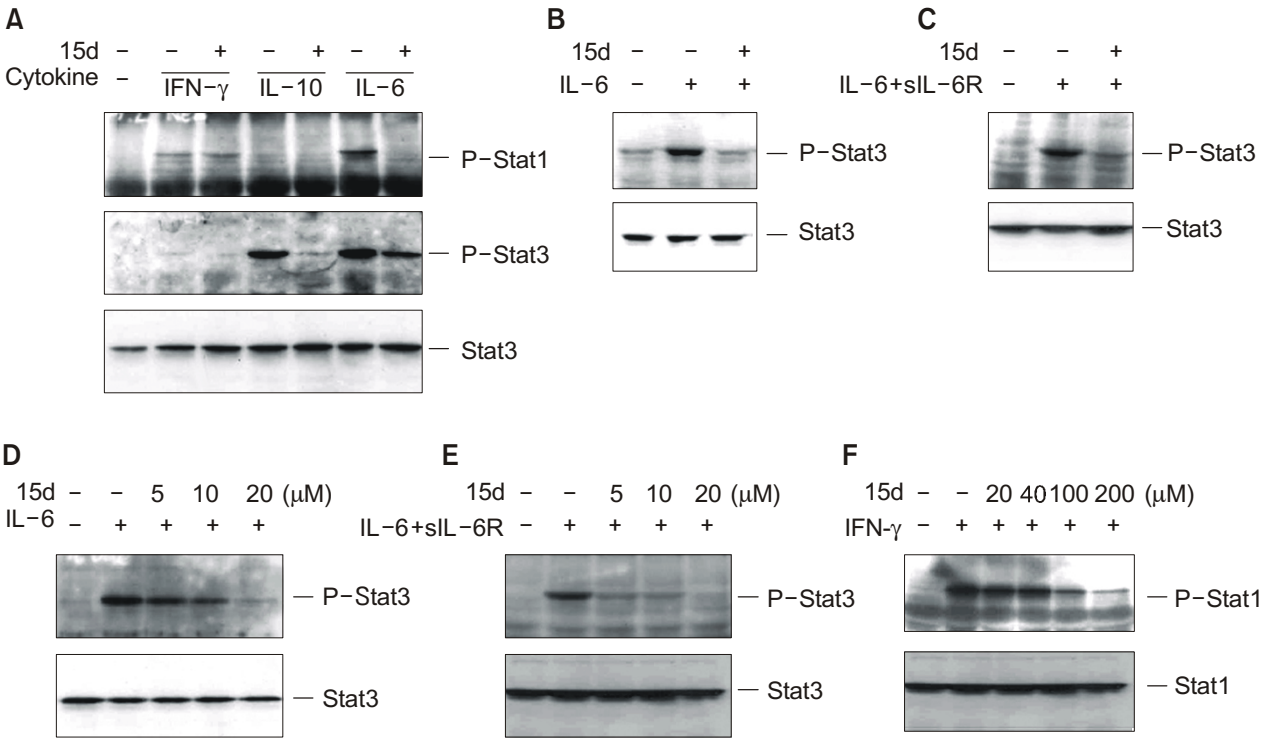

Figure 1. IL-6 (20 ng/ml) and IL-10 (20 ng/ml) induced-Stat activation is inhibited by 10 μM 15d-PGJ$_2$ in primary human lymphocytes (A), in immortalized rheumatoid arthritis B cells (B) and in Jurkat T cell lines (C). However, 10 μM 15d-PGJ$_2$ did not inhibit IFN-γ-induced stat activation in primary lymphocytes (A). 15d-PGJ$_2$ inhibited IL-6 signaling in a dose-dependent manner in primary human lymphocytes (D) and in Jurkat T cell lines (E). High concentrations of 15d-PGJ$_2$ inhibit IFN-γ-induced stat activation in primary lymphocytes (F). Stat activation was measured by immunoblotting. Extracts corresponding to 2×10^5 cells were used in each lane. The experiments are representative of three independent experiments all displaying similar results.

cells (data not shown). So we think cell death did not influence the decreased Stat activation.

15d-PGJ_2 induced-inhibition of IL-6 signaling occurs rapidly and is independent of *de novo* gene expression

We investigated the mechanism about 15d-PGJ_2 induced inhibition of IL-6 signaling. The currently known mechanisms for inhibition of Jak-Stat signaling are via de novo expression of SOCS (Yasukawa *et al.,* 2000) and a rapid, direct inhibitory pathway that is dependent upon protein kinase C (PKC) or mitogen-activated protein kinases (MAPK) (Lee *et al.,* 1999; Ahmed and Ivashkiv, 2000; Ji *et al.,* 2003). The requirement for *de novo* production of IL-6 signaling inhibitors was investigated by examining the kinetics of inhibition, and by using actinomycin D and cycloheximide to inhibit *de novo* transcription and translation. To determine the time course of 15d-PGJ_2 inhibitory effects on IL-6-induced Stat3 phosphorylation, primary lymphocytes were incubated with 10 μM 15d-PGJ_2 for various times. 15d-PGJ_2 inhibited IL-6 signaling strongly when added just 15 min prior to IL-6 (Figure 2A), suggesting that 15d-PGJ_2 stimulates a direct, very rapid inhibitory pathways or induces a inhibitor within 15 min. Inhibition of IL-6 signaling was preserved when *de novo* gene expression was blocked using actinomycin D (Figure 2B). However, when we used cycloheximide for blocking *de novo* protein synthesis, 15d-PGJ_2 induced-inhibition of IL-6 signaling was partially reversed by cycloheximide (Figure 2B). Recent report shows that cycloheximide can activate p38 in monocytes (Ahmed *et al.,* 2002). Therefore, we can guess that the reversal of inhibited IL-6 signaling by cycloheximide does not occur secondary to inhibition of de novo protein synthesis, but instead we can suggest that the effect of 15d-PGJ_2 on IL-6 signaling may be directly inhibited by cycloheximide via other pathway, such as p38.

Inhibition of Jak-Stat signaling has been proposed to occur by rapid and direct inhibitory pathway dependent upon MAPKs, phosphatase or PKC. Recent study suggests that 15d-PGJ_2 stimulates the expression of IL-8 gene in THP-1 macrophages through a MAPK signaling pathway (Fu *et al.,* 2002). The role of MAPKs, phosphatase or PKC in inhibition of IL-6 signaling was investigated using specific inhibitors of these kinases. Inhibitor of PKC (GF109203X) by itself suppressed IL-6-induced Stat3 phosphorylation (Figure 2C). So we could not definitively rule out the possibility of PKC involvement in 15d- PGJ_2 induced

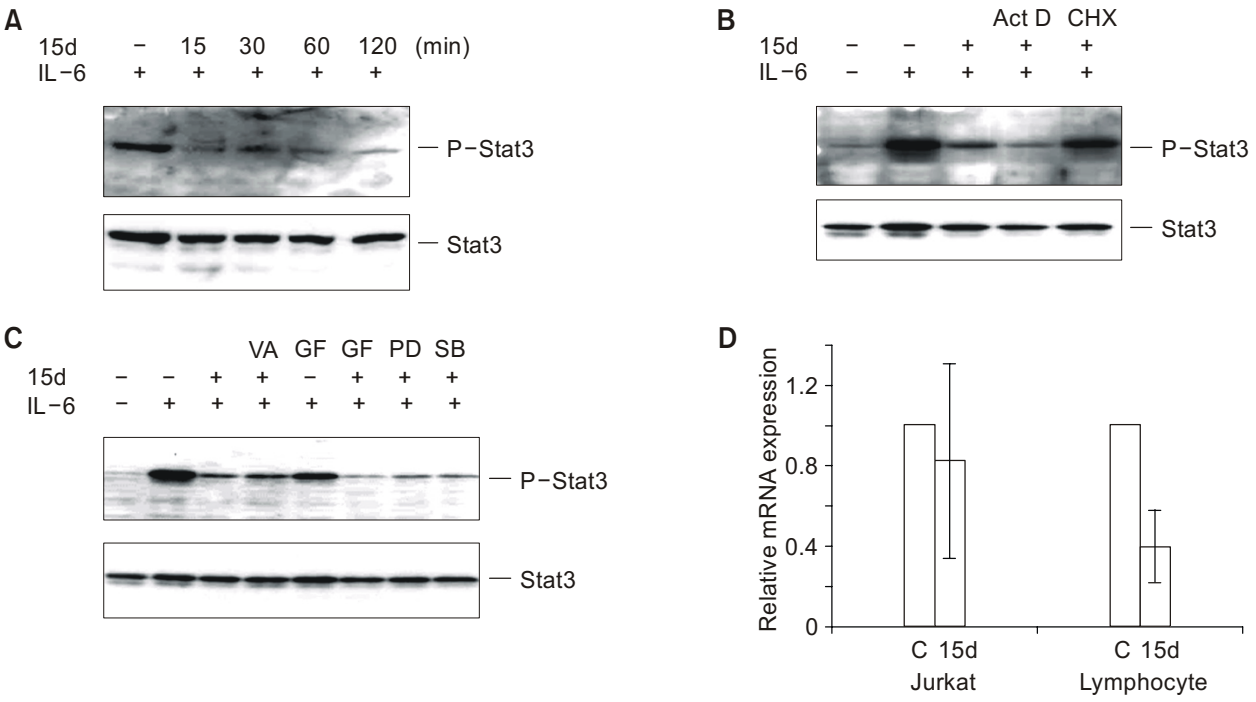

Figure 2. 15d-PGJ_2-mediated Inhibition of IL-6 signaling occurs rapidly, within 15 min in primary lymphocytes and inhibition of Stat3 activation may be independent of *de novo* protein synthesis in primary lymphocytes. (A) Time course of inhibition of IL-6 signaling. The primary lymphocytes were incubated in 10 μM 15d-PGJ_2 for indicated times, prior to IL-6 (20 ng/ml) treatment. (B) Reversal of inhibition of IL-6 signaling by cycloheximide (15 μg/ml), but lack of reversal by actinomycin D (5 μg/ml). (C) Lack of reversal of inhibition of IL-6 signaling when mitogen-activated protein kinases (MAPKs), protein kinase C (PKC), or phosphatase were blocked using inhibitors of ERKs (PD98059, 50 μM), p38 (SB203580, 10 μM), phosphatase (sodium orthovanadate, 5 mM) and PKC (GF109203X, 10 μM). STAT activation was measured by immunoblotting. (D) Lack of induction of SOCS3 gene by 15d-PGJ_2 in primary human lymphocytes and Jurkat cells, detected by real time PCR (*$P < 0.05$). Thew data are representatives of three independent experiments all displaying similar results.

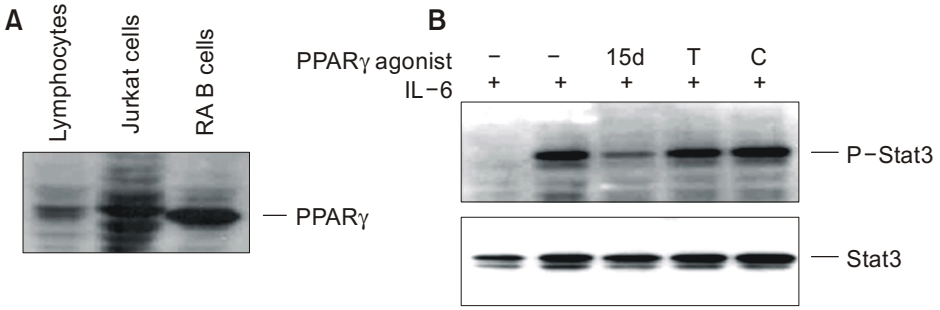

Figure 3. (A) Expression of PPARγ in primary lymphocytes, immortalized rheumatoid arthritis B cells and Jurkat cells. (B) 15d-PGJ$_2$ but not other PPARγ agonists (40 μM ciglitazone (C) and 30 μM troglitazone (T)) inhibit IL-6 signaling in primary human lymphocytes. Thus 15d-PGJ$_2$-mediated Inhibition of IL-6 signaling may be independent of PPARγ in primary lymphocytes and immortalized rheumatoid arthritis B cells. STAT activation was measured by immunoblotting. The data are representatives of three independent experiments all displaying similar results.

inhibition of IL-6 signaling. But inhibitors of ERKs (PD98059), p38 (SB203580), and phosphatase (sodium orthovanadate) had no effect on the IL-6-induced Stat3 phosphorylation by themselves (data not shown). PD98059, SB203580, and sodium orthovanadate did not influence 15d-PGJ$_2$ induced inhibition of IL-6 signaling (Figure 2C), indicating that MAPKs and phosphatase may be not involved in 15d-PGJ$_2$-induced inhibition of IL-6 signaling.

Also we tested 15d-PGJ$_2$-induced SOCS3 expression in lymphocytes, using real time PCR. Recently, Yu *et al.* (2003) reported that SOCS3 are constitutively expressed in murine naive T cells. 15d-PGJ$_2$ did not induce SOCS3 expression in primary lymphocytes and Jurkat cells (Figure 2D). In contrast, 15d-PGJ$_2$ inhibited SOCS3 expression in primary human lymphocytes (Figure 2D). These results indicate that 15d-PGJ$_2$-induced-inhibition of IL-6 signaling is independent of the expression of new genes, such as SOCS3.

15d-PGJ$_2$ inhibits IL-6-induced Jak-Stat signaling *via* PPARγ-independent mechanism

Previous studies demonstrated that 15d-PGJ$_2$ exerts anti-inflammatory action *via* PPARγ-independent mechanism (Hinz *et al.,* 2003), and inhibition of IFNγ-induced Jak-Stat signaling is independent of PPARγ (Chen *et al.,* 2003; Park *et al.,* 2003). So we investigated whether PPARγ mediates the inhibitory action of 15d-PGJ$_2$ on IL-6-induced Jak-Stat signaling. First we determined the presence of PPARγ in primary human lymphocytes, Jurkat human T cells and immortalized rheumatoid arthritis B cells. PPARγ could be detected in all three cell types (Figure 3A). Then we compared the inhibitory effects of different PPARγ agonists on IL-6 signaling. 15d-PGJ$_2$ but not other PPARγ agonists inhibits IL-6 signaling in primary human lymphocytes (Figure 3B). Our results suggest that inhibition of IL-6 signaling by 15d-PGJ$_2$ in lymphocytes is mediated via PPARγ-independent mechanism.

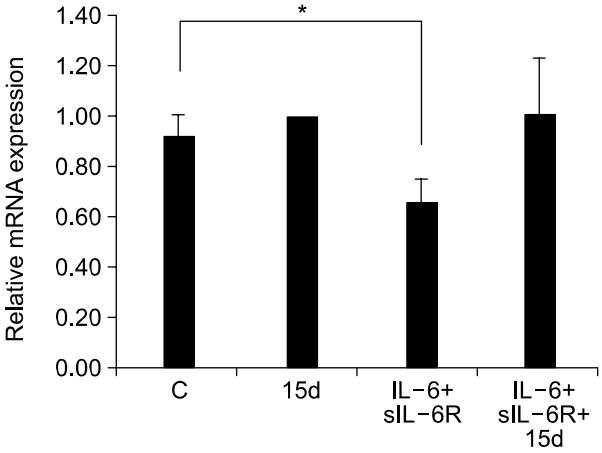

Figure 4. Reversal of IL-6-induced inhibition of SOCS3 gene by 15d-PGJ$_2$ in Jurkat cells, detected by real time PCR (*$P < 0.05$).

IL-6 inhibits SOCS3 expression in Jurkat cells and this inhibition is reversed by 15d-PGJ$_2$

We assessed the functional significance of 15d-PGJ$_2$ -induced inhibition of IL-6 signaling, by examining the effects of 15d-PGJ$_2$ on IL-6-induced change of SOCS3 expression. SOCS3 gene was expressed in Jurkat cells without any stimulation. Previous reports demonstrated that IL-6 induced SOCS3 expression in HepG2 cells and mouse liver (Starr *et al.,* 1997; Senn *et al.,* 2004). Unexpectedly, in our results, treatment of IL-6 for 3 h decreased the expression of SOCS3. IL-6 induced inhibition of SOCS3 expression was reversed by pretreatment of 15d-PGJ$_2$ (Figure 4). Thus, this result showed that inhibition of IL-6 signaling by 15d-PGJ$_2$ resulted in suppression of IL-6 functional activity.

Discussion

After two reports about anti-inflammatory effects of

PPARγ were published (Jiang *et al.,* 1998; Ricote *et al.,* 1998), PPARγ agonists have been focused on research fields studying inflammation. The inhibitory effect of PPARγ agonists on Stat molecules was previously suggested (Ricote *et al.,* 1998). However, most studies about anti-inflammatory action of PPARγ have been focused on inhibiting NFκB signaling pathway. Thus we studied the effects of PPARγ agonists on Jak-Stat signaling pathway. Park *et al.* (2003) reported that 15d-PGJ_2 and rosiglitazone are potent inducers of SOCS expression in primary astrocytes, and that the inhibition of IFN-γ signaling by 15d-PGJ_2 is dependent of induction of SOCS expression. But here we show that 15d-PGJ_2-induced inhibition of IL-6 signaling is independent of *de novo* gene expression and 15d-PGJ_2 can not induce SOCS3 in lymphocytes. Also, in our experiments, other well known mechanisms for inhibition of Jak-STAT signaling, such as MAPKs, phosphatase were not involved in 15d-PGJ_2-induced inhibition of IL-6 signaling. Therefore, we cannot rule out the possibility that unknown rapid, direct inhibitory pathway may be involved in 15d-PGJ_2-induced inhibition of IL-6 signaling, and further experiments about the unknown mechanisms of 15d-PGJ_2-induced inhibition of IL-6 signaling need to be done.

Many studies described a role of PPARγ for control of inflammation in the monocyte/macrophage. However, as yet, there have been very few reports about a role for PPARγ in the lymphocytes. Clark *et al.* (2000) described the expression and function of PPARγ in T lymphocytes. They demonstrated that murine T cell expresses PPARγ. In this study, they also demonstrated 15d-PGJ_2 and ciglitazone mediate significant inhibition of proliferative responses of both the T cell clones and the freshly isolated splenocytes, but did not inhibit IL-2-induced proliferation of such clones. They suggest 15d-PGJ_2 and ciglitazone may affect signaling pathway involved in T cell receptor stimulation but not IL-2-induced signaling pathway. In our study, 15d-PGJ_2 inhibits IL-6-induced Stat activation but not IFN-γ induced Stat activation at the same concentration. So we can suggest that 15d-PGJ_2 modulate specifically Jak-Stat signaling of individual cytokines in lymphocytes. Yang *et al.* (Yang *et al.,* 2000) showed that PPARγ mRNA was expressed in human peripheral blood T lymphocytes and 15d-PGJ_2 inhibited IL-2 production and phytohemagglutinin-inducible proliferation in human peripheral blood T-cells in a dose-dependent manner. Also they showed wild-type Jurkat cells expressed little detectable PPARγ mRNA and PPARγ-transfected Jurkat cells but not wild-type were inhibited in IL-2 secretion by PPARγ ligands. These results indicated that in T cells, 15d-PGJ_2 and troglitazone mediate their effects only through PPARγ-dependent pathways. In our study, we detected a strong PPARγ expression in Jurkat cells by Western blot and 15d-PGJ_2 inhibited IL-6 signaling in wild-type Jurkat cells. Also we showed that other PPARγ agonists did not inhibit IL-6 signaling in lymphocytes, so these result suggest 15d-PGJ_2 maybe mediate its effect through PPARγ-independent mechanism. We need further studies about PPARγ-dependent and independent pathway of several PPARγ ligands in lymphocytes

The demonstration that 15d-PGJ_2 specifically inhibits Jak-STAT signaling pathway in lymphocytes may expand the possible immunoregulatory role of 15d-PGJ_2 and other PPARγ agonists. Taken together with the effects of 15d-PGJ_2 and other PPARγ agonists on macrophage function, our results suggest that 15d-PGJ_2 may play a significant role in the lymphocyte. Also our findings demonstrated that 15d-PGJ_2 can regulate inflammatory reactions through the modulation of different signaling pathway other than NF-κB.

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