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Prostaglandin E₂ Augments IL-10 Signaling and Function

HyeonJoo Cheon,* Young Hee Rho, † Seong Jae Choi, † Young Ho Lee, † Gwan Gyu Song, † Jeongwon Sohn, ‡ Nam Hee Won,* and Jong Dae Ji††

In inflamed joints of rheumatoid arthritis, PGE₂ is highly expressed, and IL-10 and IL-6 are also abundant. PGE₂ is a well-known activator of the cAMP signaling pathway, and there is functional cross-talk between cAMP signaling and the Jak-STAT signaling pathway. In this study, we evaluated the modulating effect of PGE₂ on STAT signaling and its biological function induced by IL-10 and IL-6, and elucidated its mechanism in THP-1 cells. STAT phosphorylation was determined by Western blot, and gene expression was analyzed using real-time PCR. Pretreatment with PGE₂ significantly augmented IL-10-induced STAT3 and STAT1 phosphorylation, as well as suppressors of cytokine signaling 3 (SOCS3) and IL-1R antagonist gene expression. In contrast, PGE₂ suppressed IL-6-induced phosphorylation of STAT3 and STAT1. These PGE₂-induced modulating effects were largely reversed by actinomycin D. Pretreatment with dibutyryl cAMP augmented IL-10-induced, but did not change IL-6-induced STAT3 phosphorylation. Misoprostol, an EP2/3/4 agonist, and butaprost, an EP2 agonist, augmented IL-10-induced STAT3 phosphorylation and SOCS3 gene expression, but sulprostone, an EP1/3 agonist, had no effect. H89, a protein kinase A inhibitor, and LY294002, a PI3K inhibitor, diminished PGE₂-mediated augmentation of IL-10-induced STAT3 phosphorylation. In this study, we found that PGE₂ selectively regulates cytokine signaling via increased intracellular cAMP levels and de novo gene expression, and these modulating effects may be mediated through EP2 or EP4 receptors. PGE₂ may modulate immune responses by alteration of cytokine signaling in THP-1 cells. The Journal of Immunology, 2006, 177: 1092–1100.

The mediator PGE₂ is produced during inflammatory responses, and increased levels of PGE₂ mediate some of the cardinal features of inflammation, including pain, edema, and fever (1). In contrast, several studies suggest that in addition to its proinflammatory actions, PGE₂ may also exert strong anti-inflammatory effects such that PGE₂ suppresses the production of proinflammatory cytokines and enhances the synthesis of anti-inflammatory cytokines (2, 3). PGE₂ also promotes humoral and Th2-type immune responses and inhibits Th1-type immune responses (4). Therefore, PGE₂ is regarded not simply as a proinflammatory molecule but as a modulator of immune responses. The biological actions of PGE₂ are mediated by four distinct G protein-coupled E prostanoid (EP)³ receptors, termed EP1, EP2, EP3, and EP4 (5). The EP1 receptor is a well-known activator of a G protein, Gq, which enhances intracellular Ca²⁺ levels. The EP3 receptor inhibits adenylate cyclase via G₀, reducing cAMP levels. The EP2 and EP4 receptors activate G proteins that stimulate cAMP production via adenylate cyclase activation.

IL-10 is a strong anti-inflammatory cytokine that plays an important role in limiting tissue injury during infection by regulating the duration and intensity of immune and inflammatory reactions (6). The IL-10R system consists of two subunits, IL-10R1 and IL-10R2, both of which are members of the IFN receptor family. Ligation of the heterodimeric IL-10R results in activation of receptor-associated protein tyrosine kinases, Jak1 and Tyk2, and leads to subsequent tyrosine residue phosphorylation of STAT3 and STAT1. In myeloid cells, IL-10 predominantly activates STAT3 and activated STAT3 is primarily involved in the negative regulation of macrophage activation (6). The role of STAT1 in IL-10 biology and its signal transduction remains unclear.

IL-6 is a pleiotropic cytokine required for immune and inflammatory responses (7). IL-6 binds to its receptor (IL-6Rα), and the IL-6-IL-6R complex induces gp130 homodimerization. The dimerized gp130 activates receptor-associated protein tyrosine kinases Jak1, Jak2, and Tyk2, leading to subsequent tyrosine phosphorylation and DNA binding of STAT3 and STAT1 (8). In many cells, IL-6 activates predominantly STAT3, although STAT1 may also be activated.

Several reports have demonstrated a functional cross-talk between the cAMP signaling pathway and the Jak-STAT signaling pathway in various cell types. Activators of the cAMP signaling pathway, such as PGE₂, inhibit STAT1 DNA binding activity in mononuclear cells (9), and also inhibit IL-6-induced STAT activation in NFS-60 cells (10). In addition, PGE₂ activates IL-6 signaling via stimulation of the soluble IL-6R release in prostatic intraepithelial neoplasia (PIN) cells (11).

In the inflamed joints of rheumatoid arthritis, PGE₂ is highly expressed (12), and IL-10 and IL-6 are also abundant (13). In this study, we evaluate whether PGE₂ modulates STAT signaling and its biological function induced by IL-10 and IL-6, and identify the mechanisms by which PGE₂ regulates cytokine signaling in THP-1 cells.

Materials and Methods

Materials

Actinomycin D, H89, and PGE₂ were purchased from Sigma-Aldrich. Dibutyryl cAMP and LY294002 were purchased from Calbiochem. Sulprostone, misoprostol, and butaprost were purchased from Cayman Chemicals. Phospho-specific (Tyr²⁰⁵) STAT3 Ab and phospho-specific (Tyr²¹²) STAT1 Ab were purchased from Cell Signaling Technology. Monoclonal...
STAT3 and STAT1 Ab were purchased from BD Transduction Laboratories.

Cell isolation and culture

Monocytes were obtained from PBMC by positive selection, using anti-CD14 magnetic beads, as recommended by the manufacturer (Miltenyi Biotec). Monocytes were used fresh in RPMI 1640 medium supplemented with 10% FBS. THP-1 cells were cultured at 37°C in 5% CO2 using RPMI 1640 medium with 10% FBS.

Protein extraction and immunoblotting

Total cellular protein was extracted from several conditioned cells using Pro-prep protein extraction solution (Intron). Extracts corresponding to 2 x 10^5 cells were separated on 7.5% SDS-polyacrylamide gels, transferred to polyvinylidene difluoride membranes by semidry electrophoretic transfer, and incubated with phospho-specific (Tyr705) STAT3 Ab, phospho-specific (Tyr701) STAT1 Ab, and with monoclonal STAT3 and STAT1 Ab. ECL Western blotting detection reagent (Amersham Biosciences) was used for detection. For quantitative analysis, the density of the blots for phosphorylated STAT3 and total STAT3 was measured and expressed as the ratio of phosphorylated STAT3 to total STAT3.

RNA extraction and real-time PCR

Total cellular RNA was isolated using TRIzol reagent (Invitrogen Life Technologies) according to the manufacturer’s instruction. cDNA was prepared by reverse transcription with a random hexamer according to the manufacturer’s protocol (TaqMan Reverse Transcription Reagent; Applied Biosystems). Oligonucleotide primers used are as follows: GAPDH, AAG GTG AAG GTC GGA GTC AAC G and CCT TCT CCA TGG TGA AGA C; EP1, ATG GTG GGC CAG CTT GTC and GCC ACC AAC ACC AGC ATT G; EP2, CTG CTG CTG CTTCTC ATT GT and ATG CGG ATG AGG TTG AGA AT; EP3, GGT CTC CGC TCC TGA TAA TG and CTC CGT GTG TGT CTT GTA GCC GT; EP4, CGA CCT TCT ACA CGC TGG TAT G and CCG GGC TCA CCA ACA AAG T; IL-1R antagonist, GAA GAT GTG CCT GTC CTG GTG GTG CTG ACC TT and suppressors of cytokine signaling 3 (SOCS3), CAC TCT TGA TCT CTG TCG GAA G and CAT AGG AGT CCA GGT GGC CGT TGA C. Real-time PCR was performed in a SYBR Green PCR Master mix (Applied Biosystems) using the GeneAmp 7000 Sequence Detection System (Applied Biosystems) with the following protocol: initial activation at 95°C for 10 min, 40 cycles at 95°C for 15 s, and 60°C for 1 min. The mRNA amount of the target gene was normalized relative to GAPDH using the threshold cycle (ΔCt) method as described in the manufacturer’s protocol.

Flow cytometric detection of IL-10R or IL-6R on cell surfaces

The binding of IL-10 or IL-6 to cell surface receptors was measured using a Fluorokine kit according to the manufacturer’s protocol (R&D Systems). Analyses were done using a FACScan flow cytometer with CellQuest software (BD Biosciences).

Results

PGE2 modulates cytokine-induced STAT phosphorylation in THP-1 cells

PGE2 has been shown to inhibit STAT1 DNA binding activity in mononuclear cells and to inhibit IL-6-induced STAT activation in NFS-60 cells (9, 10). We began the present study by examining whether PGE2 modulates cytokine-induced STAT activation in THP-1 cells. IL-10 and IL-6 (20 ng/ml) were treated for 10 min, and PGE2 (1 μM) was pretreated 2 h before cytokine stimulation. IL-10-induced STAT activation was augmented and IL-6-induced STAT activation was inhibited by pretreatment with PGE2. E. In THP-1 cells, IL-10 and IL-6 (20 ng/ml) were treated for 10 min, and PGD2 (1 μM) was pretreated 2 h before cytokine stimulation. F. In primary monocytes, IL-10 and IL-6 (20 ng/ml) were treated for 10 min, and PGE2 (1 μM) was pretreated 2 h before cytokine stimulation. All sample incubations were followed by immunoblot analysis as described in Materials and Methods. PGE2 itself did not induce STAT3 phosphorylation. These experiments are representative of three independent experiments, all displaying similar results.
STAT1. In contrast, pretreatment with PGE$_2$ significantly augmented IL-10-induced phosphorylation of STAT3 and STAT1. PGE$_2$ had no effect on the protein expression of STAT3 and STAT1 (Fig. 1A). PGE$_2$ modulated cytokine-induced STAT3 activation at 0.01–1 μM (Fig. 1, B and C). Because it has been reported that PGE$_2$ increases production of both IL-10 and IL-6 in macrophages (14), and activates the STAT3 signaling pathway via release of IL-6 and soluble IL-6R in PIN cells (11), we examined whether PGE$_2$ itself could augment IL-10-induced STAT3 phosphorylation by increased production of cytokines. Fig. 1D shows that PGE$_2$ itself did not activate STAT3 phosphorylation, so we excluded the possibility that PGE$_2$ may augment IL-10 signaling via PGE$_2$-induced cytokine production.

To examine the specificity of PGE$_2$ on modulation of cytokine signaling, THP-1 cells were pretreated with PGD$_2$. Cytokine-induced phosphorylation of STAT3 was not changed by PGD$_2$, indicating that PGE$_2$ specifically modulates cytokine-induced phosphorylation of STAT3 (Fig. 1E).

To examine the biological significance of these modulating effects in physiological condition, we used primary human monocytes for evaluating the effects of PGE$_2$ on cytokine signaling. Disappointingly, PGE$_2$ did not influence the cytokine signaling in primary human monocytes (Fig. 1F). Based on this result, we can conclude PGE$_2$ did not change the cytokine signaling in physiological condition. However, we cannot exclude the possibility that PGE$_2$ influences the cytokine-induced STAT phosphorylation in other pathological condition, similar to the results in THP-1 cells. Therefore, we need additional experiments to evaluate the PGE$_2$ effects on cytokine signaling in various conditioned monocytes and macrophages.

Fig. 2 shows the time course during which PGE$_2$ modulates cytokine-induced STAT3 phosphorylation in THP-1 cells. Augmentation of IL-10-induced STAT3 phosphorylation was detectable after 2 h of PGE$_2$ pretreatment, and reached a maximal level after 6 h of PGE$_2$ pretreatment (Fig. 2A). In contrast, inhibition of IL-6-induced STAT3 phosphorylation was induced very rapidly, occurring after 30 min of PGE$_2$ pretreatment, and was maintained for 6 h of PGE$_2$ pretreatment (Fig. 2B).

All four EP receptors are expressed in THP-1 cells

PGE$_2$ exerts its action through four distinct membrane-associated G protein-coupled receptors, EP1, EP2, EP3, and EP4. We examined the expression of EP receptors in THP-1 cells using real-time PCR. Fig. 3 shows that all four EP receptor genes were expressed and that the EP4 receptor was expressed at a higher level when compared with EP1, EP2, and EP3 in THP-1 cells.

PGE$_2$ augments IL-10 biological activity

It has been previously reported that IL-10 up-regulates gene expression of SOCS3 and of the IL-1R antagonist in human monocytes (15). SOCS3 and IL-1R antagonist have been suggested to be mediators of anti-inflammatory and immune-modulating effects in several studies (16–18). We assessed the biological significance of PGE$_2$-mediated augmentation of IL-10 signaling by examining the effects of PGE$_2$ on IL-10-induced SOCS3 and IL-1R antagonist gene expressions. IL-10 increased SOCS3 gene expression by 2.3-fold, and the addition of PGE$_2$ enhanced IL-10-induced SOCS3 gene expression by 52.8-fold (Fig. 4A). PGE$_2$ also synergistically increased IL-10-induced IL-1R antagonist gene expression (Fig. 4B).

Because it has been reported that IL-6 also up-regulates SOCS3 gene expression (19), we examined the effect of PGE$_2$ on IL-6-induced SOCS3 expression. IL-6 increased SOCS3 gene expression by 7.7-fold in THP-1 cells. However, surprisingly PGE$_2$ induced an additional increase of IL-6-induced SOCS3 gene expression, in contrast to its effect upon STAT3 phosphorylation (Fig. 4C). This result is inconsistent with a previous report, which found that inhibition of STAT3 activation contributes to inhibition of IL-6-induced SOCS3 gene expression (19). Our result suggests that IL-6-induced SOCS3 gene expression is not mediated by STAT3 phosphorylation, and this is supported by a recent report which shows that IL-6-induced SOCS3 expression is mediated by p38 MAPK activation (20).

The cAMP signaling pathway is involved in PGE$_2$-induced augmentation of IL-10-induced STAT3 phosphorylation by PGE$_2$

Intracellular cAMP production by adenylate cyclase is involved in many biological activities of PGE$_2$. To analyze the mechanism for the modulation of cytokine signaling induced by PGE$_2$, we examined whether cAMP mediates the effects of PGE$_2$ on cytokine signaling. Pretreatment with dibutyryl cAMP, which increases intracellular concentration of cAMP, augmented IL-10-induced STAT3 phosphorylation in THP-1 cells (Fig. 5A). STAT3 phosphorylation levels are quantified by densitometry and normalized to STAT3 levels. Pretreatment with dibutyryl cAMP (50 μM) significantly augmented IL-10-induced phosphorylation of STAT3 (Fig. 5B). As with PGE$_2$ treatment, dibutyryl cAMP augmented IL-10-induced STAT3 phosphorylation after 2 h of treatment (Fig. 5C). In contrast to IL-10, IL-6-induced STAT3 phosphorylation was not affected by exogenous cAMP (Fig. 5, A and B). These results indicate that PGE$_2$ augments IL-10-stimulated STAT3 phosphorylation through a cAMP-dependent pathway.
Modulation of cytokine-induced STAT3 phosphorylation by PGE2 is not mediated by changes in cytokine receptor expression

We also determined whether PGE2 modulates proximal events in the IL-10 or IL-6 signaling pathway. Cell surface expressions of IL-10R or IL-6R were measured by flow cytometry after staining with biotinylated IL-10 or IL-6. Treatment with PGE2 for 2, 4, or 6 h did not result in an increased cell surface expression of IL-10R (Fig. 6A). Also treatment with PGE2 for 2 h did not result in a decreased cell surface expression of IL-6R (Fig. 6B).

Modulation of cytokine-induced STAT3 phosphorylation is dependent on de novo gene expression

It is currently well known that modulation of the Jak-STAT signaling pathway is mediated via de novo gene expressions, including SOCSs (21). The requirement for newly produced molecules for PGE2-induced modulation of cytokine signaling was investigated by using actinomycin D. PGE2-induced modulations of IL-6- and IL-10-stimulated STAT3 phosphorylation were greatly reversed when de novo gene expression was blocked by the addition of actinomycin D (Fig. 7, A and B), indicating that PGE2-induced modulation of cytokine signaling is dependent on new gene expressions.

To examine the possibility that PGE2 modulates cytokine signaling by increasing synthesis of new proteins, we performed experiments using the protein synthesis inhibitor cycloheximide. A recent report showed that cycloheximide can inhibit IL-6 signaling, and this inhibition by cycloheximide was dependent on the p38 stress kinase (22). Also we reported that cycloheximide itself inhibits IL-10-induced STAT3 phosphorylation in THP-1 cells (23). PGE2-induced augmentation of IL-10-stimulated STAT3 phosphorylation was greatly reversed by pretreatment with actinomycin D (Fig. 7C). Consistent with previous our report (23), cycloheximide itself inhibits IL-10-induced STAT3 phosphorylation. So we cannot rule out the possibility that cycloheximide directly inhibits PGE2-induced augmentation of IL-10-stimulated STAT3 phosphorylation, not by inhibiting the synthesis of new proteins.

Also studies using cycloheximide on IL-6 signaling did not yield informative data, as cycloheximide treatment alone significantly blocked IL-6 signaling (Fig. 7D).

Because SOCS3 is a major feedback inhibitor of IL-6-induced STAT signaling (20, 24, 25), we suspected that SOCS3 may be involved in the PGE2-mediated inhibition of IL-6-stimulated STAT3 activation. PGE2 rapidly induced SOCS3 mRNA expression within 30 min (Fig. 7F), and this PGE2-induced SOCS3 expression was completely suppressed by pretreatment with actinomycin D (Fig. 7F). Thus, it is possible that the PGE2-induced inhibition of IL-6 signaling might be due to de novo synthesis of SOCS3. In our studies, IL-10-induced STAT3 phosphorylation was not affected by PGE2-mediated induction of SOCS3, which is consistent with another report suggesting that IL-10-induced STAT3 activation is much less sensitive to the inhibitory activity of SOCS3 (25).

EP2 and/or EP4 receptor mediates PGE2 modulation of IL-10-induced STAT3 phosphorylation in THP-1 cells

Next, we examined which EP receptor mediates the effect of PGE2 on IL-10-induced STAT3 phosphorylation by using agonists specific for one or more subtypes of EP receptors, such as misoprostol (EP2/EP3/EP4 agonist), sulprostone (EP1/EP3 agonist), and butaprost (EP2 agonist). Misoprostol augmented IL-10-induced STAT3 phosphorylation, suggesting a dependence on EP2, EP3, and EP4 receptors (Fig. 8, A and B). In contrast to misoprostol and PGE2, sulprostone had no effect on IL-10-induced STAT3 phosphorylation (Fig. 8A); thus, we ruled out involvement of the EP1 and EP3 receptors in the modulation of IL-10-induced STAT3 phosphorylation. Because butaprost has a lower affinity for the EP receptor compared with misoprostol and PGE2, it was used at a higher concentration (10 μM) than the other agonists (1 μM). Butaprost augmented IL-10-induced STAT3 phosphorylation, but its modulating effect was much less potent than misoprostol and PGE2 (Fig. 8A). Misoprostol and butaprost each augmented IL-10-induced SOCS3 expression. The effect of misoprostol was similar to that of PGE2 (Fig. 8B), but butaprost was less potent than
misoprostol and PGE2. The SOCS3 gene expression increase induced by the addition of 10 μM butaprost was only 15–20%, compared with the level of gene expression induced by 1 μM misoprostol and PGE2 (Fig. 8C). These results suggest that PGE2-induced modulation of IL-10 signaling occurs via the EP2 and/or EP4 receptors.

\textbf{PGE2-induced augmentation of IL-10 signaling involves PI3K and protein kinase A (PKA)}

EP2 and EP4 receptors activate adenylate cyclase, leading to cAMP production and activation of a cAMP-dependent protein kinase, known as PKA (26). In addition to this PKA signaling pathway, stimulation of the EP4 receptor leads to activation of the PI3K-dependent pathway (27). We therefore examined the effect of H89, a specific PKA inhibitor, and LY294002, a PI3K inhibitor, on PGE2-induced modulation of cytokine signaling. When THP-1 cells were pretreated with H89 for 30 min before PGE2 treatment, PGE2 augmentation of IL-10-induced STAT3 phosphorylation was greatly reversed (Fig. 9A). In addition, LY294002 partially diminished the augmentation of the IL-10-induced STAT3 phosphorylation by PGE2 (Fig. 9B). Pretreatment with H89 diminished the augmentation of IL-10-induced SOCS3 gene expression in a dose-dependent fashion, and combined pretreatment with LY294002 and H89 greatly suppressed it when compared with H89 alone (Fig. 9C). LY294002 did not reverse the PGE2-induced augmentation of SOCS3 gene expression in this experiment, which is inconsistent with our other results of STAT3 phosphorylation. The reason for this discrepancy is unclear at present and will need further evaluation in future studies. These findings indicate that the PKA and PI3K signaling pathways are involved in PGE2-mediated augmentation of IL-10-induced STAT3 phosphorylation.

\section*{Discussion}

Our study demonstrates that PGE2 augments IL-10-induced STAT3 phosphorylation and its biological functions. We were surprised to find that PGE2 augments IL-10-induced STAT3 phosphorylation and gene expression because previous studies demonstrated that PGE2 and cAMP usually inhibit cytokine-induced STAT activation (9, 10, 28). To our knowledge, these data are the first demonstration that PGE2 augments IL-10 signaling and function. Our results suggest the possibility that PGE2 may cooperate with IL-10 to promote anti-inflammatory responses in inflammatory diseases in which there is a high expression of PGE2.

In human macrophages, IL-10 induces SOCS3 expression and this expression absolutely requires phosphorylation of STAT3 (18). In our study, we observed that PGE2 enhanced synergistically IL-10-induced SOCS3 gene expression. Another interesting finding is that the induction of SOCS3 gene expression by PGE2 alone is stronger than by IL-10 alone. We cannot exclude the possibility that augmentation of SOCS3 gene expression is the effect of IL-10 on PGE2-induced SOCS3 expression. However, recently published data from Antoniv et al. (29) reported that IL-10 induces increased degradation of PGE2 and suppresses PGE2-mediated gene expression. Gasperini et al. (30) have recently shown that PGE2 not only directly induces SOCS3 mRNA and protein expression in human leukocytes, but also cooperates with IL-10, via the mechanism that PGE2 prolongs the stability of SOCS3 mRNA. In contrast to our results, expression level of SOCS3 mRNA induced by PGE2 is lower than those induced by IL-10. Also another report demonstrated that PGE2 increases erythropoietin-dependent STAT5 regulatory genes, such as SOCS3 (31). This stimulatory effect of PGE2 on erythropoietin-mediated STAT5 transactivation is mediated by the cAMP/PKA/CREB pathway, without affecting the STAT5 tyrosine phosphorylation or STAT5 DNA binding. Thus, we can suggest that PGE2 modulates cytokine function via several different mechanisms, according to cell types and individual cytokines.

The molecular mechanisms for negative regulation of cytokine-induced Jak-STAT signaling have been extensively investigated. In contrast, its augmentation is poorly understood. In this study we have examined several possible mechanisms for augmented cytokine-induced STAT signaling, such as increased cell surface expression of cytokine receptors (11, 32), induction of cytokine to activate STAT phosphorylation (11, 33), and increased STAT protein expression (34).

First, we investigated whether PGE2-derived augmentation of IL-10 signaling is due to the increased cell surface expression of cytokine receptors. Crepaldi et al. (32) demonstrated that IL-10 induced STAT3 phosphorylation not seen in freshly isolated human neutrophils, but is found in LPS-treated neutrophils, thus demonstrating that IL-10R1 mRNA and protein expression are substantially increased in LPS-stimulated neutrophils. Another report demonstrated that PGE2 stimulates soluble IL-6R release, gp130 dimerization, and STAT3 phosphorylation (11). However,
in our experiments, pretreatment with PGE2 had no discernible effect on cell surface IL-10R expression in THP-1 cells, ruling out this possibility in our study. Next, we examined whether PGE2 augments STAT3 phosphorylation via production of new cytokines. It has been reported that PGE2 stimulates IL-6 secretion, as well as soluble IL-6R release, resulting in STAT3 activation in a PIN cell line (11). PGE2 also enhances IL-10 production in LPS-stimulated peripheral blood monocytes via an increase in intracellular cAMP. In activated murine macrophages, PGE2 increases the levels of both IL-10 and IL-6 via the p38 MAPK pathway (14). In our experiments, PGE2 itself did not activate STAT3 phosphorylation, suggesting that augmentation of IL-10 signaling is not mediated via PGE2-induced cytokine production, which results in STAT3 phosphorylation.

![Graphs and images](http://www.jimmunol.org)
We then checked another possible mechanism for augmenting cytokine signaling, that of increased STAT3 protein expression. Low concentrations of IFN-γ augmented STAT1 protein expression, and subsequently enhanced STAT1 activation and IFN-γ-dependent gene expression (34). Prolonged insulin treatment has been shown to reduce STAT3 protein expression and resulting STAT3 phosphorylation (35). These studies suggest that altered STAT protein expression can have an influence in Jak-STAT signaling. However, in our experiments PGE2 did not change STAT3 protein expression in THP-1 cells.

The diverse and specific effects of PGE2 depend on different EP receptor subtypes. In THP-1 cells, all four EP receptor genes are expressed, and EP4 is expressed at a higher level when compared with EP1, EP2, and EP3. Misoprostol, an EP2/EP3/EP4 agonist, augmented IL-10-induced STAT3 phosphorylation, producing an effect similar to that of PGE2. Sulprostone, an EP1/3 agonist, had no effect on IL-10-induced STAT3 phosphorylation. These results suggest that PGE2-induced modulation of cytokine signaling occurs via EP2 and/or EP4 receptors. Because EP2/EP4 receptors are activated by increased cAMP levels, results showing that exogenous cAMP augmented IL-10-induced STAT3 phosphorylation corroborate this finding.

In PGE2 EP2/EP4 signaling, increased cAMP levels result in the activation of PKA. EP4 receptors lead to the activation of the PI3K-dependent pathway, in addition to PKA pathway. H89, a PKA inhibitor, strongly reversed PGE2-derived augmentation of IL-10-induced STAT3 phosphorylation and SOCS3 gene expression, suggesting that PKA mediates PGE2-induced augmentation of IL-10 signaling. LY294002, a PI3K inhibitor, partially diminished PGE2-derived augmentation of IL-10-induced STAT3 phosphorylation. Combined pretreatment with H89 and LY294002 provided a more effective suppression of PGE2-enhanced SOCS3 expression, when compared with H89 alone. These findings indicate that PI3K also mediates the PGE2 effect in the modulation of IL-10 signaling. Because inhibitors of PI3K, such as wortmannin and LY294002, inhibit relaxin-mediated cAMP production in THP-1 cells (36), PI3K is thought to be involved in cAMP production. We suggest that increased cAMP production and subsequent activation of PKA are a possible mechanism by which PGE2 modulates IL-10 signaling.

PGE2 augments or inhibits IL-6-induced STAT3 activation according to cell type (10, 11). In PIN cells, PGE2 stimulates soluble IL-6R release, STAT3 phosphorylation, and DNA binding activity. In the promyelocytic cell line, NFS-60, PGE2 suppresses IL-6R

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**FIGURE 7.** Effect of actinomycin D on PGE2-induced modulation of cytokine signaling in THP-1 cells. A and B, THP-1 cells were pretreated with actinomycin D (2.5 μg/ml) 30 min before PGE2 (1 μM) treatment. After 2 h, IL-10 (A) or IL-6 (B) (20 ng/ml) was added for 10 min. STAT3 phosphorylation was measured by immunoblot assay. These data are representative of three independent experiments all displaying similar results. C and D, THP-1 cells were pretreated with cycloheximide (CHX, 50 μg/ml) 30 min before PGE2 (1 μM) treatment. After 2 h, IL-10 (A) or IL-6 (B) (20 ng/ml) was added for 10 min. STAT3 phosphorylation was measured by immunoblot assay. These data are representative of three independent experiments all displaying similar results. E, THP-1 cells were treated with PGE2 for the indicated time, followed by RNA extraction and real-time PCR with SOCS3 primer. PGE2 induced SOCS3 gene expression after 30 min of stimulation. F, Actinomycin D (2.5 and 5 μg/ml) was added 30 min before PGE2 (1 μM) treatment, followed by RNA extraction and real-time PCR with SOCS3 primer. Actinomycin D completely reversed PGE2-induced SOCS3 expression. Data represent relative mRNA amounts of SOCS3 normalized to GAPDH mRNA amounts. The mean value of three independent experiments are presented, and error bars indicate the SD.
expression and STAT3 activation. In our current study, down-regulation of IL-6R became evident following 6 h of PGE2 treatment. In contrast to previous reports, our results show that PGE2 inhibited IL-6-induced STAT3 phosphorylation within 30 min, but it did not change the cell surface expression of IL-6R. These results suggest that PGE2 stimulates a direct and prompt inhibitory pathway, which can induce inhibitors within 30 min. Our experiments show that actinomycin D reversed PGE2-derived inhibition of IL-6-induced STAT3 phosphorylation, suggesting dependence on synthesis of new inhibitory molecules. PGE2 induced SOCS3 gene expression within 30 min, and this was completely suppressed by actinomycin D. These results indicate that SOCS3 may be involved in the PGE2-induced inhibition of IL-6 signaling. It remains to be determined which intracellular signaling pathway is involved in PGE2-derived inhibition of IL-6 signaling and PGE2-induced SOCS3 gene expression.

In primary monocytes, PGE2 did not influence cytokine-induced STAT3 phosphorylation. These results suggest that PGE2 regulates cytokine-induced STAT3 phosphorylation in a cell-specific manner. We have not further addressed these findings in this article. Further study in various conditioned monocytes and macrophages will be necessary to prove the modulating effects of PGE2 on cytokine signaling in the inflammatory diseases, such as rheumatoid arthritis.

In this study, we found that PGE2 differentially regulates Jak-STAT signaling from different cytokines in THP-1. Our results demonstrate a previously unrecognized function of PGE2, the augmentation of IL-10-induced STAT3 phosphorylation and gene expression. It is also a novel finding that PGE2 can modulate effects on the immune response via the regulation of cytokine signaling, as well as of cytokine production.

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Disclosures
The authors have no financial conflict of interest.