Prostaglandin E2 mediates growth arrest in NFS-60 cells by down-regulating interleukin-6 receptor expression

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Interleukin-6 (IL-6), a potent myeloid mitogen, and the immunosuppressive prostanoid prostaglandin E2 (PGE₂) are elevated following thermal injury and sepsis. We have previously demonstrated that bone marrow myeloid commitment shifts toward monocytopoiesis and away from granulocytopoiesis during thermal injury and sepsis and that PGE₂ plays a central role in this alteration. Here we investigated whether PGE₂ can modulate IL-6-stimulated growth in the promyelocytic cell line, NFS-60, by down-regulating IL-6 receptor (IL-6r) expression. Exposure of NFS-60 cells to PGE₂ suppressed IL-6-stimulated proliferation as well as IL-6r expression. Receptor down-regulation is functionally significant since IL-6-induced signal transduction through activators of transcription (STAT)-3 is also decreased. Down-regulation of IL-6r correlated with the ability of PGE₂ to arrest cells in the G1/G0 phase of the cell cycle. PGE₂ appears to signal through EP2 receptors. Butaprost (EP2 agonist) but not sulprostone (EP3 agonist) inhibited IL-6-stimulated proliferation. In addition, an EP2 antagonist (AH6809) alleviated the anti-proliferative effects of PGE₂. NFS-60 cells express predominantly EP2 and EP4 receptors. While PGE₂ down-regulated both the IL-6r protein and mRNA expression, it had no influence on EP2 or EP4 mRNA expression. The present study demonstrates that PGE₂ is a potent down-regulator of IL-6r expression and thus may provide a mechanistic explanation for the granulocytopenia seen in thermal injury and sepsis.

Key words: bone marrow, myelopoiesis, promyelocytic cell, sepsis.

INTRODUCTION

Despite advances in critical care, sepsis and the systemic complications arising from sepsis have remained the major cause of death of critically injured patients [1,2]. Central to the pathology of sepsis is the systemic inflammatory response syndrome that is characterized by an elevation of proinflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor-α [3–6]. In particular, IL-6 levels have shown a positive correlation with increased mortality in severely injured patients, including those who have sustained massive burns [7]. Severely injured patients [8–10] and patients presenting with symptoms of sepsis have high circulating levels of IL-6. A major source of the IL-6 appears to be the monocyte/macrophage population, as evidenced by the increased IL-6 mRNA levels in peripheral blood monocytes in these patients [7]. IL-6 is a multifunctional cytokine, which promotes the proliferation and maturation of haematopoietic progenitor cells [11]. Recently it has been shown to be a major stimulator of granulopoiesis in vivo, independent of granulocyte colony-stimulating factor (CSF) action [12]. Despite the elevated levels of IL-6 during sepsis, profound myeloid maturation arrest resulting in neutropenia persists.

We have previously demonstrated that thermal injury superimposed with Gram-negative bacterial sepsis precipitates a critical shift in bone marrow myeloid commitment towards monocytosis, and away from granulocytopoiesis causing a drastic reduction in the number of circulating neutrophils [13]. We were also able to show that granulocytic maturation arrest leads to an accumulation of promyelocytes in the bone marrow [14]. Further, we demonstrated that one of the key mediators of this myeloid shift is the immunosuppressive prostanoid prostaglandin E₂ (PGE₂) [15]. PGE₂ is also a powerful inhibitor of both bone marrow myeloid and lymphoid lineages [16–23]. In addition, PGE₂ suppresses colony formation of bone marrow cells in vitro [21] and when injected intravenously significantly reduces bone marrow cellularity [24]. Similar to IL-6, PGE₂ levels have also been shown to remain elevated following severe injury and sepsis both in animal models and in human patients [25–28].

We have previously shown that in burn sepsis neutropenia and granulocytopenic maturation arrest persist despite high circulating levels of granulocyte-CSF, which is considered essential for granulocytopenic maturation [14]. Interestingly, the inability of the myeloid cells to respond to granulocyte-CSF appears to be due to down-regulation of granulocyte-CSF receptor expression. Therefore we hypothesized that the inability of myeloid cells to respond to the elevated IL-6 levels may be due to a down-regulation of IL-6 receptor (IL-6r) expression and PGE₂ may play a critical role in this process. Here we tested this premise in the murine promyelocytic NFS-60 cell line and demonstrate that PGE₂ is indeed a powerful down-regulator of IL-6r expression and mediates its effects through an EP2-receptor-mediated cAMP signalling pathway.

EXPERIMENTAL

Cell culture

The murine promyelocytic NFS-60 cell line was cultured in RPMI 1640 medium containing 10% fetal bovine serum (FBS) and 1% medium obtained from WEHI-3 cell cultures (a source of IL-3). For experimental procedures cells were suspended in RPMI 1640 medium containing 10% FBS. Recombinant mouse IL-6 (R&D Systems, Minneapolis, MN, U.S.A.) was added at a concentration of 10 ng/ml to NFS-60 cells.

Proliferation assays

Cell proliferation was assessed based on the method of Cory et al. [29]. Cells (5 × 10⁵) were incubated with various compounds at

Abbreviations used: Bt2cAMP, dibutyryl cAMP; CSF, colony-stimulating factor; FBS, fetal bovine serum; IL-6, interleukin-6; IL-6r, IL-6 receptor; PGE₂, prostaglandin E₂; RT-PCR, reverse transcriptase PCR; STAT, signal transduction and activators of transcription.

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37 °C in 5% CO₂ for 72 h. Inhibitors and antagonists were added 15 min prior to the addition of PGE₂. At the end of the incubation period 20 μl of a 20:1 solution of [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium; Promega, Madison, WI, U.S.A.] and phenazine methosulphate (‘PMS’; Sigma, St. Louis, MO, U.S.A.) was added per well, incubated at 37 °C for up to 60 min and absorbance read at 495 nm.

Preparation of cell lysates
Cells were lysed in RIPA buffer (150 mM NaCl/1% Nonidet P-40/0.5% deoxycholic acid/0.1% SDS/50 mM Tris, pH 8.4) containing a protease inhibitor cocktail (Sigma). Samples were incubated at 4 °C for up to 30 min. Protein concentration in the supernatant was measured using a commercially available kit utilizing biocinchonic acid (Pierce, Rockford, IL, U.S.A.) according to manufacturer’s instructions.

Immunoprecipitation
Prior to immunoprecipitation, 1 mg of cell lysate was precleared with 0.25 μg of rabbit IgG (Accurate, Westbury, NY, U.S.A.) and Protein A–agarose beads (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) for 30 min at 4 °C. The beads were pelleted and the supernatant was incubated with 0.8 μg of IL-6r antibody (Santa Cruz Biotechnology) for 1 h and then overnight with Protein A–agarose beads at 4 °C. Pellets were washed four times and resuspended in 30 μl of RIPA buffer containing protease inhibitors for Western blotting.

Western blotting
Equal amounts of protein from cell lysates (100 μg) or immunoprecipitated protein were mixed with Laemmli’s buffer, heated at 100 °C for 5 min and resolved by SDS/PAGE (8% gel) along with prestained protein molecular-mass markers (Bio-Rad, Hercules, CA, U.S.A.). The gels were electrotransferred (15 V for 60 min) to a nitrocellulose membrane (Hybond-c extra: Amersham Biosciences, Little Chalfont, Bucks, U.K.). Membranes were incubated overnight at 4 °C with 1% casein in 11.5 mM NaH₂PO₄/10 mM Na₂HPO₄/150 mM NaCl/0.1% Tween 20/0.01% thimerosal (blocking solution), for 1 h at room temperature with IL-6r antibody (M-20 for NFS-60; Santa Cruz Biotechnology) in blocking solution and then with horseradish peroxidase-conjugated IgG (Bio-Rad) under similar conditions. Immunoblots were visualized using the enhanced chemiluminescence detection system (Pierce) following the manufacturer’s instructions.

Measurement of cAMP
Cells were treated as described previously [30]. Briefly, 10⁶ cells were pretreated with 1 mM isobutylmethylxanthine (‘IBMX’) for 10 min at 37 °C prior to the addition of various compounds. Cells were resuspended in 500 μl of cold ethanol and cell debris removed by centrifugation. The supernatant was dried by vacuum and exposed to X-ray film (Fuji, Stamford, CT, U.S.A.) for up to 24 h at −70 °C.

Reverse transcriptase PCR (RT-PCR) analysis for the expression of prostaglandin receptors
PGE receptor expression was studied by RT-PCR [32]. Total RNA from NFS-60 cells that had been treated with PGE₂ (1 μM) for 0–8 h was isolated and RT-PCR was carried out for the expression of PGE₁, PGE₆, PGE₂, and PGE₆ receptors using the following primers: EP1, sense primer, 5’-TTA ACC TGC TAG CGG ATG-TG-3’, and antisense primer, 5’-CGC TGA GCG TAT TGC ACA CTA-3’; EP2, sense, 5’-GTG GCC CTG GCT CCCC GAA AGT-3’, and antisense, 5’-G GCC AAC CAT ATG GCG AAG GTG-3’; EP3-α, sense, 5’-TGA CTT CCT GCT GCA ACC TG-3’, and antisense, 5’-AGC TGG AAG CAT AGT TG TG-3’; EP3-β, antisense, 5’-GAC CCA GGG AAA CAG GTA CT-3’; EP3-γ, antisense, 5’-AGA CAA TGA GAT GCC CTG CC-3’; EP4, sense, 5’-AGT AAC TGG AAG GAA CAG GTA CT-3’, and antisense, 5’-AAC AGG AAA CAG GTA CT-3’; EP4-β, antisense, 5’-TTA ACC TGA GCC ATG GCG AAG GAT CTT-3’, and antisense, 5’-AAC ACT TTG GGC TGA ACT TGT-3’; β-Actin primers were sense, 5’-GTT ACC AAC TGG GAC GAC ATG G-3’, and antisense, 5’-CAA AGA AAG GGT GTA AAA CGC CAG C-3’. PCR conditions for EP2 were 94 °C for 30 s, 70 °C for 1 min and 72 °C for 2 min with a final extension of 10 min at 72 °C; the total number of cycles was 30. PCR conditions for EP4 were 94 °C for 30 s, 58 °C for 1 min and 72 °C for 2 min with a final extension of 10 min at 72 °C; total number of cycles, 30. PCR conditions for β-actin were 94 °C for 30 s, 59 °C for 1 min and 72 °C for 2 min with a final extension of 10 min at 72 °C; total number of cycles, 30.

Northern analysis
Total RNA was separated from NFS-60 cells 0–6 h following treatment with PGE₂ (1 μM). RNA was separated on a formaldehyde gel and transferred to a nylon membrane. The membrane was then hybridized to a ³²P-labelled murine IL-6r cDNA and to ³²P-labelled β-actin cDNA. Murine IL-6r cDNA (GenBank accession no. X51975) was cloned from murine liver RNA by RT-PCR using the following primers: sense primer, 5’-TGATGACTGAATAGAGATGCCCG-3’; antisense primer, 5’-GCTGCTTTCCAGATGTTGAGAAGG-3’. A 244 bp product was isolated and subcloned into the pTA vector (Clontech, Palo Alto, CA, U.S.A.). The cloned cDNA was sequenced and homology with the GenBank murine IL-6r sequence was confirmed.

RESULTS
In initial experiments, using immunoblot analysis, we were able to demonstrate that PGE₂ (1 μM) was a potent down-regulator
Prostaglandin E<sub>2</sub> down-regulates interleukin-6 receptor expression

Figure 1  Effect of PGE<sub>2</sub> on IL-6r expression in NFS-60 cells

NFS-60 cells were incubated in the presence and absence of 1 µM PGE<sub>2</sub> for up to 8 h. Immunoprecipitation (IP) and Western blotting (WB) were carried out on cell lysates as described in the Experimental section. n = 3. Data represent a typical Western blot.

8 h the inhibition was almost complete. During this period the cells remained viable and did not show any evidence of either apoptosis or necrosis. To verify whether PGE<sub>2</sub>-induced down-regulation of IL-6r in NFS-60 cells is biologically significant, we next assessed the efficacy of IL-6 to stimulate cellular proliferation and the capacity of PGE<sub>2</sub> to block proliferation (Figure 2). Proliferation assays corroborated the results of immunoblot analysis. IL-6 (10 ng/ml) stimulated maximal proliferation of NFS-60 cells and PGE<sub>2</sub> (0.01 nM–1 µM) inhibited IL-6-induced proliferation in a dose-dependent manner. Since immunoblot analysis of IL-6r represents the status of both the cell-surface as well as the intracellular IL-6r, we wanted to verify if PGE<sub>2</sub> treatment was in fact capable of significantly diminishing IL-6-stimulated intracellular signals. To this end, we utilized mobility-shift assay to assess IL-6-stimulated STAT-3 activation. NFS-60 cells were incubated in the presence or absence of PGE<sub>2</sub> (1 µM) for 8 h at 37 °C and then washed and exposed to IL-6 (10 ng/ml) for 15 min at 37 °C. Nuclear extracts were made from all the samples and the extent of STAT-3 activation was determined by mobility-shift assay using radiolabelled duplex oligonucleotides specific for STAT-3. Our results clearly demonstrated that IL-6 treatment was able to induce robust STAT-3 activation, as indicated by the mobility shift. Prior treatment of NFS-60 cells with PGE<sub>2</sub> abolished this response to a large extent (Figure 3). We were able to ascertain the specificity of the response by effective competition with unlabelled STAT-3-specific nucleotides.

Figure 2  Proliferation of NFS-60 cells

Main panel: NFS-60 cells were incubated in RPMI containing 10% FBS in the presence or absence of IL-6 (10 ng/ml) and PGE<sub>2</sub> (1 µM) for 72 h. Proliferation was measured by a colorimetric assay as described in the Experimental section and is expressed as absorbance at 495 nm. Inset: dose–response curve for PGE<sub>2</sub>. Proliferation is shown as the percentage absorbance of cells incubated in the presence of IL-6 alone (untreated control). Data represent means ± S.D. (n = 4).
Figure 3 Effect of PGE₂ on IL-6-induced signal transduction

NFS-60 cells were incubated in the presence or absence of 1 μM PGE₂ for 8 h, washed and then treated with or without 10 ng/ml IL-6 for 15 min. Nuclear extracts were subjected to electrophoretic mobility-shift assay with [γ-³²P]ATP-labelled STAT-3 consensus DNA as described in the Experimental section. Excess (50 ‰) unlabelled probe was incubated with nuclear extracts to ensure specificity of binding. Data represent a typical electrophoretic mobility-shift assay.

We then investigated if the down-regulation in IL-6r protein expression is paralleled by changes in IL-6r mRNA expression. NFS-60 cells were treated with PGE₂ (1 μM) for 0–6 h prior to RNA extraction. Northern blot analysis showed that the steady-state levels of IL-6r mRNA were reduced by 46 and 90 ‰ compared with the untreated control 2 and 4 h after PGE₂ treatment. There was no further decrease in IL-6r expression when cells were treated with PGE₂ for 8 h. Taken together, these lines of evidence support our premise that PGE₂ is indeed a powerful inhibitor of IL-6r expression in the murine promyelocytic cell line NFS-60.

Having established the potency of PGE₂ in IL-6r regulation, we queried the receptor subtype through which PGE₂ signals to accomplish its inhibitory effects. Four receptors (EP₁–EP₄) that mediate the effects of E-type prostaglandins have been described [33]. EP₁ receptors are involved in the mobilization of intracellular calcium. EP₂ and EP₄ receptors have similar activity in that they stimulate adenylylate cyclase but can be differentiated by distinct ligand specificity. EP₃ receptor action is mainly via inhibition of adenylylate cyclase although at times has been shown to signal via calcium mobilization. The potent EP₁/EP₃ receptor agonist sulprostone had no effect on IL-6-induced proliferation of NFS-60 cells (Figure 4). In contrast, the specific EP₂ receptor agonist butaprost inhibited IL-6-stimulated proliferation of NFS-60 cells (Figure 4). Butaprost is not as potent as PGE₂ [33] and required a 10–50-fold higher concentration to suppress IL-6-stimulated proliferation to a similar extent as PGE₂. We also observed that the EP₂ antagonist AH6809 was able to alleviate the anti-proliferative effect of PGE₂ by 30–60 ‰ (n = 3). In addition, exogenous cAMP [in the form of dibutyryl cAMP (Bt₂cAMP)] inhibited IL-6-stimulated proliferation of NFS-60 cells (Figure 4). Taken together, these results indicate that PGE₂ may inhibit IL-6-stimulated proliferation of NFS-60 cells through its ability to generate cell signals by the EP₂ receptor.

Since currently the status of the PGE receptors on NFS-60 cells remains undocumented and the response of PGE receptor expression to PGE₂ treatment is yet to be determined, we investigated the distribution and response of PGE receptors (EP₁–EP₄) by RT-PCR analysis in NFS-60 cells. NFS-60 cells had no detectable levels of EP₁ and EP₃ receptors (results not shown). However, NFS-60 cells expressed both EP₂ and EP₄ receptors robustly. PGE₂ treatment for 0–8 h did not significantly change the level of expression of either EP₂ or EP₄ receptors (Figure 5). During the same period, IL-6rs on NFS-60 cells were significantly down-regulated. Involvement of EP₂/EP₄ receptors in PGE₂ signalling in NFS-60 cells was further confirmed by intracellular cAMP measurements. Treatment of NFS-60 cells with 1 μM PGE₂ increased intracellular cAMP from 17.2 ± 12.6 to 233.2 ± 75.1 pmol/10⁶ cells (n = 3).

PGE₂ is known to mediate its anti-proliferative effect through generation of cAMP, which leads to cell-cycle arrest at the G₁ phase [34,35]. Therefore we questioned whether, during this
PGE$_2$-mediated anti-proliferative effect on NFS-60 cells, there is any correlation between cell-cycle arrest and IL-6r down-regulation. NFS-60 cells exposed to PGE$_2$ or Bt$_2$cAMP for 8 h were stained with propidium iodide and subjected to flow-cytometric analysis. We demonstrate that exposure to PGE$_2$ or exogenous cAMP results in the accumulation of NFS-60 cells in the G$_0$/G$_1$ phase of the cell cycle (Table 1). Thus the reduction in IL-6r expression occurs in a similar time frame to cell-cycle arrest.

**DISCUSSION**

In this study we have shown that PGE$_2$ is a potent down-regulator of IL-6r expression in the murine promyelocytic cell line N60-60. This inhibition is manifested in the inability of IL-6 to stimulate either proliferation or initiate intracellular signalling in the presence of PGE$_2$. Previous studies from our laboratory have revealed that in thermally injured animals with superimposed sepsis bone marrow myeloid commitment shifts away from granulocytopenia and toward monocytopenia [13] and this shift occurs despite elevated levels of the stimulators of granulocytopenia granulocyte-CSF and IL-6 [14,36]. Abrogation of PGE$_2$ production or PGE$_2$ receptor blockade was effective in improving survival and normalizing the myeloid commitment following burn sepsis [15,37]. In the current work, we have tried to explore the mechanisms behind these observations through an in vitro cell-culture model system. While such approaches provide valuable mechanistic information, they have limitations in terms of providing a total explanation for the in vivo observations. Nevertheless our work clearly indicates how PGE$_2$ might impede the ability of IL-6 to promote myeloid progenitor cell growth.

In NFS-60 cells we observed a reduction in IL-6r expression after 6–8 h of exposure to PGE$_2$. We chose to monitor nuclear STAT-3 accumulation in order to determine whether this reduction in total cellular IL-6r expression was functionally significant. Within minutes of IL-6 binding to its receptor the signal-transducing moiety gp130 associates with this complex [38]. Subsequently, members of the Jak family of tyrosine kinases associated with the receptor complex become activated and phosphorylate gp130, which creates docking sites for STAT-1 and STAT-3. The STATs are phosphorylated, dimerize and are translocated into the nucleus where they associate with nuclear response elements and regulate the transcription of target genes. We demonstrated that exposure of NFS-60 cells to PGE$_2$ for 8 h did indeed abolish the ability to induce nuclear STAT-3 accumulation following stimulation with IL-6, indicating that the reduction in IL-6r expression was functionally significant. Thus, when elevated levels of IL-6 and PGE$_2$ co-exist, as in thermal injury, the effects of PGE$_2$ may be able to eliminate proliferative signals through the IL-6r, resulting in impaired granulocytopenia. Similar to the results we present here, PGE$_2$-induced cAMP inhibits human T-cell activation and proliferation by down-regulating IL-2 receptor z mRNA as well as cell-surface expression of the IL-2 receptor [16,39]. In contrast, in another in vitro cell system, PGE$_2$ has been shown to inhibit macrophage-CSF-dependent proliferation of bone marrow-derived macrophages without affecting expression of the CSF-1 receptor or early signal transduction mediated by ligand binding [34,40]. A potential explanation for the lack of effect on CSF-1 receptor expression is perhaps due to the time frame in the CSF-1 studies. These investigators examined the very early effects of PGE$_2$ (1 h), whereas in our study and in studies involving T-cell proliferation and IL-2 receptor expression much longer-term effects (8–24 h) were studied.

We confirm previous reports [34,35] that the anti-proliferative effects of PGE$_2$ are mediated by cAMP (Figures 2 and 4) and show for the first time that in NFS-60 cells these effects are mediated through the EP2 receptor. EP2 receptors are associated with adenylate cyclase and mediate signal transduction by increasing intracellular cAMP levels [33]. We demonstrated that the EP2-receptor-specific agonist butaprost inhibits IL-6-induced proliferation of NFS-60 cells. In addition, the EP2 receptor antagonist AH6809 relieves PGE$_2$-induced inhibition of IL-6-stimulated proliferation of these cells.

Previous studies have established that elevating cAMP levels inhibit mitogen-dependent DNA synthesis and proliferation of myeloid cells [34,35]. cAMP also halts progression of the cell cycle by down-regulating D-type cyclins and cyclin-dependent kinase 4 (cdk4) [41]. Other anti-proliferative effects of cAMP include suppressing phosphorylation of retinoblastoma protein and expression of c-Myc and proliferating cell nuclear antigen [35]. There is evidence that similar effects may occur following thermal injury. In an animal model intracellular cAMP levels are elevated in splenocytes following thermal injury and cAMP suppresses mitogen-stimulated proliferation of normal splenocytes [42].

The PGE$_2$-induced decrease in IL-6r expression coincides with the accumulation of NFS-60 cells in the G$_0$/G$_1$ phase of the cell cycle and our studies with exogenous Bt$_2$cAMP demonstrate that cAMP is the secondary signal which mediates this cell-cycle

**Table 1** Cell-cycle distribution of NFS-60 cells following exposure to PGE$_2$ or cAMP

<table>
<thead>
<tr>
<th>Cell-cycle phase</th>
<th>Untreated</th>
<th>1 μM PGE$_2$</th>
<th>5 μM Bt$_2$cAMP</th>
<th>50 μM Bt$_2$cAMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apoptotic</td>
<td>0.05 ± 0.01</td>
<td>0.7 ± 0.1</td>
<td>1.6 ± 0.15</td>
<td>3.9 ± 0.23</td>
</tr>
<tr>
<td>G$_0$/G$_1$</td>
<td>45.1 ± 1.2</td>
<td>62.9 ± 2.1</td>
<td>66.7 ± 3.0</td>
<td>74.5 ± 2.6</td>
</tr>
<tr>
<td>S</td>
<td>39.8 ± 1.5</td>
<td>21.1 ± 1.6</td>
<td>15.0 ± 2.1</td>
<td>7.3 ± 0.9</td>
</tr>
<tr>
<td>G$_0$/M</td>
<td>14.9 ± 2.2</td>
<td>15.1 ± 1.8</td>
<td>13.5 ± 1.1</td>
<td>12.2 ± 1.3</td>
</tr>
</tbody>
</table>

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arrest (Table 1). These results are consistent with similar findings in the current literature. For example, raising intracellular cAMP by either exogenous addition or stimulation with PGE_2 has been shown to arrest macrophage growth by arresting the cells in the mid-G_1 phase of the cell cycle [43]. Similar results were also reported in airway smooth muscle cells [44]. Nonetheless, no study until now has shown both PGE_2 and cAMP-mediated cell-cycle arrest and receptor down-regulation in the same cell type. However, it is not known whether the down-regulation of the IL-6r and cell-cycle arrest are related or are independent events initiated by PGE_2.

Our studies also provide the PGE_2 receptor distribution in NFS-60 cells. These cells predominantly express EP2 and EP4 receptors. Exposure to PGE_2 for 0–8 h did not significantly down-regulate EP2 and EP4 receptors. Recent studies on mouse calvaria have demonstrated that PGE_2 causes bone resorption through cAMP generated by the engagement of EP2 and EP4 receptors [45,46]. Furthermore, using knockout mice specific for each type of EP receptor, Nataraj et al. [32] have demonstrated that the anti-proliferative effect is independent of the EP1 and EP3 receptors. They further showed that PGE_2 activated EP4 receptors on macrophages. Similar agonist-induced up-regulation of EP4 receptor expression in macrophages has also been demonstrated by Hubbard et al. [47]. Our studies, however, demonstrate that EP receptor expression in NFS-60 cells remains unchanged for the duration of the experiment. PGE_2 appears, however, to down-regulate IL-6r during the similar time period at both the protein and mRNA levels and this results in inhibition of the IL-6-mediated intracellular signalling.

In summary, the current study demonstrates that PGE_2 is a potent down-regulator of IL-6r expression in NFS-60 cells and is likely to mediate this effect through either EP2 and/or EP4 receptor activation. Furthermore, the current study provides a potential mechanism for the inhibition of granulocytopoiesis and the resulting neutropenia in animals with burn injury and sepsis even in the presence of elevated levels of myeloid mitogens such as IL-6.

This work was supported by National Institutes of Health grants R01 GM56424 (to R.S.) and R01 GM42577 (to R.L.G.). We thank Patricia Simms for flow-cytometric data collection and analysis.

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