Biochemical and Biophysical Research Communications **290,** 249–255 (2002)
doi:10.1006/bbrc.2001.6188, available online at http://www.idealibrary.com on IDEAL®

Prostaglandin E_2 Stimulates Prostatic Intraepithelial Neoplasia Cell Growth through Activation of the Interleukin-6/GP130/STAT-3 Signaling Pathway

Xin-Hua Liu,*[,1] Alexander Kirschenbaum,† Min Lu,* Shen Yao,* Adam Klausner,† Chad Preston,* James F. Holland,* and Alice C. Levine*

**Department of Medicine and †Department of Urology, Mount Sinai School of Medicine, New York, New York 10029*

Received November 26, 2001

Cyclooxygenase (COX)-2 expression and prostaglandin E_2 (PGE_2) secretion are increased in prostatic intraepithelial neoplasia (PIN) and prostate cancer. PGE_2 biosynthesis by cyclooxygenase (COX)-2 plays a pivotal role in inflammation and carcinogenesis. One of the critical proinflammatory cytokines in the prostate is interleukin-6 (IL-6). We hypothesized that increased expression of COX-2, with resultant increased levels of PGE_2 in human PIN cells, activates the IL-6 signaling pathway. We demonstrate an autocrine upregulation of PGE_2 mediated by IL-6 in a human PIN cell line. We further demonstrate that PGE_2 stimulates soluble IL-6 receptor (sIL-6R) release, gp130 dimerization, Stat-3 protein phosphorylation, and DNA binding activity. These events, induced by PGE_2, lead to increased PIN cell growth. Treatment of PIN cells with a selective COX-2 inhibitor decreases cell growth. Finally, PGE_2-stimulated PIN cell growth was abrogated by the addition of IL-6 neutralizing antibodies. These data provide mechanistic evidence that increased expression of COX-2/PGE_2 contributes to prostate cancer development and progression via activation of the IL-6 signaling pathway.

Key Words: **COX-2; gp130; IL-6; soluble IL-6 receptor; PGE_2; prostate cancer; Stat-3.**

Proliferation associated with longstanding, chronic inflammation has been implicated in the development of cancer in a variety of tissues including skin, urinary bladder, gastric mucosa, liver and large bowel (1). Cyclooxygenase (COX)-2 is the highly inducible form of COX which catalyzes the conversion of arachidonic acid to prostaglandins. COX-2 expression is induced by inflammation. Increased biosynthesis of PGE_2, a major eicosanoid product of the COX-2-catalyzed reaction, plays a pivotal role in inflammation (2). Recent studies have also demonstrated enhanced expression of COX-2 and elevated PGE_2 production in human cancer of several lineages, including prostate cancer (3). Forced overexpression of COX-2 in nonneoplastic colon cells promotes neoplastic transformation and resistance of those cells to apoptosis (4). Clinical studies revealed that patients treated with nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit both isoforms of COX, are at a reduced risk of developing prostate cancer (5).

The human prostate gland is a common site of inflammation. Immunohistochemical staining of noncancerous prostates demonstrates that luminal epithelial cells surrounded by inflammation are induced to express COX-2 (6). It has also been reported that focal prostatic atrophy, which is associated with chronic inflammation, is highly proliferative (7). We demonstrated increased expression of COX-2 in prostate cancer, as well as in prostatic intraepithelial neoplasia (PIN) (8), the most likely precursor of prostate cancer (9). In addition, inflammatory cytokines, notably tumor necrosis factor-α (TNF-α), and IL-6, induce the expression of COX-2 and the secretion of PGE_2 in human prostate cancer cell lines (10, 11). These data indicate that COX-2 activity is induced in both prostatic inflammation and neoplasia, and suggest that increased PGE_2 derived from increased COX-2 activity may be involved in inflammation-associated prostatic carcinogenesis.

The effects of COX-2 are believed to be partially mediated by inflammatory cytokines (12). One of the critical proinflammatory cytokines in the prostate is

Abbreviations used: COX, cyclooxygenase; Gp130, glycoprotein 130; PG, prostaglandin; PIN, prostatic intraepithelial neoplasia; sIL-6R, soluble IL-6 receptor; Stat, signal transducers and activators of transcription.

[1] To whom correspondence and reprint requests should be addressed at Department of Medicine, Box 1055, Mount Sinai School Medicine, One Gustave L. Levy Place, New York, NY 10029. Fax: (212) 241-4218. E-mail: liux0l@doc.mssm.edu.

0006-291X/02 $35.00

IL-6. IL-6 has been implicated in prostate cancer development and progression (13–15). It binds to either the cognate IL-6 receptor (IL-6Rα) or, in most cases, soluble IL-6 receptor (sIL-6R) and consequently induces dimerization of gp130, a signal-transducing element, previously identified as IL-6 receptor-β (16). These events lead to activation of Stat signaling and result in the induction of proteins, such as bcl-2 and cMyc, both of which are critical regulators of cell cycle progression and apoptosis (17). Clinical studies have demonstrated that IL-6 and its receptors are upregulated in human high grade PIN and prostate cancer compared to benign tissue (18). IL-6, sIL-6R, and gp130 are also expressed in human prostate cancer cell lines (14). Recent studies have demonstrated that IL-6 stimulates prostate cancer cell growth through activation of the Stat-3 signaling pathway (19). However, the complex interactions between PGE_2, IL-6 and IL-6-induced signaling pathway and the mechanisms whereby these interactions may promote prostatic neoplasia have not yet been elucidated.

In the present report, we demonstrate autocrine upregulation of PGE_2 production induced by IL-6 in a human PIN cell line. We further demonstrate that PGE_2 stimulates soluble IL-6 receptor (sIL-6R) release, gp130 dimerization, Stat-3 protein phosphorylation and DNA binding activity. These events induced by PGE_2 lead to increased growth of PIN cells. Finally, we observed that PGE_2-stimulated PIN cell growth can be abrogated by the addition of IL-6 neutralizing antibodies. These data provide mechanistic evidence that elevated PGE_2 levels, induced by inflammatory cytokines, contribute to prostate cancer development and progression through activation of the IL-6 signaling pathway.

MATERIALS AND METHODS

Cell line and cell culture. A human high grade PIN cell line was provided as a gift by Dr. M. Stearns (MCP-Hahnemann University, Philadelphia, PA). This cell line was established by human papilloma virus-18 immortalization of PIN cell areas of radical prostatectomy specimens. It was previously demonstrated that these cells express prostate specific antigen and cytokeratin 34βE12, thereby establishing their PIN cell origin. The cell line has been characterized with respect to its growth potential, colony formation and androgen responsiveness (20). PIN cells were cultured in keratinocyte medium (KTM) supplemented with 10% FBS. Before treatment with various compounds, cells were washed with PBS and serum-free medium containing 0.1% BSA was replaced overnight. For the cell growth studies, PIN cells were plated in 12-well cluster dishes with 1 ml of 10% FBS-supplemented KTM for 24 h. Incubations were continued with or without various treatments for 1, 3, or 5 d. Cells were then harvested, and the number of living cells (identified by trypan blue staining) was determined by counting in a hemacytometer.

Immunoprecipitation and immunoblotting. PIN cells cultured under the desired conditions were lysed as described previously (21). Briefly, cells were rinsed twice with ice-cold PBS and scraped with 1.5 ml of PBS containing 4 mM iodoacetate. After centrifugation, the pellets were resuspended in Chaps extraction solution (10 mM Chaps, 2 mM EDTA, pH 8.0, and 4 mM iodoacetate in PBS) with protease inhibitors. The samples were then incubated for 30 min on ice and centrifuged at 15,000*g* for 10 min. The supernatants were collected and stored at −70°C. For Western blotting, samples were electrophoresed on SDS–PAGE, transferred to a polyvinylidene difluoride (PVDF) membrane (DuPont, NEN), and incubated with target antibodies overnight at 4°C. For immunoprecipitation, the lysates were precleared with protein A–Sepharose beads for 2 h at 4°C. The precleared lysates were incubated with 1 μg polyclonal anti-phospho-Tyr_{705}-Stat-3 antibody (Calbiochem, San Diego, CA) followed by the addition of protein A–Sepharose beads. The immunoprecipitated proteins were then subjected to SDS–PAGE and electrophoretically transferred to a PVDF membrane. Immunoreactive proteins were visualized with an enhanced chemiluminescence (ECL) detection system (Amersham) using horseradish peroxidase-linked IgG (Amersham, Arlington Heights, IL) as secondary antibody.

Preparation of conditioned medium for ELISA. PIN cells were plated at 1×10^5 cells/well in six-well cluster dishes with 2 ml medium containing 10% FBS. After washing with PBS, serum-free medium was replaced. Incubations were continued under the desired conditions. The culture medium was collected, centrifuged at 800*g* for 10 min to remove suspended cells, and stored at −70°C for assays. ELISA kits for IL-6 and sIL-6R detection were purchased from R & D Systems (Minneapolis, MN), and the kits for PGE_2 assay were obtained from Cayman Chemical Co. (Ann Arbor, MI).

Nuclear extract preparation and electrophoretic mobility shift assay (EMSA). PIN cells were plated in 150-mm culture dishes with 20 ml KTM containing 10% FBS for 24 h. Cells were then washed with PBS and serum-free medium containing 0.1% BSA was replaced. The culture was continued with or without the following treatment: 1 μM PGE_2, or 10 ng/ml IL-6, or 10 ng/ml IL-6 plus 20 ng/ml sIL-6R for 6 h. Nuclear extracts were prepared using a kit from Pierce Inc. (Rockford, IL). Protein concentration was measured by the Bio-Rad protein assay. Specific Stat-3 oligonucleotide, formed by annealing the oligonucleotide sequence with the antisense strand, was purchased from Santa Cruz Inc. (Santa Cruz, CA). The probe was purified and labeled with [γ-^{32}P]ATP (Dupont, NEN, Boston, MA) using T4 polynucleotide kinase. The binding of the probes (10,000 cpm) to nuclear extracts (5 μg protein) was performed in a 20-μl mixture containing 5 μg of poly(dI–dC)–poly(dI–dC), 15 mM Tris–HCl, pH 7.5, 1 mM EDTA, 100 mM KCl, 5 mM $MgCl_2$, 12% glycerol, 0.5 mM phenylmethylsulfonyl fluoride, and 0.5 mM dithiothreitol. The mixture was incubated at 25°C for 30 min, and applied to 5% polyacrylamide gels containing 7.5% glycerol in 0.5× Tris-borate EDTA buffer at a constant 200 V for 2 h. Gels were fixed in 10% methanol, 10% acetic acid, and 80% water for 30 min, dried, and autoradiographed.

RESULTS

Interactive and Reciprocal Effects of COX-2/PGE_2 and IL-6 in PIN Cells

Increased COX-2 expression and elevated levels of PGE_2 and IL-6 have been demonstrated in human prostate tissue and prostate cancer cell lines. We initially determined the maximal stimulatory dose of PGE_2 on IL-6 secretion by PIN cells (data not shown). We next performed time course studies. As shown in Fig. 1A, PGE_2 increased IL-6 secretion by PIN cells in a time-dependent manner. This effect was first noted at 4 h with a peak induction 16 h after PGE_2 addition.

The human COX-2 gene contains a putative NF-IL-6 element in the 5′-flanking promoter region which can be activated by IL-6 (22). Recent studies have demon-

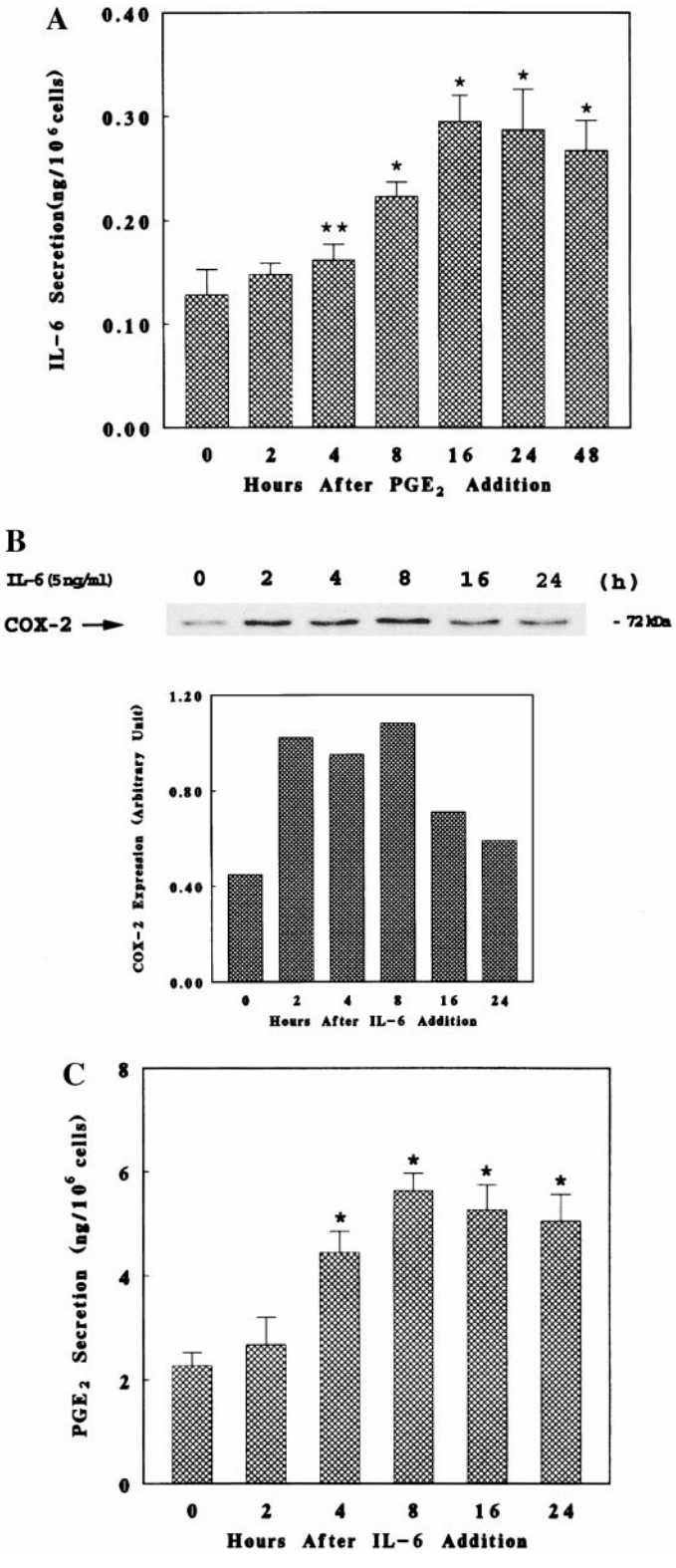

FIG. 1. Interactive and reciprocal effects of COX-2/PGE_2 and IL-6. PIN cells were cultured in serum-free KTM with 0.1% BSA. (A) Cells were treated with either vehicle as control or 1 μM PGE_2, for various times as indicated. Culture medium in various conditions was collected, clarified, and subjected to ELISA for the determinations of IL-6 secretion. (B) Cells were treated with either vehicle as control or 10 ng/ml IL-6, for various times as indicated. Total protein was then isolated and subjected to Western blot analysis with an anti-COX-2 antibody. The result shown in the upper panel is a representative from triplicate experiments. The quantitative analysis of COX-2 protein expression is shown in the lower panel. (C) Cells were treated with either vehicle as control or 10 ng/ml IL-6, for various times as indicated. Culture medium under various conditions was collected, clarified, and subjected to ELISA for the determinations of PGE_2 secretion. Results from ELISA were normalized to cell number. Data represent means ± SEM from three separate determinations, $^{**}P < 0.05$, $^{*}P < 0.01$.

strated that IL-6 mediates COX-2 induction by serum in cultured endothelial cells (23). We, therefore, examined the effect of IL-6 on COX-2 expression and PGE_2 secretion by PIN cells. As shown in Fig. 1B, PIN cells expressed basal levels of COX-2, and this expression was increased in the presence of IL-6 (5 ng/ml). The IL-6 induction of COX-2 expression was time-dependent, with a peak increase noted 8 h after IL-6 treatment. Figure 1C demonstrates that IL-6 induced PGE_2 release by PIN cells, confirming a stimulatory effect of IL-6 on COX-2 activity.

PGE_2 Stimulates sIL-6R Release and Facilitates gp130 Dimerization

The receptor mediating the biological activities of IL-6 is composed of two subunits: an 80-kDa alpha chain (gp80, IL-6Rα) and a 130-kDa glycoprotein beta chain (gp130, IL-6-Rβ). Additionally, a soluble form of IL-6R (sIL-6R) with a molecular weight of approximately 50 kDa was reported, which has been identified as a central element in the mediation of IL-6 signaling (24). We were initially unable to detect the expression of IL-6Rα (gp80) in PIN cells (data not shown). In contrast, PIN cells do secrete sIL-6R and the secretion is significantly increased following treatment with 1 μM PGE_2. This effect began at 1 h and was maximal at 4–8 h (Fig. 2A).

The effect of PGE_2 on gp130 expression was examined using Western blot analysis under reducing and nonreducing conditions. Representative results are illustrated in Fig. 2B. Under reducing conditions, PIN cells expressed low basal levels of gp130 monomers which were not significantly influenced by PGE_2. Under non-reducing conditions, however, gp130 dimers were detected as a protein band with a molecular weight of 260 kDa (25) and the level of gp130 dimers was significantly increased by the addition of 1 μM PGE_2. The induction was first noted at 1 h and reached peak levels at 8 h after PGE_2 treatment. The level gradually decreased thereafter, with a return to basal levels by 16 h. These results demonstrate that PGE_2 activates gp130 by promoting its dimerization.

PGE_2 Activates the Stat-3 Signaling Pathway

Stat-3 is a latent transcription factor that plays a central role in transmitting IL-6 signals from the mem-

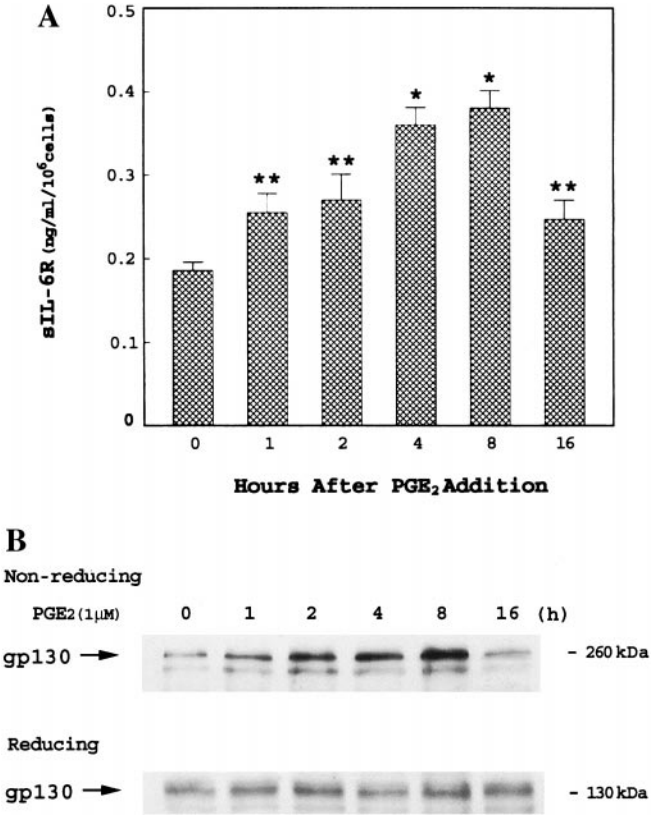

FIG. 2. PGE_2 upregulates sIL-6R release and gp130 dimerization. PIN cells were cultured in serum-free KTM, and treated with either vehicle or 1 μM PGE_2 for various times as indicated. (A) Culture medium was collected, clarified, and subjected to ELISA for the determinations of sIL-6R release. The results were normalized to cell number. Data represent means ± SEM from three separate determinations, $^{**}P < 0.05$, $^{*}P < 0.01$. (B) Total protein was isolated and subjected to Western blot analysis using antibodies against gp130 either under a nonreducing (top) or reducing condition (bottom). The band with molecular weight of 260 kDa represents the homodimers of gp130, and the lower molecular weight band (130 kDa) represents the monomers of gp130. Data shown are representative of three independent experiments.

brane to the nucleus. Activation of stat-3 is essential for IL-6/gp130-mediated transformation and cell survival (17, 26). Stat-3 is activated by the IL-6/sIL-6R/gp130 complex through phosphorylation on a single tyrosine residue (Tyr 705) (16, 25). The observed stimulatory effects of PGE_2 on IL-6 secretion, sIL-6R release, and gp130 dimerization led us to further examine the potential effect of PGE_2 on Stat-3 activation. As shown in Fig. 3A, PGE_2 induced a significant elevation of phospho-Stat-3 level, with a peak induction at 6 h after treatment. By 16 h after PGE_2 addition, phospho-Stat-3 levels had declined and were sustained at 50% of peak values. In contrast, PGE_2 showed no effect on endogenous Stat-3 protein expression (Fig. 3A).

Phosphorylation of Stat-3 leads to enhancement of Stat-3 DNA binding activity. To test the effects of PGE_2 on Stat-3 DNA binding, nuclear extracts from PGE_2-treated or non-treated PIN cells were analyzed by EMSA utilizing a specific Stat-3 probe. Results of representative experiments are demonstrated in Fig. 3B. PIN cells expressed detectable basal levels of Stat-3 activity. While the addition of IL-6 alone had no significant effect, combination treatment with both IL-6 and sIL-6R stimulated Stat-3 DNA binding activity in PIN cells. In addition, a 2.2-fold increase in Stat-3 DNA binding activity was observed when PIN cells were treated with 1 μM PGE_2 alone, confirming that PGE_2 activates Stat-3 biologic activity in human PIN cells (Fig. 3B).

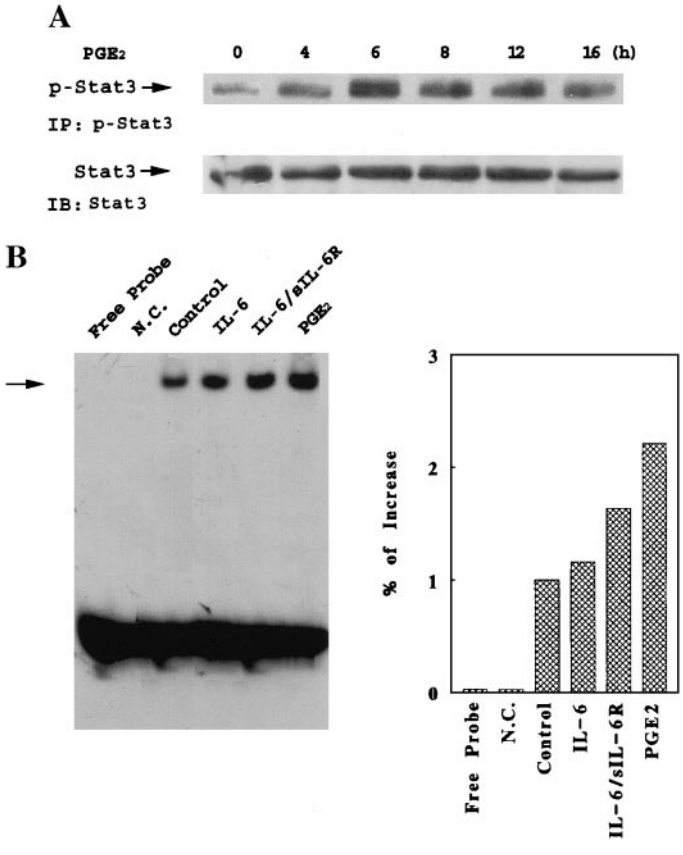

FIG. 3. PGE_2 stimulates Stat-3 protein phosphorylation and DNA binding activity. (A) PIN cells were cultured in serum-free KTM, and treated with either vehicle or PGE_2 (1 μM) for various times as indicated. Total protein was isolated and subjected to either immunoprecipitation (IP) with an antibody against phospho-Stat-3 (Tyr 705) followed by separation on SDS–PAGE, or immunoblotting (IB) with an antibody against endogenous Stat-3. Phosphorylated and endogenous Stat-3 proteins were visualized by peroxidase-linked IgG with ECL detection system. (B) Stat-3 DNA-binding activity in cultured PIN cells was determined by EMSA. Lane 1, free probe; lane 2, no cells (N.C.). Cells were treated with either vehicle as control (lane 3) or 10 ng/ml IL-6 for 24 h (lane 4); or combination of IL-6 (10 ng/ml) and sIL-6R (20 ng/ml) for 24 h (lane 5); or 1 μM PGE_2 for 6 h (lane 6). Arrow indicates position of Stat-3 protein/DNA complex (left). The amount of binding protein was quantitated by densitometry and expressed as percentage of increase vs control (right).

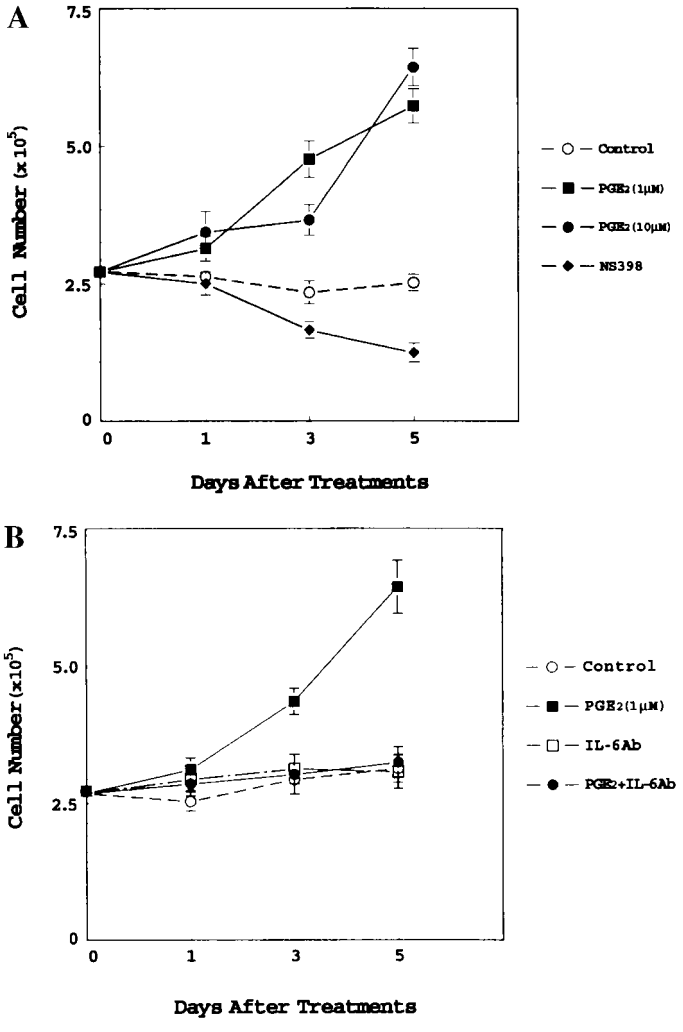

FIG. 4. PGE_2 increased PIN cell growth. PIN cells were cultured in serum-free KTM with refeeding after 3 days. (A) Cells were treated with either vehicle as control, or two doses of PGE_2 (1 and 10 μM), or 50 μM NS398 for the time as indicated. (B) Cells were treated with either vehicle as control or 1 μM PGE_2, or 5 μg/ml neutralizing anti-IL-6 antibodies, or a combination of PGE_2 (1 μM) and neutralizing anti-IL-6 antibodies (5 μg/ml) for various times as indicated. Number of living cells was counted with a hemacytometer. Data are means ± SE of three separate experiments with triplicate wells each.

IL-6 Neutralizing Antibodies Block PGE_2-Induced PIN Cell Growth

Stat-3 is an important transcription factor responsible for the induction of c-*myc* and bcl-2 family of genes, which are critical in the regulation of cell cycle progression and apoptosis (17, 26). To determine the biological consequences of PGE_2-induced Stat-3 activation, we next examined the effect of PGE_2 on PIN cell growth *in vitro*. As demonstrated in Fig. 4A, the addition of PGE_2 (1 or 10 μM) increased PIN cell growth significantly over control in a time-dependent fashion. A significant increase in cell number was detectable 3 days after the addition of PGE_2. In addition, the effect of NS398, a selective COX-2 inhibitor, on PIN cell growth was tested. Treatment with 50 μM NS398 significantly inhibited PIN cell growth compared to control (Fig. 4A). Finally, we tested whether IL-6 neutralizing antibodies would prevent the stimulatory effects of PGE_2 on PIN cell growth. Neutralizing antibodies to IL-6 completely abrogated the PGE_2-induced PIN cell growth (Fig. 4B).

DISCUSSION

Chronic inflammation of longstanding duration has been linked to the development of human cancers in several organ systems, including the prostate (7). One of the critical mediators of these processes is COX-2, the expression of which is induced by proinflammatory stimuli (2). Our immunohistochemical data indicate that non-cancerous prostate luminal epithelial cells do not normally express COX-2, but are induced to express the enzyme when surrounded by inflammatory cells. Interestingly, these same cells also expressed the anti-death gene, bcl-2 (6). Overexpression of COX-2 in a variety of tissues leads to resistance to apoptosis, tumor development and progression (4, 27). COX-2, therefore, is a stress gene, which is overexpressed in states of inflammation and malignancy, to protect cells from destruction.

Although many reports confirm that inflammatory cytokines induce COX-2 expression, the signaling pathways which mediate the consequent effects of COX-2 overexpression on cell survival are less well studied. Elevated level of cytokines in response to inflammation can induce a network which may influence cell survival, mutation, proliferation and differentiation (1). We herein report that IL-6 promotes COX-2 expression and PGE_2 secretion in a human PIN cell line. In addition, we demonstrate that PGE_2 activates PIN cell proliferation via several effects on the IL-6 system. PGE_2 stimulates IL-6 secretion and sIL-6R release by this cell line. These events lead to increased formation of IL-6/sIL-6R complexes and stimulation of gp130 dimerization with resultant activation of Stat-3 (16).

Gp130 has been identified as the signal-transducing element of the IL-6 receptor. Binding of IL-6 to sIL-6R induces disulfide-linked homodimerization of gp130, a critical step leading to Stat-3 activation (16, 25). Gp130 is a widely expressed protein which is not subject to major regulation (16). Our data demonstrate that PGE_2 does not regulate the endogenous expression of gp130 in PIN cells but, rather, promotes its activation via homodimerization.

It has been reported that cells which do not express IL-6Rα on their surface can be stimulated only by the IL-6/sIL-6R complex and are insensitive to IL-6 alone (24). In the present study, we demonstrate that PIN

cells, which do not express IL-6Rα, are not stimulated by IL-6 alone to induce Stat-3 binding activity. However, the addition of both IL-6 and sIL-6R resulted in increased Stat-3 binding activity. These results are in agreement with the report by Peters and Tamura *et al.* (24, 28) that sIL-6R, in addition to acting synergistically with IL-6, is critical in controlling IL-6 responses in cells lacing IL-6Rα expression.

Stat-3 is a transcription factor with oncogenic properties which has been demonstrated to play a role in cell survival, proliferation and differentiation (17, 26). The c-*myc* gene is induced in response to the proliferative signals elicited by IL-6 via Stat-3 activation (17). Stat-3 also induces bcl-2 or bcl-XL gene (29), thereby regulating cell death/survival. The present studies demonstrate a stimulatory effect of PGE_2 on PIN cell growth and further reveal that the growth induced by PGE_2 can be blocked with neutralizing antibodies to IL-6. Moreover, TUNEL assay of cultured PIN cells revealed that the decreased number of PIN cells noted after NS398 treatment (COX-2 inhibition) was largely due to the induction of apoptosis.[2] These data suggest that PGE_2-induced PIN cell growth is mediated, at least in part, by the IL-6 signaling pathway.

Previous studies have demonstrated the involvement of both COX-2 and IL-6 in human prostate cancer. Inhibition of COX-2 (21) and IL-6 (30) have been reported to decrease human prostate tumor growth *in vivo*. The COX-2 inhibitor induced cancer cell apoptosis (31) and decreased tumor vascular endothelial growth factor (VEGF) levels and microvessel density (21). However, most of the previous work has focused on prostate cancer cell lines derived from advanced, hormone refractory disease (10, 14, 19, 21, 30, 31). The expression of both COX-2 and IL-6 signals in PIN cells is not surprising, in light of previous reports demonstrating their expression in tissue sections of PIN (8, 18). Our finding that the COX-2/PGE_2 and IL-6 systems have reciprocal and synergistic effects on the growth of a human PIN cell line demonstrate the interaction of these factors at an earlier stage of disease progression. These novel data provide a rationale for the use of anti-inflammatory agents, particularly COX-2 inhibitors, in the early stages of prostate cancer.

ACKNOWLEDGMENTS

This work was supported by Grants-in-Aid from the T. J. Martell Foundation for Leukemia, Cancer and Aids Research, and a Ph.D. Research Scholarship (PhD 9905) from the American Foundation for Urological Disease. The PIN cell line was a generous gift from Dr. Mark Stearns.

[2] Xin-Hua Liu, A. Kirschenbaum, and A. C. Levine, unpublished data.

REFERENCES

1. Balkwill, F., and Mantovani, A. (2000) Inflammation and cancer: Back to Virchow? *Lancet* **357,** 539–545.
2. DuBois, R. N., Abramson, S. B., Crofford, L., Gupta, R. A., Simon, L. S., Van DePutte, I. B., and Lipsky, P. E. (1998) Cyclooxygenase in biological and disease. *FASEB J.* **12,** 1063–1073.
3. Taketo, M. M. (1998) Cyclooxygenase-2 inhibitor in tumorigenesis (Part II). *J. Natl. Cancer Inst.* **90,** 1609–1620.
4. Tsujii, M., and Dubois, R. N. (1995) Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase-2. *Cell* **83,** 493–501.
5. Norrish, A. E., Jackson, R. T., and McRae, C. U. (1998) Non-steroidal anti-inflammatory drugs and prostate cancer progression. *Int. J. Cancer* **77,** 511–515.
6. Kirschenbaum, A., Liu, X. H., Yao, S., and Levine, A. C. (2001) The role of cyclooxygenase-2 in prostate cancer. *Urology* **58**(Suppl. 2A), 127–131.
7. De Marzo, A. M., Marchi, V. L., Epstein, J. I., and Nelson, W. G. (1999) Proliferative inflammation atrophy of the prostate: Implications for prostatic carcinogenesis. *Am. J. Pathol.* **155,** 1985–1992.
8. Kirschenbaum, A., Klausner, A. P., Lee, R., Unger, P., Yao, S., Liu, X. H., and Levine, A. C. (2000) Expression of cyclooxygenase-1 and cyclooxygenase-2 in the human prostate. *Urology* **56,** 671–676.
9. Bostwick, D. G. (1996) Prospective origins of prostate carcinoma: Prostatic intraepithelial neoplasia and atypical adenomatous hyperplasia. *Cancer* **78,** 330–336.
10. Subbarayan, V., Sabichi, A. L., Liansa, N., Lippman, S. M., and Menter, D. G. (2001) Differential expression of cyclooxygenase-2 and its regulation by tumor necrosis factor-α in normal and malignant prostate cells. *Cancer Res.* **61,** 2720–2726.
11. Hughes, F. J., Buttery, L. D. K., Hukkanen, M. V. J., O'Donnell, A., Maclouf, J., and Polak, J. M. (1999) Cytokine-induced prostaglandin E_2 synthesis and cyclooxygenase-2 activity are regulated both by a nitric oxide-dependent and -independent mechanism in rat osteoblasts *in vitro. J. Biol. Chem.* **274,** 1776–1782.
12. Hong, S. H., Ondrey, F. G., Avis, I. M., Chen, Z., Loukinova, E., Cavanaugh, P. F., Jr., Waes, C. V., and Mulshine, J. L. (2000) Cyclooxygenase regulates human oropharyngeal carcinomas via the proinflammatory cytokine IL-6: A general role of inflammation? *FASEB J.* **14,** 1499–1507.
13. Smith, P. C., Hobisch, A., Lin, D. L., Culig, Z., and Keller, E. T. (2001) Interleukin-6 and prostate cancer progression. *Cytokine Growth Factor Rev.* **12,** 33–40.
14. Okamoto, M., Lee, C., and Oyasu, R. (1997) Interleukin-6 as a paracrine and autocrine growth factor in human prostatic carcinoma cells *in vitro. Cancer Res.* **57,** 141–146.
15. Chung, T. D. K., Yu, J. J., Spiotto, M. T., Bartkowski, M., and Simons, J. W. (1999) Characterization of the role of interleukin-6 in the progression of prostate cancer. *Prostate* **38,** 199–207.
16. Taga, T., and Kishimoto, T. (1997) Gp130 and the interleukin-6 family of cytokines. *Annu. Rev. Immunol.* **15,** 797–819.
17. Hirano, T., Ishihara, K., and Hibi, M. (2000) Roles of Stat-3 in mediating the cell growth, differentiation and survival signals relayed through the IL-6 family of cytokine receptors. *Oncogene* **19,** 2548–2556.
18. Hobisch, A., Rogatsch, H., Hittmair, A., Fuchs, D., Bartsch, G., Jr., Klocker, H., Bartsch, G., and Culing, Z. (2000) Immunohistochemical localization of interleukin-6 and its receptor in benign, premalignant and malignant prostate tissue. *J. Pathol.* **191,** 239–244.
19. Ni, Z, Lou, W., Leman, E. S., and Gao, A. C. (2000) Inhibition of

constitutively activated Stat3 signaling pathway suppresses growth of prostate cancer cells. *Cancer Res.* **60,** 1225–1228.

20. Wang, M., Liu, A., Garcia, F. U., Rhim, J. S., and Stearns, M. E. (1999) Growth of HPV-18 immortalized human prostatic intraepithelial neoplasia cell line, influence of IL-10, follistatin, activin-A and DHT. *Int. J. Oncol.* **14,** 1185–1195.
21. Liu, X. H., Kirschenbaum, A., Yao, S., Lee, R., Holland, J. F., and Levine, A. C. (2000) Inhibition of cyclooxygenase-2 suppresses angiogenesis and growth of prostate cancer *in vivo. J. Urol.* **164,** 820–825.
22. Akira, S., Isshiki, H., Nakajima, T., Kinoshita, S., Nishio, Y., Hashimoto, S., Natsuka, S., and Kishimoto, T. (1990) A nuclear factor for the IL-6 gene (NF-IL-6). *Chem. Immunol.* **51,** 299–322.
23. Akarasereenont, P., Techatrisak, K., Chotewuttakorn, S., and Thaworn, A. (1999) The induction of cyclooxygenase-2 in cultured endothelial cells treated with serum from preeclampsia is mediated by interleukin-6. *J. Med. Assoc.* **82,** 876–884.
24. Peters, M., Muller, A. M., and Rose-John, S. (1998) Interleukin-6 and soluble interleukin-6 receptor: Direct stimulation of gp130 and hematopoiesis. *Blood* **92,** 3495–3504.
25. Murakami, M., Hibi, M., Nakagawa, N., Nakagawa, T., Yasukawa, K., Yamanishi, K., Taga, T., and Kishimoto, T. (1993) IL-6-induced homodimerization of gp130 and associated activation of a tyrosine kinase. *Science* **260,** 1808–1810.
26. Bromberg, J. F., Wrzeszczynska, M. H., Devgan, G., Zhao, Y., Pestell, R. G., Albanese, C., and Darnell, J. E., Jr. (1999) Stat-3 as an oncogene. *Cell* **98,** 295–303.
27. Sheng, H. M., Shao, J. Y., Washington, M. K., and DuBois, R. N. (2001) Prostaglandin E_2 increases growth and motility of colorectal carcinoma cells. *J. Biol. Chem.* **276,** 18075–18081.
28. Tamura, T., Udagawa, N., Takahashi, N., Miyaura, C., Tanaka, S., Yamada, Y., Koishihara, Y., Ohsugi, Y., Kumaki, K., Taga, T., Kishimoto, T., and Suda, T. (1993) Soluble interleukin-6 receptor triggers osteoclast formation by interleukin-6. *Proc. Natl. Acad. Sci. USA* **90,** 11924–11928.
29. Catlett-Falcone, R., Landowski, T. H., Oshiro, M. M., Turkson, J., Levitzki, A., Savino, R., Ciliberto, G., Moscinski, L., Fernandez-Luna, J. L., Nunez, G., Dalton, W. S., and Jove, R. (1999) Constitutive activation of Stat-3 signaling confers resistance to apoptosis in human U266 myeloma cells. *Immunity* **10,** 105–115.
30. Smith, P. C., and Keller, E. T. (2001) Anti-interleukin-6 monoclonal antibody induces regression of human prostate cancer xenografts in nude mice. *Prostate* **48,** 47–53.
31. Liu, X. H., Yao, S., Kirschenbaum, A., and Levine, A. C. (1998) NS398, a selective cyclooxygenase-2 inhibitor, induces apoptosis and down-regulates Bcl-2 expression in LNCaP cells. *Cancer Res.* **58,** 4245–4249.