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Interleukin-6 Is an Autocrine Growth Factor in Human Prostate Cancer

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Prostate cancer is the most common cancer in American men and the second leading cause of cancer deaths in this group. We have found that interleukin (IL)-6 protein concentrations are increased ~18-fold in clinically localized prostate cancers when compared to normal prostate tissue. Normal and neoplastic prostatic epithelial cells in culture, with the exception of LNCaP cells, secrete IL-6. Addition of exogenous IL-6 to primary epithelial cells in culture or the LNCaP prostate cancer cell line leads to phosphorylation of Stat-3 and increases in net cell proliferation. The concentration of IL-6 receptor is increased eightfold in the prostate cancer tissues and is increased in the cancer cells by immunohistochemistry. The increased expression of IL-6 receptor is correlated with increased proliferation of prostate cancer cells *in vivo* as assessed by Ki67 immunohistochemistry. These findings strongly support the hypothesis that IL-6 acts as a significant autocrine growth factor *in vivo* for primary, androgen-dependent prostate cancers. *(Am J Pathol 2001, 159:2159–2165)*

Interleukin-6 (IL-6) is a multifunctional cytokine that is secreted by both lymphoid and nonlymphoid cells and plays a key role in immune responses and hematopoiesis. The role of IL-6 in human malignancy is most clearly established in multiple myeloma, where autocrine stimulation of growth by IL-6 is common.[1] Inhibition of this autocrine growth stimulation using monoclonal antibodies seems to enhance the effectiveness of chemotherapy in this disease.[2] There is also *in vitro* evidence that IL-6 can act as an autocrine growth factor in a number of human epithelial malignancies including renal,[3] lung,[4] and prostate cancer.[5–8]

Prostate cancer is the most common cancer in American men and the second leading cause of cancer deaths in this group. There is evidence that IL-6 may play an important role as an autocrine growth factor in metastatic prostate cancer. It has been demonstrated by several laboratories that all of the commonly used prostate cancer cell lines (PC3, DU145, and LNCaP) express high-affinity receptors for IL-6.[5–8] In addition, it has been shown that the PC3 and DU145 cell lines secrete IL-6,[6–8] leading to a potential autocrine growth loop, and that neutralizing antibody to IL-6 decreases growth of these two cell lines.[7] LNCaP does not secrete IL-6[6–8] and the effect of exogenous IL-6 on this cell line is controversial, with some groups showing growth stimulation[7,9,10] and other groups showing growth inhibition.[6,11,12] Thus, there is evidence that IL-6 can act as an autocrine growth factor for prostate cancer cell lines *in vitro*. However, all of these cell lines were established from metastatic prostate cancers and both PC-3 and DU145 are androgen-independent, so the extent to which IL-6 acts as a growth factor for primary human prostate cancers *in vivo* is not clear. Hobisch and colleagues[13] have shown by immunohistochemistry that both IL-6 and IL-6 receptors are expressed at increased levels in clinically localized prostate carcinomas. However, the actual magnitude of the increased expression of IL-6 and IL-6 receptor in the tissues cannot be assessed quantitatively by immunohistochemistry and the biological consequences of this potential autocrine loop have not been established *in vivo*.

We have found that IL-6 protein concentrations are increased ~18-fold in clinically localized prostate cancers when compared to normal prostate tissue and that IL-6 is secreted by normal and neoplastic prostatic epithelial cells. IL-6 can act as a growth factor for both normal prostatic epithelial cells and the LNCaP prostate cancer cell line. In addition, the concentration of IL-6 receptor is increased eightfold in the prostate cancer tissues. Most importantly, increased expression of IL-6 receptor is correlated with increased proliferation *in vivo* as assessed by Ki67 immunohistochemistry. These findings demonstrate for the first that there is a quantitatively significant increase in IL-6 and its receptor in prostate cancer *in vivo* and that this increase is strongly correlated with a biological significant parameter, ie, proliferation. Thus IL-6 seems to be an important autocrine growth factor for androgen-dependent human prostate cancer *in vivo*.

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Materials and Methods

Tissue Acquisition and Analysis

Samples of the benign peripheral and transition zone of the prostate or prostate carcinoma were taken from radical prostatectomies performed for treatment of clinically localized prostate cancer. Tissues were received fresh and portions snap-frozen in liquid nitrogen or, if from benign peripheral zone areas, used to establish primary cell cultures (see below). The frozen tissues were then analyzed by frozen section to confirm the presence or absence of carcinoma. The carcinoma tissues were 30 to 80% carcinoma by frozen section analysis.

Cell Culture, Production of Conditioned Medium, and Cell Growth Assays

Primary prostatic epithelial cultures were established using prostatic tissue samples from areas in the peripheral zone of radical prostatectomy specimens that were free from carcinoma as described previously.[14] To prepare conditioned medium, primary epithelial cells were plated in 10-cm tissue culture dishes. When the cells were subconfluent epithelial growth medium was replaced with 8 ml of MCDB 153 medium containing insulin, transferrin, selenium, bovine serum albumin, and oleic acid (1% ITS+2; Sigma Chemical Co., St. Louis, MO). Conditioned medium was collected after 72 hours. The epithelial cells tolerated this treatment well and appeared healthy after this period.

PNT2, an immortalized but nontumorigenic prostate epithelial cell line, and LNCaP, DU145, and PC3 prostate cancer cells were maintained in RPMI 1640 containing 10% fetal bovine serum. Conditioned medium was collected by incubating subconfluent cultures of each cell line in 8 ml of RPMI 1640 containing 1% ITS for 72 hours.

For growth assays, primary epithelial cultures were plated at 1×10^5 cells per 35-mm dish and the next day refed with MCDB 153 with 1% ITS. Control plates were incubated in this medium and experimental plates were treated with 1 or 10 ng/ml of recombinant IL-6 (R&D Systems, Minneapolis, MN). Cells were harvested by trypsinization after 1 to 3 days and cell number determined by counting using a Coulter counter. LNCaP cells were plated at 5×10^4 cells per 35-mm dish and the next day refed with RPMI 1640 with 1% ITS. Control plates were incubated in this medium and experimental plates were treated with 10 or 100 ng/ml of recombinant IL-6. Cells were harvested by trypsinization after 1 to 3 days and cell number determined by counting using a Coulter counter.

Cell Cycle Analysis

LNCaP cells (2×10^5) were plated in 100-mm dishes and, after overnight incubation, medium was changed to RPMI 1640 containing 1% ITS with or without 100 ng/ml of IL-6 and incubated for 24, 48, and 72 hours. Both floating and attached cells were harvested and stained with propidium iodide for DNA cell-cycle analysis following a standard protocol. DNA content was measured using a flow cytometer (Epics XL-MCl; Beckman Coulter, Miami, FL) and cell cycle analysis was performed using Multi Cycle for Windows version 3.0 software (Phoenix Flow Systems, San Diego, CA).

Analysis of Stat-3 and Phosphorylated Stat-3

Prostatic primary epithelial cells or LNCaP cells (1×10^6 cells/10-cm plate) were treated with 10 ng/ml of recombinant IL-6 at 37°C. After treatment, the cells were washed with cold phosphate-buffered saline (PBS) and suspended in 0.2 ml of lysis buffer (10 mmol/L HEPES, pH 7.9, 10 mmol/L KCl, 0.1 mmol/L ethylenediaminetetraacetic acid, 0.1 mmol/L EGTA, 1 mmol/L dithiothreitol, 0.5 mmol/L phenylmethyl sulfonyl fluoride, 2 μg/ml leupeptin, 2 μg/ml aprotinin, and 0.5 mg/ml benzamidine). The cells were allowed to swell on ice for 15 minutes after which 6 μl of 10% Nonidet P-40 was added. The tube was then vigorously vortexed for 20 seconds and the homogenate centrifuged for 1 minute in an Eppendorf microcentrifuge at 4°C. The supernatant (cytoplasmic extract) was removed and stored at −80°C. The nuclear pellet was resuspended in 30 μl of ice-cold nuclear extraction buffer (20 mmol/L HEPES, pH 7.9, 0.4 mol/L NaCl, 1 mmol/L ethylenediaminetetraacetic acid, 1 mmol/L EGTA, 1 mmol/L dithiothreitol, 1 mmol/L phenylmethyl sulfonyl fluoride, 2 μg/ml leupeptin, 2 μg/ml aprotinin, and 0.5 mg/ml benzamidine) and the tubes incubated on ice for 30 minutes with intermittent vortexing. The tubes were then centrifuged for 5 minutes in an Eppendorf microcentrifuge at 4°C and the supernatant (nuclear extract) stored at −80°C. Western blotting was performed as described previously[15] using 30 μg of extract protein per lane. Blots were incubated with either 0.1 μg/ml of anti-phospho-Stat3 (Y704) mouse monoclonal antibody (Upstate Biotechnology, Lake Placid, NY) or 0.2 μg/ml rabbit polyclonal anti-Stat-3 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) at 4°C for 12 hours and then incubated with the appropriate secondary antibody and antigen-antibody complexes visualized by enhanced chemiluminescence (Amersham, Arlington Heights, IL).

Immunohistochemistry (IHC)

Frozen tissue sections were fixed in acetone for 10 minutes and stored at −80°C. All sections were treated with Autoblocker (R&D Systems) to inhibit endogenous peroxidase and avidin/biotin (Vector Laboratories, Burlingame, CA) to block endogenous biotin. The sections were incubated with 0.5 μg/ml of anti-phospho-Stat3 (Y704) mouse monoclonal antibody (Upstate Biotechnology) or 10 μg/ml of anti-IL-6 receptor mouse monoclonal antibody (Sigma) at 4°C for 12 hours. After liberal washing with PBS, pH 7.4, sections were then incubated with biotinylated horse anti-mouse antibody at 7.5 μg/ml for 45 minutes at room temperature (Vector Laboratories). Sections were then washed with PBS containing 0.1% Tween 20 and incubated with avidin-biotin complex (Vec-

tastain Elite, Vector Laboratories) for 15 minutes. The antigen-antibody reaction was demonstrated using diaminobenzidine as substrate and the sections then counterstained with hematoxylin. Ki67 immunohistochemistry was performed and staining quantitated as described previously.[16]

Enzyme-Linked Immunosorbent Assay (ELISA)

Cell extracts were prepared from snap-frozen tissues as described previously.[15] Determination of the IL-6 and IL-6R concentration in the media and tissue extracts was performed by ELISA based on a quantitative sandwich immunoassay technique using kits from R&D Systems as described by the manufacturer. An aliquot of 100 μl of conditioned medium or 25 μl of tissue extract made to 100 μl with diluent (0.1% bovine serum albumin, 0.05% Tween 20, 10 mmol/L Tris, and 150 mmol/L sodium chloride, pH 7.3) was added per well. The IL-6 ELISA was linear between 3 to 300 pg/ml whereas the IL-6 receptor ELISA was linear between 2 to 200 pg/ml.

Results

Increased IL-6 Concentration in Human Prostate Cancer

To determine whether IL-6 may act as an autocrine growth factor in clinically localized prostate cancer the concentration of IL-6 was determined in tissue extracts of 40 prostate cancers and 10 normal, noncancerous tissue samples from radical prostatectomy specimens. In all cases the presence or absence of cancer was assessed by frozen section analysis before preparation of extracts and all cancer tissues contained at least 30% cancer based by frozen section. Figure 1 shows the IL-6 content of prostate cancers and benign prostatic tissue as determined by ELISA.

The benign tissues contained between 0 and 3 ng of IL-6/g wet weight of tissue, with a mean concentration of 0.51 ng/g wet weight. Five of the 10 tissues had undetectable IL-6 (<0.1 ng/g). The cancer tissues were more variable, with IL-6 concentrations ranging from 0 to 52.8 ng/g wet weight, with a mean concentration of 9.26 ng/g wet weight. This difference in tissue concentration of IL-6 was statistically significant ($P < 0.04$, *t*-test). Twenty of the 40 cancer tissues contained IL-6 within the normal concentration range (0 to 3 ng/g), whereas the other 20 samples contained moderately to markedly elevated tissue IL-6. It should be noted that tissue IL-6 concentration was not correlated with the presence of inflammatory infiltrates on frozen section.

Expression of IL-6 by Normal and Neoplastic Prostatic Epithelial Cells

To determine whether normal prostatic epithelial cells can produce IL-6, conditioned medium was collected from six different primary cultures of prostatic epithelial cells and from an immortalized but nontransformed prostatic epithelial cell line (PNT2) after 72 hours incubation and the cells counted. The concentration of IL-6 in the conditioned medium was then determined by ELISA. The primary cells secreted 609 ±90 (SD) pg of IL-6/24 hours/10^6 cells. The PNT2 cells secreted lower but still significant quantities of IL-6 (53 pg/24 hours/10^6 cells). In agreement with reports of other laboratories,[6–9] LNCaP cells did not secrete detectable IL-6 into the media, whereas both PC3 and DU145 secreted quantities similar to the primary epithelial cells.

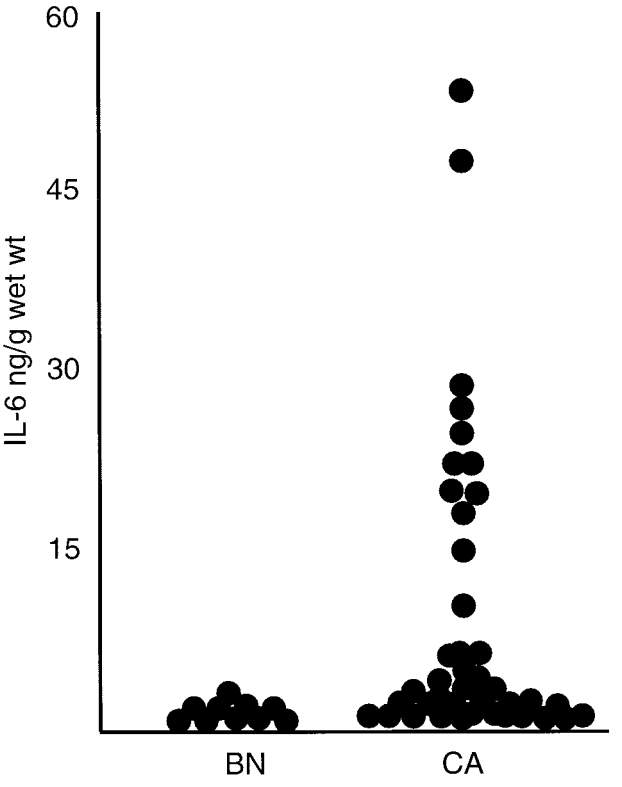

Figure 1. IL-6 content of benign and neoplastic prostate tissues. Protein extracts were prepared from prostate tissue samples from radical prostatectomy specimens that contained only benign prostate (BN) or 30 to 80% carcinoma (CA) by frozen section analysis. IL-6 content was then determined by ELISA and is expressed as ng per gram wet weight of tissue.

Phosphorylation of Stat-3 in Response to IL-6 in Prostatic Epithelial Cells

To determine whether functional IL-6 receptors are present on LNCaP cells, these cells were treated with recombinant IL-6 cells, fractionated into cytoplasmic and nuclear fractions, and total Stat-3 and phosphorylated Stat-3 assessed by Western blotting in each fraction. As can be seen in Figure 2, Stat-3 protein is primarily cytoplasmic in untreated LNCaP cells and no phospho-Stat3 protein is present, consistent with the lack of IL-6 secretion by these cells. After treatment with recombinant IL-6 there is loss of cytoplasmic Stat-3 protein accompanied by an increase in nuclear Stat-3 protein and nuclear phospho-Stat-3 protein. Thus LNCaP cells express functional IL-6 receptor complexes that can phosphorylate

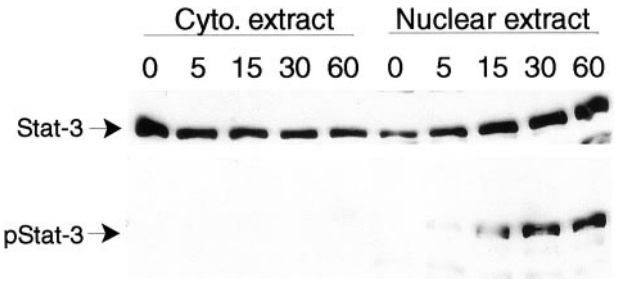

Figure 2. Phosphorylation of Stat-3 after treatment of LNCaP prostate cancer cells with IL-6. LNCaP prostate cancer cells were treated with recombinant IL-6 for 0 to 60 minutes and cells were separated into nuclear and cytoplasmic fractions at the indicated times and lysates prepared. Equal quantities of protein were then analyzed by Western blot using antibodies specific for Stat-3 protein (Stat-3) or phosphorylated Stat-3 (pStat-3).

Stat-3 protein. Similar experiments with primary epithelial cells show that IL-6 induces both translocation of Stat-3 into the nucleus and increased phosphorylation of Stat-3 protein (data not shown).

Stimulation of Net Cell Proliferation by IL-6

To determine the biological effect of IL-6 receptor complex activation on prostatic epithelial cells, we determined the effect of exogenous recombinant IL-6 on cell proliferation of both primary prostatic epithelial cells and the LNCaP prostate cancer cell line. Exogenous IL-6 at either 1 or 10 ng/ml lead to a statistically significant increase in net cell proliferation in primary prostatic epithelial cells after 3 days of treatment when compared to untreated control cells (Figure 3A). Similarly, IL-6 at 10 or 100 ng/ml lead to a statistically significant increase in net proliferation of LNCaP cells after only 24 hours of treatment (Figure 3B). The range of IL-6 concentrations used (1 to 100 ng/ml) are similar to the concentration of IL-6 found in prostate cancer tissues (0 to 53 ng/g) and thus are biologically relevant to this disease process.

The increased net proliferation might be because of increased cell division, decreased cell death, or both. To distinguish these possibilities we performed flow cytometry on LNCaP cells treated with 100 ng/ml of IL-6 or maintained in 1% ITS after 24, 48, and 72 hours of treatment. IL-6 treatment led to a decrease in the percentage of dead cells under these conditions at all times after treatment (Figure 4A). The percentage of cells in the S phase of the cell cycle was similar in treated and untreated cells for the first 48 hours but decreased markedly in untreated cells after 72 hours when compared to treated controls (Figure 4B). Thus the increase in net cell proliferation in LNCaP cells treated with IL-6 under these conditions is both because of a decrease in cell death and an increase in the percentage of dividing cells at later times after treatment.

Presence of Phosphorylated Stat-3 in Human Prostate Cancer Cells

To determine whether Stat-3 is phosphorylated in prostate cancer cells *in vivo* immunohistochemistry with anti-

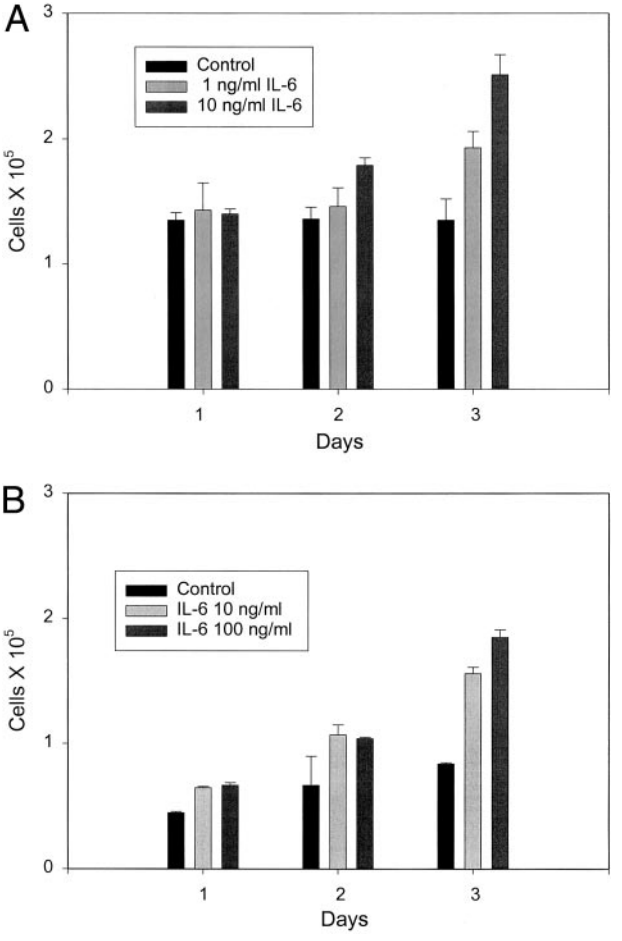

Figure 3. Proliferation of prostate epithelial cells and LNCaP prostate cancer cells in response to exogenous IL-6. **A:** Primary epithelial cultures were plated at 1×10^5 cells per 35-mm dish and the next day refed with MCDB 153 containing insulin as the only growth factor. Control plates were incubated in this medium and experimental plates were treated with 1 or 10 ng/ml of recombinant IL-6. Cells were harvested by trypsinization after 1 to 3 days and cell number determined by counting using a Coulter counter. Standard deviations of triplicate determinations are shown. **B:** LNCaP cells were plated at 5×10^4 cells per 35-mm dish and the next day refed with RPMI 1640 containing insulin as the only growth factor. Control plates were incubated in this medium and experimental plates were treated with 10 or 100 ng/ml of recombinant IL-6. Cells were harvested by trypsinization after 1 to 3 days and cell number determined by counting using a Coulter counter. Standard deviations of triplicate determinations are shown.

bodies to phospho-Stat-3 was performed on frozen sections of 12 prostate cancer tissues from radical prostatectomy specimens. Variable nuclear staining was identified in the prostate cancer epithelial cells in 10 of 12 prostate cancer tissues examined. This varied both between sections and in different areas of each section. In some cases staining was extensive and in other cases only focal areas of staining of neoplastic epithelial cells were seen. An example of extensive staining using antibodies to phospho-Stat-3 is shown in Figure 5. Some staining of normal epithelial cells was seen that was also variable but was less than that seen in the neoplastic epithelial cells in most cases. Our results are quite similar to those reported by Campbell and colleagues.[17] These investigators also reported significantly higher levels of phosphorylated Stat-3 in prostate cancer cells compared to benign prostatic epithelial cells.

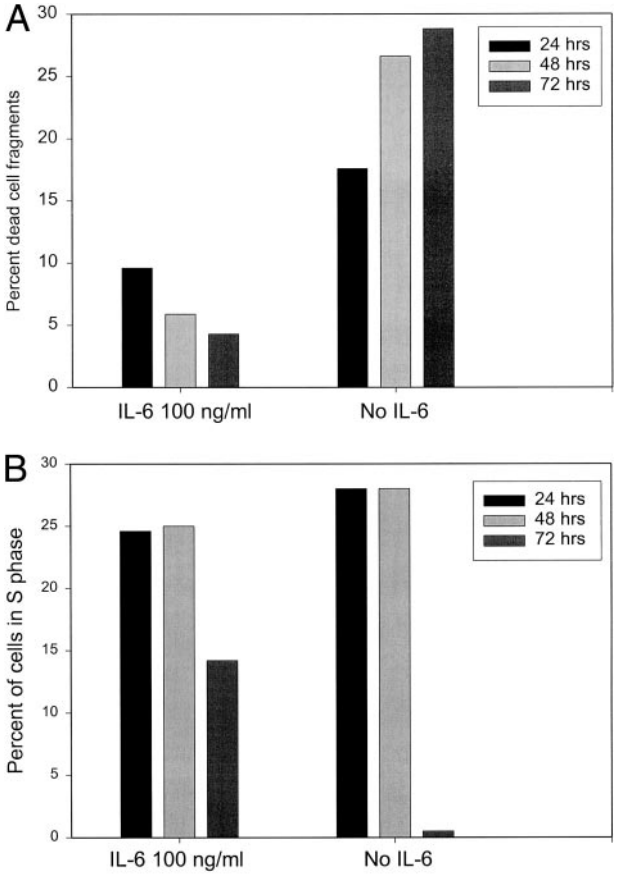

Figure 4. Cell cycle analysis of LNCaP prostate cancer cells treated with exogenous IL-6. LNCaP cells were plated at 2×10^5 cells per 100-mm dish and the next day refed with RPMI 1640 containing insulin as the only growth factor. Control plates were incubated in this medium and experimental plates were treated with 100 ng/ml of recombinant IL-6. Cells were harvested after 1 to 3 days and the number of dead cell fragments and the distribution within the cell cycle of viable cells determined by flow cytometry after staining with propidium iodide. **A:** Percentage of total events that are dead cell fragments at 24, 48, and 72 hours with or without 100 ng/ml IL-6. **B:** Percentage of viable cells in the S phase of the cell cycle after 24, 48, and 72 hours with or without 100 ng/ml IL-6.

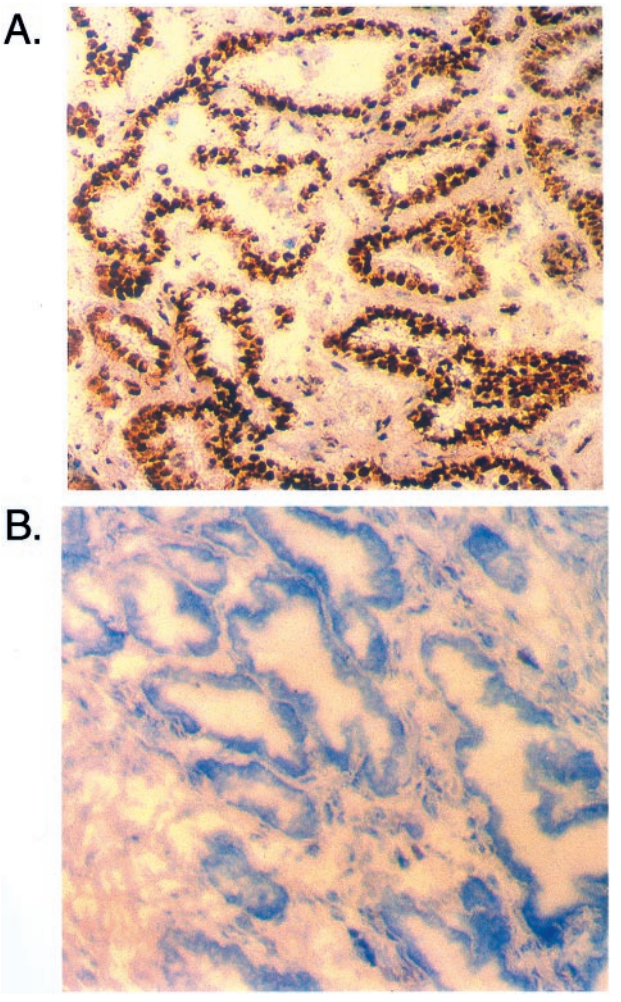

Figure 5. Immunohistochemistry with anti-phospho-Stat-3 antibody. Frozen sections of prostate cancer tissues were analyzed by immunohistochemistry using an anti-phospho-Stat-3 antibody. Variable staining of prostate cancer nuclei was seen in 10 of 12 cases. **A:** Anti-phospho-Stat-3 antibody. **B:** Control. Original magnifications, ×200.

Increased Expression of IL-6 Receptor in Human Prostate Cancers

To determine whether IL-6 receptor was increased in human prostate cancer we determined the quantity of IL-6 receptor in protein extracts of 12 human prostate cancer and 4 samples of normal prostate tissue by ELISA. The normal tissue contained 5.4 ± 2.3 (SEM) ng IL-6 receptor per g wet of tissue whereas the cancer tissues contained 44.7 ± 9.5 (SEM) ng per g wet weight. This difference was statistically significant ($P < 0.03$, *t*-test). To determine which cells in the benign and malignant prostate express IL-6 receptor we performed immunohistochemistry with anti-IL-6 receptor antibody on six prostate cancers and two normal prostatic tissue samples. In the normal prostate we observed moderate staining of basal epithelial cells and weak staining of the secretory epithelial cells. The stromal cells also expressed moderate levels of IL-6 receptor. Prostate cancer cells expressed variable levels of IL-6 receptor, with some cancers showing intense staining and others having weak or no staining (data not shown). These results are very similar to the results reported by Hobisch and colleagues.[13] Therefore the increased levels of IL-6 receptor in prostate cancer tissue are because of increased IL-6 receptor expression in a subset of the prostate cancers.

To determine the biological significance of increased IL-6 receptor expression in human prostate cancer, we correlated the level of IL-6 receptor expression in the cancers and proliferation, as assessed by Ki67 immunostaining, in the same cancers using frozen sections obtained before protein extraction. The cancers were divided into three groups of four cases each based on the IL-6 receptor protein concentration: low (mean 12.5 ng/g wet weight), intermediate (38.0 ng/g), and high (82.5 ng/g). As seen in Figure 6, the prostate cancers with high levels of IL-6 receptor expression had significantly higher rates of proliferation. This difference was statistically significant by analysis of variance ($P < 0.02$). It should be noted that there was no significant difference between

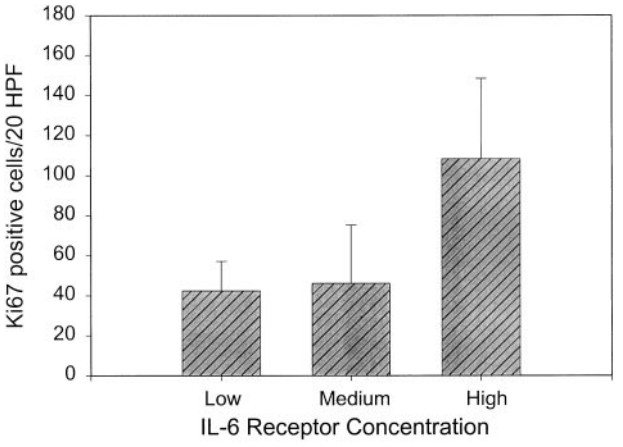

Figure 6. Correlation of tissue IL-6 receptor levels and proliferation. IL-6 receptor concentration was determined by ELISA of protein extracts of 12 human prostate cancer tissues and proliferation assessed by Ki67 immunostaining in the same cancers using frozen sections obtained before protein extraction. Total Ki67-positive nuclei were counted in 20 high-power (×400) fields of tumor. The cancers were divided into three groups of four cases each based on the IL-6 receptor protein concentration: low (12.5 ng/g wet weight), intermediate (38.0 ng/g), and high (82.5 ng/g). The standard deviation of the number of Ki67-positive nuclei per 20 high-power fields for each group is indicated.

the three groups in the average Gleason score or in the percent carcinoma in the tissue.

Discussion

In this study we have shown that the mean IL-6 concentration of prostate cancer tissues is increased 18-fold when compared to benign prostatic tissue. Seven of the 10 benign tissues had undetectable or extremely low levels (<0.3 ng/g) of IL-6, whereas half of the prostate cancers contained elevated levels of IL-6 at concentrations of 4.4 to 53 ng of IL-6 per gram of tissue. Primary or immortalized prostatic epithelial cell cultures and two of three prostate cancer cell lines secrete IL-6 *in vitro*, so that it is likely that much of IL-6 present in the cancer tissues is derived from the neoplastic epithelial cells. This conclusion is supported by studies of Hobisch and colleagues,[13] which revealed that IL-6 was expressed in normal basal epithelial cells and in cancer cells but not in stromal cells in cancer tissues from radical prostatectomies analyzed by immunohistochemistry with anti-IL-6 antibodies.

We have also demonstrated that normal and neoplastic prostatic epithelial cells *in vitro* have functional IL-6 receptor complexes that lead to Stat-3 phosphorylation and increased net cell proliferation when cells are treated with IL-6. Thus the increased IL-6 concentration in clinically localized prostate cancer, derived from neoplastic epithelial cells, can then act as an autocrine growth factor for the cancer cells *in vivo*. The finding that there is increased Stat-3 phosphorylation *in vivo* in the prostate cancer cells is consistent with the presence of such autocrine growth, although it does not prove it, because Stat-3 can be phosphorylated by activation of other receptors that are expressed in prostate cancer such as the epidermal growth factor receptor.[18] However, if Stat-3 phosphorylation had not been seen *in vivo*, this would have been a strong argument against the existence of autocrine IL-6 activation, so this observation is of significance.

Finally we have shown by ELISA and immunohistochemistry with anti-IL-6 receptor antibody that there is a eightfold increase in IL-6 receptor expression in human prostate cancers because of increased expression of IL-6 receptor by the prostate cancer cells. This increased expression of IL-6 receptors is biologically significant because the cancers with high IL-6 receptor levels have significantly higher rates of proliferation. We did not observe a correlation between increased levels of IL-6 and increased expression of IL-6 receptor. There is obviously the potential for synergism between increased levels of IL-6 and its receptor in promoting proliferation. Indeed, the highest proliferation rate we observed was in a cancer with marked increase in both IL-6 and IL-6 receptor. However, in many cases there was either increased IL-6 or increased IL-6 receptor, so that there seems to be two independent modes of arriving at increased levels of autocrine IL-6 signal transduction in prostate cancer.

The activation of IL-6 receptors in prostate cancer cells has been shown to have a variety of effects *in vitro*. IL-6 can activate androgen receptor transcriptional activity,[11,19] at least in part via Stat-3 phosphorylation.[19] It can also lead to increased phosphatidyl-inositol 3′ kinase activity, which may have anti-apoptotic effects.[20] This is consistent with our finding of decreased cell death in LNCaP cells treated with IL-6. Finally, activation of IL-6 receptors has been shown to lead to activation of erbB2 receptors via a direct physical interaction of this receptor with the gp130 portion of the IL-6 receptor and activation of the mitogen-activated protein kinase pathway.[9] Each one of these would be expected to promote net cell proliferation, as we observed in both primary epithelial cell cultures and in the LNCaP cell line. As described previously there has been controversy regarding the effects of IL-6 on LNCaP cells, with some groups observing stimulation of growth,[7,9,10] similar to our results, and other groups observing growth inhibition.[6,11,12] Whether this represents subtle differences in the conditions in which the growth assays were performed or clonal variation of the LNCaP cell line in different laboratories is unclear.

The role of cytokines in normal prostate biology and prostate cancer is still an emerging area of investigation. IL-6 is present in substantial quantities (100 to 600 pg/ml) in human seminal fluid,[8] consistent with the finding that prostatic epithelial cells secrete IL-6 *in vitro*, although its biological function in the seminal fluid is unknown. Serum IL-6 is significantly elevated in many men with advanced, hormone-independent prostate cancer[8,21,22] and elevated IL-6 levels may constitute an independent prognostic marker for decreased survival.[22] It has also been suggested that such elevated IL-6 may directly mediate some of the morbidity associated with advanced prostate cancer such as anemia and cachexia.[8] Our finding of both increased IL-6 and IL-6 receptor in prostate cancer tissues and the correlation of increased IL-6 receptor with increased proliferation further support the idea that IL-6 may play an important role in prostate cancer progres-

sion. Thus disruption of IL-6 autocrine signaling is a potential therapeutic target in prostate cancer. In addition, our results suggest that serum levels of IL-6 soluble receptor, which arises by proteolytic cleavage[23] or alternative splicing[24] of the IL-6 receptor, may be useful as a prognostic marker either by itself or in conjunction with measurement of serum IL-6 levels.

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