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Interleukin-6 and renal cell cancer: production, regulation, and growth effects*

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Summary. Interleukin-6 (IL-6) is a recently characterized pleiotropic cytokine with antitumor activity. We investigated the production of IL-6 by renal cell cancer (RCC) and the growth effects of IL-6 on RCC. Using immunoperoxidase staining, cytoplasmic IL-6 was detected in four of four renal tumor lines and in tumor cells from freshly nephrectomized RCC. We found that IL-6 mRNA was expressed at basal culture conditions by seven of ten RCC tumor lines tested. Biologically active IL-6, as measured by the B9 assay, was produced by all ten RCC tumor lines. The addition of tumor necrosis factor α (TNFα) significantly augmented the expression of IL-6 mRNA in five RCC tumor lines (P <0.05). The combination of interferon γ IFNγ and TNFα further enhanced the augmented IL-6 mRNA accumulation seen with TNFα alone (P <0.05). TNFα also significantly stimulated the production of biologically active IL-6 (P <0.01). Furthermore, IFNγ and TNFα were found to enhance IL-6 bioactivity synergistically (P <0.05). The growth effects of IL-6 on RCC were also investigated in two experimental systems: IL-6 was found to stimulate proliferative responses in six of six RCC tumor lines as measured by thymidine-uptake assays; however, only one of six tumor lines displayed an increase in proliferative response of greater than 21% (113%). The growth effect of IL-6 was further tested in clonogenic assays. One of the tumor lines tested displayed an enhanced growth response of up to 200%. We conclude that IL-6 is produced by RCC; this production is enhanced by TNFα with synergistic effects seen with IFNγ at both mRNA and protein levels. In turn, IL-6 may have a modest stimulatory growth effect on certain RCC tumor lines.

Key words: Interleukin-6 – Renal cell cancer – Growth effect

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Introduction

Interleukin 6 (IL-6) is a pleiotropic cytokine that appears to be an important element in the regulatory network controlling cellular growth and differentiation [22]. Therefore, aberrant or uncontrolled expression of IL-6 could ostensibly contribute to tumor development. A large number of tumor types have been shown to produce IL-6. In turn, IL-6 has been shown to have differential actions on tumor growth, including a stimulatory effect on plasmocytoma/hybridoma cells and myeloma cells [10, 19], and an antiproliferative effect on malignant mammary cell lines and M1 cells [8, 29] as well as in vivo antitumor activity in mice [30].

Renal cell carcinoma (RCC) is a malignant tumor with no effective treatment once metastatic. Patients with RCC can exhibit paraneoplastic symptoms, which include fever, leukocytosis, erythrocytosis, hypercalcemia and elevation of acute-phase reactants. The pathogenic mechanisms of these phenomena are not known. Among the actions of IL-6, many parallel the paraneoplastic symptoms of RCC. IL-6 has been found to stimulate blood stem cells [23], activate osteoclasts [25], induce fever [31], and the production of acute-phase reactants [14]. Thus, an association of IL-6 with RCC is suggested.

In view of the interactions between IL-6 and various tumor types and the possible association between IL-6 and RCC, we investigated whether IL-6 is produced by RCC at both the mRNA and protein levels, and how this production is regulated by other cytokine signals. In addition, we examined the effects of IL-6 on RCC growth in vitro.

Materials and methods

Cells and culture conditions. The RCC cell lines R4, R6, and R11 (Dr. Hans Stotter, Bethesda, Md.), SK28, SK29, SK39 (Dr. Neil Bender, New York, N. Y.); J80 (Dr. Sidney Golub, Los Angeles, Calif.), and RC30 (Dr. Kyogo Itoh) were obtained as noted. TL2, TL9 and 444 were obtained from nephrectomized RCC specimens. The tumor mass was minced and trypsinized to obtain a single-cell suspension. Fibroblasts were meticulously eradicated by needle puncture, and tumor cells were

used after more than 10–15 passages in vitro. Culture media consisted of RPMI-1640 (Biofluids Inc., Rockville, Md.) with 10% fetal calf serum (FCS), penicillin, streptomycin, Fungizone, and glutamine. J82 is an established bladder tumor line known to express IL-6 mRNA (Dr. H. P. Koeffler, Los Angeles, Calif.). T24 and M397 are established melanoma tumor lines (Dr. Sidney Golub).

Recombinant cytokines and monoclonal antibodies. rIL-6 was kindly provided by the Immunex Corp. (Seattle, Wash.). Recombinant tumor necrosis factor α (rTNFα) was generously supplied by the Cetus Corp. (Emeryville, Calif.). Recombinant interferon γ (rIFNγ) was a gift from Amgen Inc. (Thousand Oaks, Calif.). mAb specific for human IL-6 was purchased from Collaborative Research Inc. (Bedford, Mass.). MOPC 21 mAb (Organon Teknika, West Chester, Pa.) was used as a control mAb that has no known hapten- or antigen-binding activity.

RNA extraction. Nephrectomized specimens were frozen in liquid nitrogen and pulverized. The samples were lysed in guanidinium isothiocyanate, and ultracentrifugation through a cesium chloride gradient was performed for 16 h. The RNA fraction was isolated with ethanol precipitation and stored in ribonuclease-free water at –70° C.

Total cellular RNA from tumor lines was extracted using the miniprep method. Briefly, 10^7 cells were suspended and washed in 1 ml ice-cold TRIS/saline (25 mM TRIS [pH 7.4], 130 mM NaCl, 5 mM KCl). The cells were centrifuged and resuspended in 400 µl TRIS/saline and 100 µl NDD buffer (1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.01% dextran sulfate in TRIS/saline). The suspension was then centrifuged and the supernatant extracted and added to 25 µl 20% sodium dodecyl sulfate (SDS) and 15 µl 5 M NaCl and further extracted for RNA with a 1:1 mixture of phenol:(chloroform and amyl alcohol at 24:1). The aqueous phase was transferred to 1 ml 100% ethanol and precipitated at –20° C for 30 min. Further centrifugation yielded a pellet, which was washed in 70% ethanol, dried, and resuspended in ribonuclease-free water at –70° C. Concentrations of RNA samples were determined by spectrophotometry at 260 nm. Polyadenylated RNA was isolated using a column [Poly(A) Quik Kit, Stratagene, La Jolla, Calif.].

Northern blot analysis. RNA (10 µg) from each sample was size-fractionated on 1% agarose gels containing 6% formaldehyde and transferred onto nylon membranes (Sure Blot, Oncor, Gaithersburg, Md.); the membranes were baked under vacuum at 80° C for 3 h and then were prehybridized for 8–12 h at 42° C. Hybridization was then undertaken with ^{32}P-labeled probes for 16 h at 42° C in 50% formamide, 2× standard saline citrate, 5× Denhardt's solution, 0.1% SDS, 10% dextran sulfate, and 20 µg/ml sheared and denatured salmon sperm DNA. Membranes were washed to a stringency of 0.1×SSC at 65° C and exposed to X-omat AR film (Eastman-Kodak, Rochester, N. Y.) at –70° C with intensifying screens. The intensity of the signals was then quantified by densitometry. The quality and equivalent loading of RNA were confirmed by ethidium bromide staining of the gel before Northern transfer. In addition, the β-actin band of hybridization was used to help confirm that similar amounts of RNA were added to each lane.

Blots were sequentially hybridized with ^{32}P-labeled IL-6 and β-actin probes. Levels of IL-6 gene expression were quantified by standardization to the amount of β-actin transcripts by a previously described method [15]. The relative intensity of RNA expression in different lanes was determined by densitometry and the level of RNA expression in the control lane was assigned to be the baseline level (= 1). The fold stimulation (mRNA expression index) was calculated by

$$\frac{\text{IL-6(experimental conditions)/IL-6(control)}}{\text{β-actin(experimental conditions)/β-actin (control)}}$$

cDNA probes. Human interleukin-6 (1.2×10^3 bases, 1.2 kb, *Xho*1) and β-actin (0.7 kb, *Eco*R1-*Bam*H1) probes were kindly provided by Dr. H. P. Koeffler. The probes were labeled with [^{32}P]CTP by the random-priming method. The specific activity was $(2-5)\times10^8$ cpm/µg.

The B9 bioassay. IL-6 levels were detected by a [^{3}H]dT uptake assay using the IL-6-dependent B9 cell line. B9 is a subclone of the murine B cell hybridoma cell line B13.29, which proliferates only in the presence of IL-6 [2]. The B9 cell line is stimulated by as little as 1 pg/ml IL-6 and is insensitive to other factors [2]. B9 cells were obtained from Dr. J. A. Norton (National Cancer Institute, Bethesda, Md.). Briefly, B9 cells were maintained in RPMI-1640 medium supplemented with 10% fetal calf serum, glutamine, penicillin, streptomycin, 2-mercaptoethanol, and 20 hybridoma growth factor units/ml (U/ml) rIL-6, where 1 U was defined as causing half-maximal B9 cell proliferation. Cell culture supernatants were stored at –70° C and thawed before assay. Triplicate sample aliquots were placed in 96-well flat-bottom plates (Costar Inc., Cambridge, Mass.) and were serially diluted 1/2 over ten dilutions. A total of 2×10^3 washed B9 cells were seeded/well and plates were incubated at 37° C and in 5% CO_2 for 72 h. Wells were then pulsed with 0.5 mCi [^{3}H]dT for 6 h, cells were harvested on a PHD cell harvester (Cambridge Technology Inc., Cambridge, Mass.) and radioactivity was measured on a beta counter. Known concentrations of rIL-6 were diluted in B9 growth media and tested concurrently as a standard. Differences in radioactivity among triplicates were less than 10%. Monoclonal antibody specific against IL-6 (Collaborative Research Inc., Bedford, Mass.) completely inhibited any bioactivity detected by this assay.

Immunoperoxidase staining. Cultured cells were cytocentrifuged onto glass slides, air-dried, and allowed to fix in 2% paraformaldehyde at room temperature for 20 min. Following a wash in phosphate-buffered saline (PBS), the samples were permeabilized with 0.1% Saponin (Sigma) for 20 min, then placed in 3% hydrogen peroxide to remove endogenous peroxidase. After blocking with normal rabbit serum, a 1:20 dilution of polyclonal goat anti-(human IL-6) (Collaborative Research Incorporated, Bedford, Mass.) was added and incubated at room temperature for 1 h followed by washing with PBS and the application of biotinylated rabbit anti-(goat IgG) (Vector Laboratories) for 20 min. After another PBS wash, the sections were treated with peroxidase-conjugated streptavidin (Zymed Lab. Inc.) at room temperature for 20 min using the labeled avidin-biotin method. This step was followed by another PBS wash and the addition of AEC substrate solution.

Treatment of tumor lines. Tumor lines R4, R6, R11, 444, and SK29 were tested for the regulatory effects of cytokines on IL-6 mRNA expression. Tumor cells were grown to 90% confluence in 175-cm^2 culture flasks (Costar) and then treated with 200 U/ml IL-2, IFNγ, TNFα, the combination of IFNγ and TNFα, or culture medium only (control). Cells were then harvested after 1, 4, or 12 h and extracted for total cellular RNA.

Renal tumor proliferation assay. Samples of 3×10^4 renal tumor cells/well were seeded in 24-well culture plates (Costar). After 12 h, supernatants were carefully withdrawn and cells were washed twice with RPMI-1640 medium. A 1-ml sample of test conditioned medium containing various concentrations of IL-6 and/or mAb against IL-6 was added to each well in triplicate along with 0.2 mCi [^{3}H]dT. After 48 h, supernatants were carefully withdrawn, cells were washed twice with RPMI-1640 medium and 0.5 ml 0.5% SDS was added to each well. When all cells had visibly disintegrated, SDS was transferred into scintillation vials containing 10 ml scintillation cocktail (ScintiVerse II, Fisher Scientific, Fair Lawn, N. J.) and counted in a beta counter.

Clonogenic assay. The methods for clonogenic assay were as previously described [16] with modifications. Briefly, the underlayer consisted of 0.5% agar with 25% FCS, 25% 1×RPMI medium, and 25% 2× RPMI medium plated on 35-mm petri dishes (Lux, Nunc Inc., Naperville, Ill.). Cells were then suspended in the upper layer in 0.3% agar with 25% 2×RPMI medium, 25% FCS, and various concentrations of IL-6 and/or mAb. Tumor cells were plated at a concentration of $(2-3)\times10^4$ cells/plate. Cultures were incubated at 37° C in 5% CO_2, and examined with an Nikon inverted microscope at 40× and 100×. Final colony counts were made from 10 to 14 days after plating by two independent investigators in a blinded fashion. Aggregates of 40 or more cells were scored as colonies. Studies of antibody neutralization of IL-6 activity in complete medium used 8 µg/ml monoclonal antibody specific against IL-6.

Statistical methods. The paired Student's *t*-test was used.

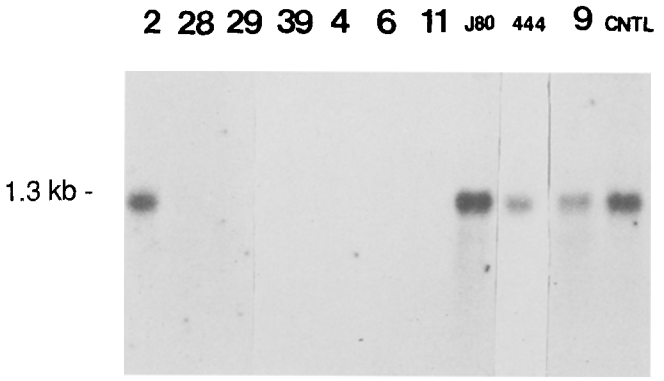

Fig. 1. Total cellular RNA from ten renal tumor lines were probed for the expression of interleukin-6 (IL-6) using Northern analysis. IL-6 expression is evident in four of ten lines. Control (*CNTL*) RNA was extracted from J82, a bladder tumor line known to express IL-6 mRNA

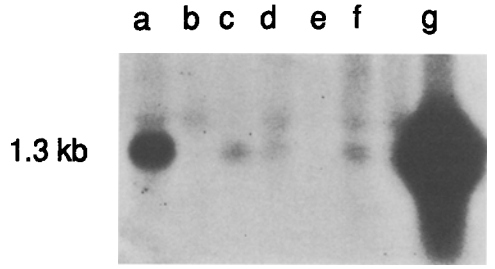

Fig. 2. Poly(A)-rich RNA from five renal tumor lines was extracted, *a*, Control total cellular RNA (5 μg) from J82, a bladder tumor line. *b*, Total cellular RNA from renal tumor line R4 (5 μg). *c*, Poly(A)-rich RNA from renal tumor line R4 (2 μg). *d*, Poly(A)-rich RNA from renal tumor line R6 (2 μg). *e*, Poly(A)-rich RNA from renal tumor line R11 (2 μg). *f*, Poly(A)-rich RNA from renal tumor line SK29 (2 μg). *g*, Poly(A)-rich RNA from renal tumor line 444 (2 μg)

Table 1. Constitutive interleukin-6 (IL-6) production by renal tumor lines[a]

Tumor line	IL-6 level (U/ml)
TL2	3886 ±287
TL9	1372 ±112
444	634 ±141
R4	963 ± 86
R6	16.3 ± 6.2
R11	35.3 ± 5.5
SK28	202 ± 12
SK29	34 ± 1.8
SK39	37 ± 3.3
J80	2574 ±224
T24	<5
M397	<5
Culture medium	<5

[a] IL-6 levels measured by the B9 bioassay. Values represent means ± SD of triplicate samples. T24 and M397 are melanoma tumor lines. Culture medium was assayed as a negative control

Results

Constitutive expression of IL-6 mRNA

IL-6 mRNA was detected by Northern analysis of total cellular RNA in four of ten renal tumor lines tested (Fig. 1). These four tumor lines were the four of the five highest (≥634 U/ml) producers of IL-6 protein amongst the RCC tumors tested. Two melanoma tumor lines tested did not produce any detectable IL-6 mRNA. In order to enhance the sensitivity of mRNA detection by Northern blotting, polyadenylated RNA was extracted from four (R4, R6, R11 and SK29) renal tumor lines in which IL-6 mRNA was not detected in their total cellular RNA, and one (444) line in which IL-6 mRNA was detected in its total RNA (Fig. 2). The analysis of poly(A)-rich RNA

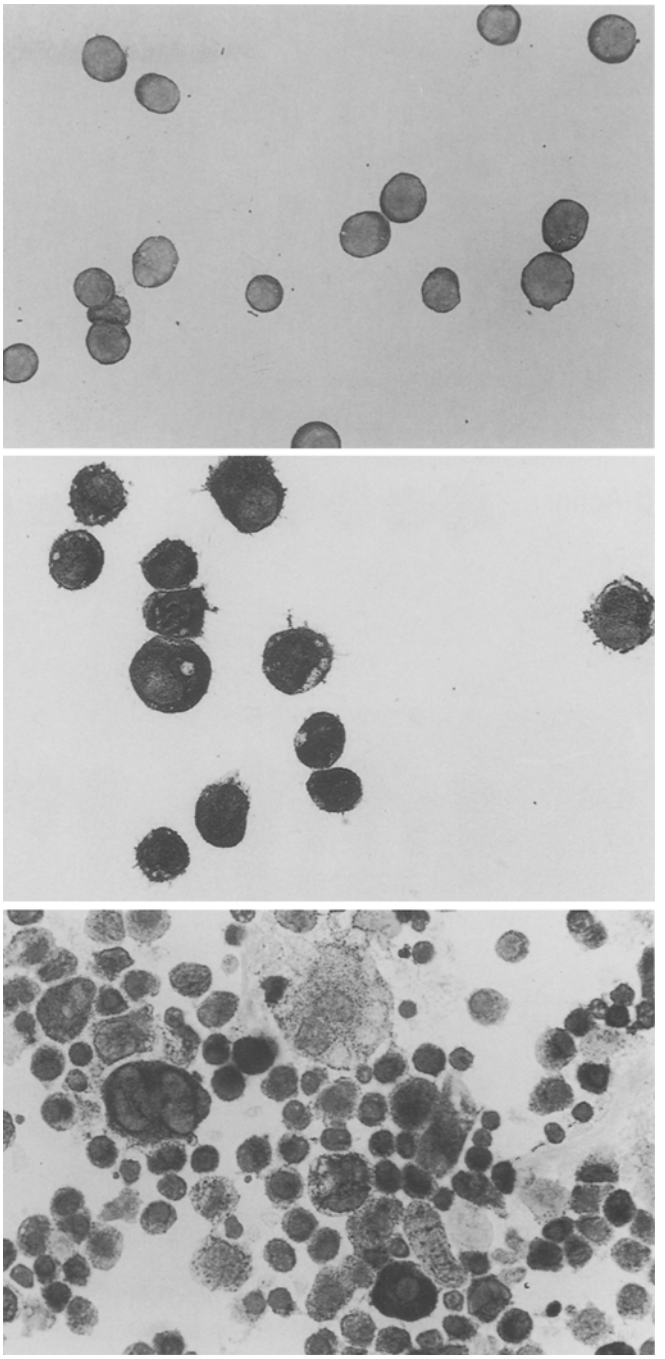

Fig. 3. Immunoperoxidase staining for IL-6 in (**A**) B9, a negative control, (**B**) SK29, a renal tumor line, and (**C**) suspensions of freshly nephrectomized renal cell carcinoma (RCC)

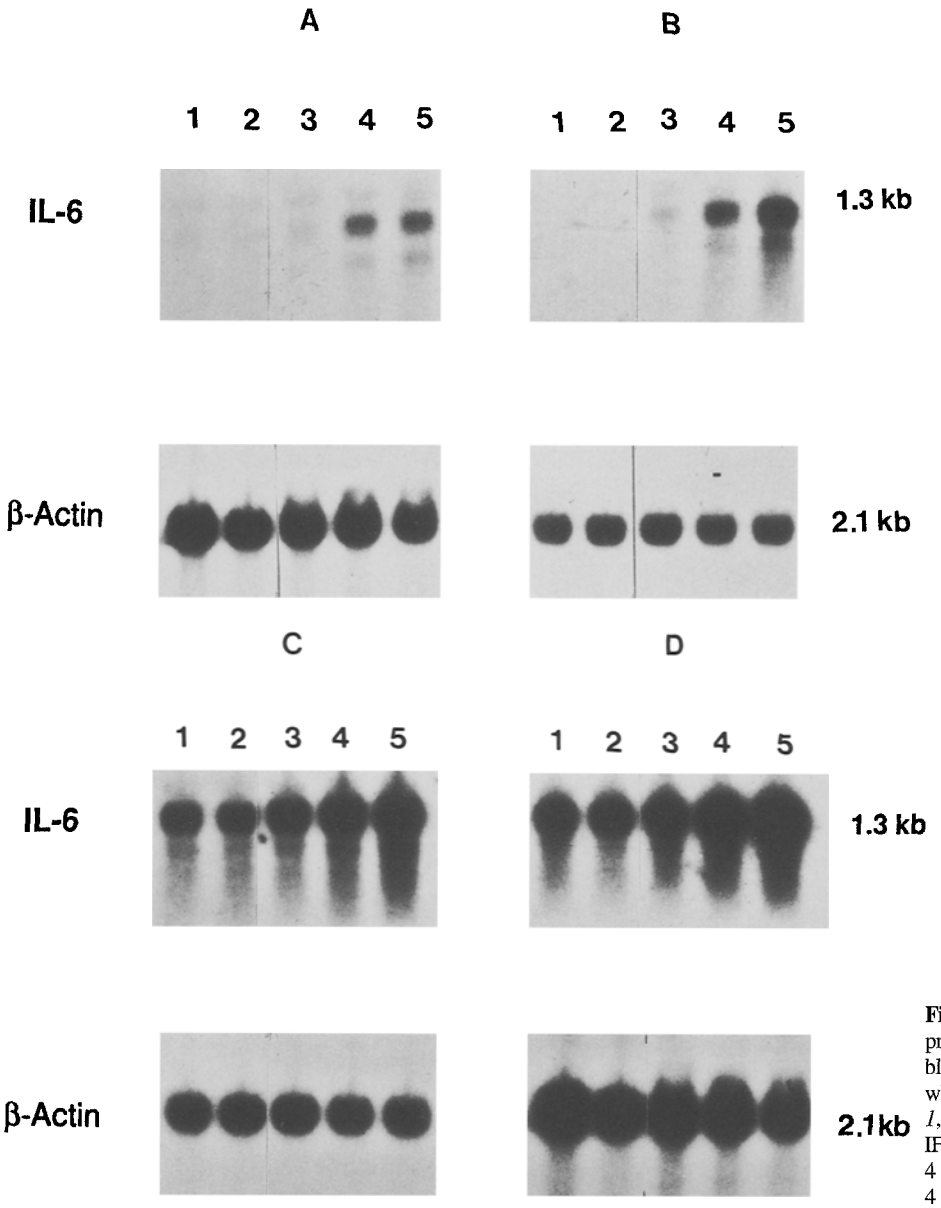

Fig. 4 A – D. Enhancement of IL-6 mRNA expression by cytokines. Representative Northern blots are shown along with β-actin bands, which controlled for even loading of RNA. *1*, Control (no cytokine added); *2*, IL-2; *3*, IFNγ; *4*, TNFα; *5*, TNFα and IFNγ. **A** R4 at 4 h after stimulation; **B** R4 at 12 h; **C** 444 at 4 h; **D** 444 at 12 h

enabled the detection of IL-6 gene expression in three of four tumor lines where IL-6 mRNA was not detected in the total cellular RNA, and the 444 tumor line was now highly positive for IL-6 gene expression. Thus, IL-6 mRNA gene expression was detected in seven of the ten renal tumor lines tested.

Constitutive production of IL-6 by renal tumor cells

From each of ten renal tumor lines, 5×10^5 cells were seeded in 25-cm^2 culture flasks in 5 ml culture media. IL-6 protein was detected in cell culture supernatants collected 12 h after seeding from ten of ten renal cell tumor lines (Table 1). IL-6 levels ranged from 16.3 U/ml to 3886 U/ml, with a mean of 975 U/ml. No IL-6 was detected in the supernatants from two melanoma tumor lines.

Immunoperoxidase staining for cytoplasmic IL-6

All four RCC lines tested (R6, R11, SK29, and 444) were found to have high degrees of cytoplasmic staining for IL-6. The B9 cell line, which does not secrete IL-6 as determined by bioassay, did not stain for cytoplasmic IL-6 (Fig. 3).

Both lymphocytes and tumor cells in single-cell suspensions from two freshly nephrectomized RCC specimens stained for cytoplasmic IL-6 (Fig. 3); however, the staining of the RCC cells was much stronger. Thus it appears that RCC cells may produce IL-6 in vivo.

Enhancement of IL-6 mRNA expression by cytokines

The RCC tumor lines (R4, R6, R11, SK29, and 444) were harvested and analyzed for IL-6 and β-actin mRNA ex-

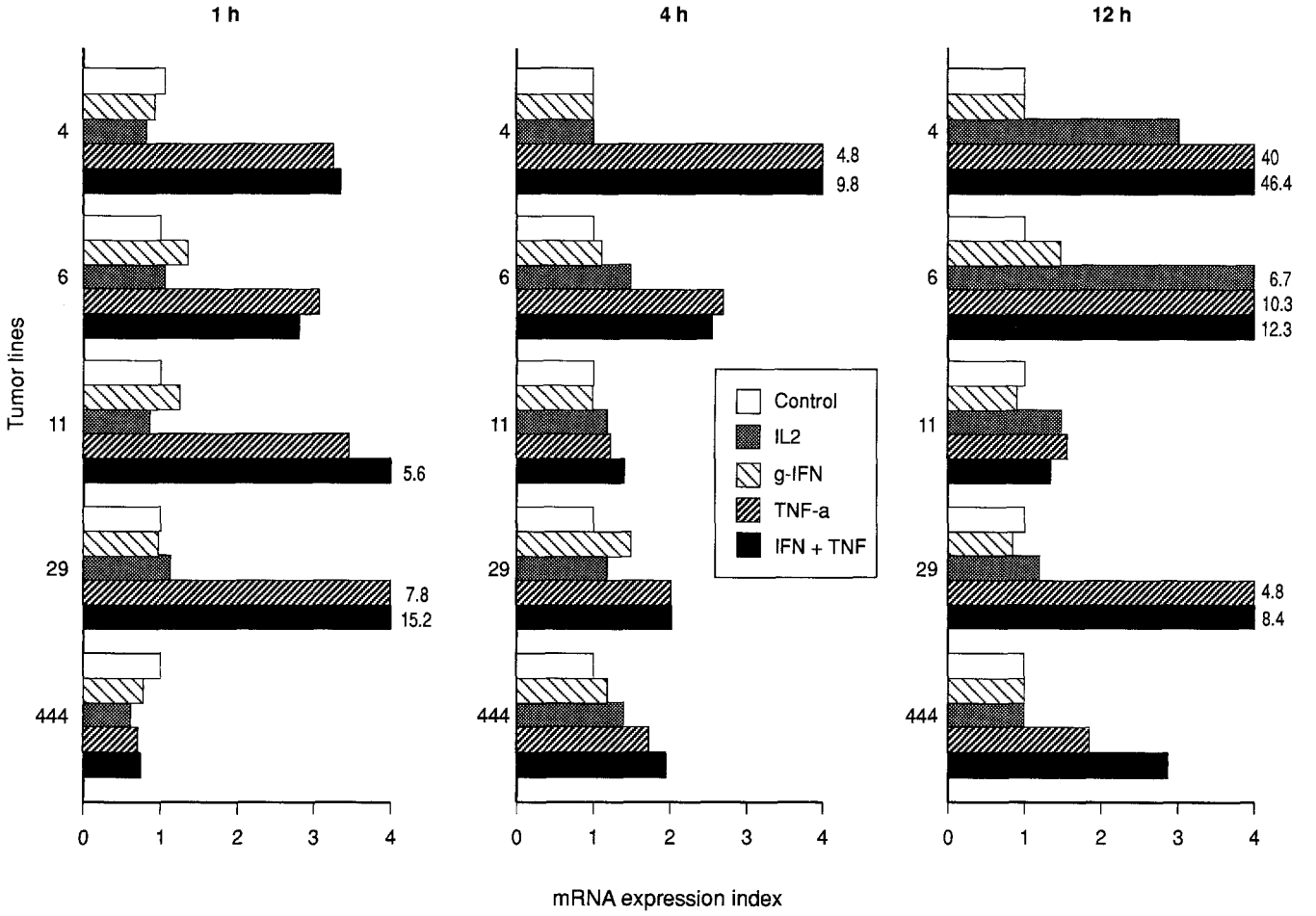

Fig. 5. IL-6 mRNA expression indexed to β-actin in five renal tumor lines at three time points

pression 1, 4, or 12 h after the addition of IL-2, IFNγ, TNFα, or both IFNγ and TNFα (200 U/ml each). Representative autoradiographs are shown in Fig. 4, and mRNA expression indices are shown in Fig. 5. IL-2 did not have much effect on IL-6 mRNA expression. IFNγ also did not alter IL-6 gene expression except in two tumor lines at 12 h. TNFα significantly enhanced IL-6 expression over the control (P <0.05) as did the combination of TNFα and IFNγ (P <0.05). When compared to TNFα alone, the combination of TNFα and IFNγ induced at least an additive and in some cases a synergistic effect in IL-6 gene expression (P <0.01).

Enhancement of IL-6 production by TNFα and IFNγ

After the addition of 200 U/ml TNFα, IFNγ, or both, to tumor cultures, supernatants were collected at two time points, 6 h or 12 h, and tested for IL-6 protein level (Table 2). At 6 h after the addition of cytokines, TNFα alone was found to augment the IL-6 protein bioactivity significantly in culture supernatants of RCC (P <0.01), while IFNγ alone was not found to have any significant effects. The addition of both cytokines was associated with an enhanced level of IL-6 bioactivity, the magnitude of which was less than that seen with TNFα alone. At 12 h, TNFα

Table 2. Enhancement of IL-6 protein production by tumor necrosis factor α (TNFα) and interferon γ (IFNγ)[a]

Time	Tumor	Protein production (U/ml) in presence of			
(h)		Control	TNFα	IFNγ	Both
6	R4	1020 ±89	1280 ±111	403 ±31	1360± 66
	R6	6.8± 3.5	35.7± 2.7	12.5± 3.4	30± 3.4
	R11	28.5± 6.1	335 ± 24	23 ± 6.4	178± 15
12	R4	963 ±86	3108 ±177	1404 ±87	9328±1100
	R6	16.3± 6.2	91.1± 7.3	16.6± 5.7	185± 16
	R11	35.3± 5.5	193 ± 15	39.6± 4.4	341± 29

[a] Values expressed in hybridoma growth factor units (U)/ml as measured by the B9 assay. Values represent means ± SD of triplicate samples

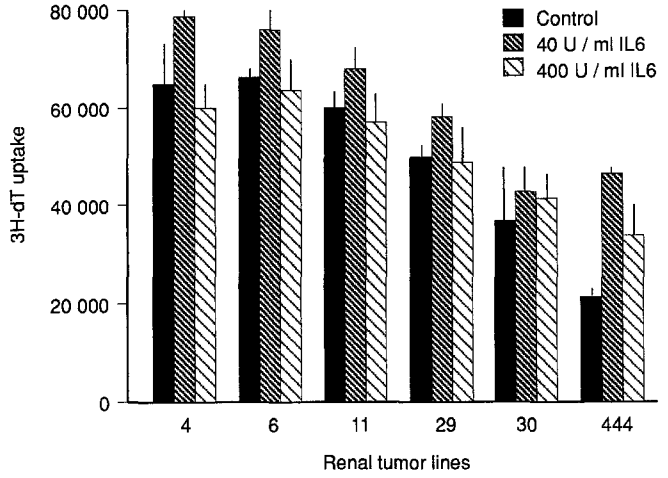

Fig. 6. Effects of IL-6 on RCC proliferation as measured by [^{3}H]dT uptake assays in six renal tumor lines. Results represent means (cpm) ± SD of triplicate samples. The experiments were repeated twice

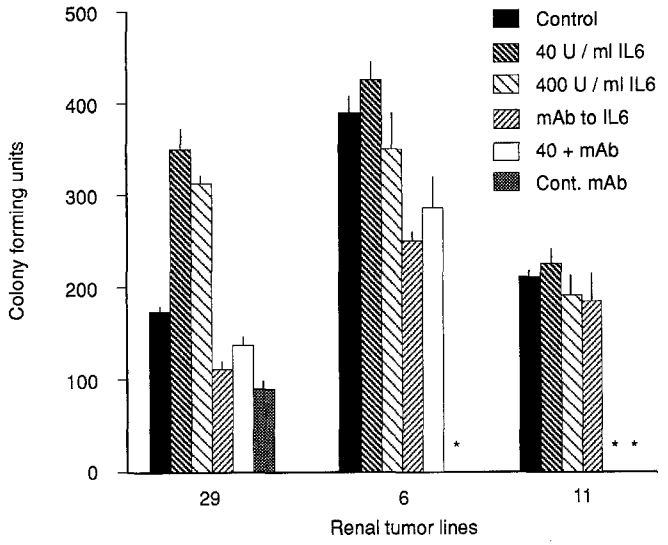

Fig. 7. Effects of IL-6 on RCC clonogenic growth in three renal tumor lines. Results represent means of triplicate samples. *mAb to IL6*, mAb specific for IL-6. *40+mAb*, 40 U/ml IL-6 and mAb specific for IL-6. *Cont. mAb*, a nonspecific mAb. The experiments were repeated twice

alone again induced a significantly higher amount of IL-6 bioactivity ($P < 0.05$), and IFNγ alone did not produce any significant change. The combination of both cytokines induced a significantly higher degree of IL-6 bioactivity over both the control cultures ($P < 0.05$) as well as cultures stimulated with TNFα alone ($P < 0.05$). Hence, 12 h after the addition of cytokines, IFNγ and TNFα were synergistic in augmenting IL-6 bioactivity as secreted by RCC tumor lines.

Effects of IL-6 on RCC proliferation

The effects of IL-6 on RCC proliferation as measured by the 48-h [^{3}H]dT uptake assay are shown in Fig. 6. IL-6 at 40 U/ml significantly enhanced the proliferative response (12%–113% increase) of six of six RCC tumor lines ($P < 0.05$). Although reaching statistical significance, the effects were very modest in five of six tumor lines (less than 21%), and in only one of six tumor lines did the proliferation increase by 113%. At 400 U/ml, IL-6 again exerted a growth-stimulatory effect in only one tumor line (444). When analyzed as a group, 400 U/ml IL-6 did not significantly alter the proliferative responses.

Effects of IL-6 on clonogenic assay

Of the six renal tumor lines tested (R4, R6, R11, SK29, RC30, and 444), only R4, R11, and SK29 were capable of forming colonies in the soft agar media. The results from these three lines are shown in Fig. 7. IL-6 at 40 U/ml induced a variable degree of increase in the number of colony-forming units among different tumor lines. In two tumor lines 40 U/ml IL-6 induced only a low degree of growth stimulation and in one tumor line (SK29), 40 U/ml IL-6 enhanced the clonogenic growth by 100%. The effects of 400 U/ml IL-6 were mixed; it enhanced the growth in one tumor line (SK29) by nearly 100%, while minimally diminishing the clonogenic growth in two other tumor lines. When mAb specific against IL-6 was added to tumor cultures, no (R11), modest (SK29), or a marked (R6) decrease in their clonogenic activity was observed. The combination of mAb and 40 U/ml IL-6 resulted in clonogenic growth activity between the values obtained when each was used alone.

Discussion

In the experiments presented, we found that IL-6 mRNA was detectable in seven of ten RCC tumor lines, and the IL-6 protein bioactivity was found in all cell line supernatants tested under basal culture conditions. Immunoperoxidase staining confirmed the cytoplasmic presence of IL-6 in RCC tumor lines as well as in freshly nephrectomized RCC tumor cells. IL-6 accumulation was enhanced by TNFα at both the mRNA and protein levels. IFNγ alone did not have any significant effect on IL-6 expression; however, when given in combination with TNFα, IFNγ synergistically enhanced IL-6 protein production by RCC and additively or synergistically enhanced IL-6 mRNA accumulation. In addition, IL-6 appeared to have a mild stimulatory growth effect on some of the RCC tumor lines tested.

IL-6 mRNA was found expressed in only four of ten RCC tumor lines when total cellular RNA was used for Northern analysis. The use of poly(A)-rich RNA enabled the detection of IL-6 mRNA in three other tumor lines. Together IL-6 gene expression was found in seven of ten RCC tumor lines while the IL-6 protein was found to be produced and secreted by all ten tumor lines. Other workers have failed to find a direct correlation between mRNA accumulation and protein activity [32, 38]. This discrepancy may be explained by the relatively low sensitivity of Northern analysis in detecting IL-6 mRNA. Schwab et al.

found that IL-6 mRNA expression could be detected by the polymerase chain reaction when Northern analysis failed to demonstrate the presence of IL-6 mRNA [33]. In addition, IL-6 has been shown to be a transiently expressed gene having an AU-rich 3′ region, which might be a target for short-acting RNases [3]. The relatively short half-life of the IL-6 mRNA may have contributed to the difficulty in its detection. The IL-6 protein product, on the other hand, was relatively more stable and therefore more easily detected.

The results found in experiments involving RCC tumor lines established that renal tumor cells indeed expressed IL-6 mRNA and produced and exported biologically active IL-6. Immunoperoxidase staining studies further demonstrated the cytoplasmic accumulation of IL-6 protein in fresh RCC tumor cells that have not been passed in vitro. This finding suggested that RCC tumor cells may produce IL-6 in vivo.

The production of IL-6 in other cell types has been shown to be regulated by cytokine signals. We found that TNFα significantly enhanced IL-6 mRNA expression ($P < 0.05$) and increased IL-6 protein production ($P < 0.05$). TNFα has been shown to be a stimulator of IL-6 production in a number of cell types [7, 9, 13, 21, 22, 26, 38, 40, 41]. It was found to have stimulatory effects on IL-6 mRNA transcript synthesis [5] and has been shown consistently to increase the stability of IL-6 mRNA [33]. It was also demonstrated that TNFα, when administered to cancer patients, could induce considerable amounts of IL-6 protein in plasma [7]. While IFNγ alone exerted little effect on IL-6 expression, the combination of TNFα and IFNγ showed an additive and sometimes synergistic effect in enhancing IL-6 mRNA accumulation and a significant synergistic effect ($P < 0.05$) on IL-6 protein production. This synergistic effect on IL-6 protein bioactivity between TNFα and IFNγ is a novel finding. IFNγ has been shown to be synergistic with TNFα in many of its actions [6, 12]. In FS-4 cells, pretreatment with IFNγ further enhanced IL-6 mRNA accumulation induced by TNF [11]. IFNγ was also found to up-regulate TNFα binding sites in ME-180 cells [24], and this may represent a possible mechanism of their synergistic actions. Alternatively, IFNγ may have independent actions on IL-6 mRNA accumulation, as we found that IFNγ alone enhanced IL-6 gene expression 12 h after stimulation in two of the five tumor lines (R4, R6). These cytokines may themselves act as or induce other protein factors to act as short-lived activators of gene expression or alter mRNA stability [5]. Regardless of the speculative mechanism involved, the regulation of IL-6 production in RCC by cytokines is consistent with observations made in other cell types.

IL-6 appears to have a dual effect on tumor growth depending on the cell type examined. It stimulates the growth of B cell hybridomas [1, 2, 18, 37]. Epstein-Barr-virus-transformed B cells [34, 35], human myeloma cells [19], mouse plasmacytoma cells [4], and human lymphoma cells [36]. IL-6 has been suggested to be an autocrine growth factor for AIDS-Kaposi-sarcoma-derived cells [28], astroglioma cell line U337, myeloid cell line HL60 [20] and two lymphoma cell lines [39]. Alternatively, IL-6 has been shown to be an autocrine growth inhibitor for breast cancer, histiocytic lymphoma, and T-cell lymphoma [8]. IL-6 was found to have in vivo antitumor activity in mice [30]. The role of IL-6 as an autocrine growth factor for RCC was suggested in a brief report by Miki et al. [27]. In the present study, we found that IL-6 at 40 U/ml stimulated the growth response of all six renal tumor lines as measured by a proliferation assay. Although the observed stimulatory effects reached statistical significance, only one cell line out of six tested clearly exhibited a growth response to IL-6. In five tumor lines, increases in [^{3}H]dT uptake were minimal (<21%). Overall, with the exception of one tumor line, IL-6 did not induce any substantial degree of enhancement in proliferative responses. Of the three cell lines able to form colonies in soft agar media, only one showed a marked increase in number of colonies in response to IL-6 at either 40 U/ml or 400 U/ml. This clonogenic growth effect was diminished when monoclonal antibody was added to the culture, thus implicating a possible role of IL-6 as a growth factor in certain RCC tumor lines. However, the low frequency and magnitude of the stimulatory responses observed with IL-6 would argue against the role of IL-6 as a prominent autocrine growth factor for most RCC tumor lines. The small autocrine growth effect observed may have been artifactually diminished by the manipulations used to purify rIL-6 [39]. Alternatively, RCC growth responses to exogenous rIL-6 may be more pronounced under conditions that minimize autocrine IL-6 availability, such as a lower cell density in culture. However, the experimental systems used prevented the testing of such conditions. Our findings suggested that in most RCC tumor lines, rIL-6 did not have any significant growth-stimulatory effects. However, rIL-6 may act as an autocrine growth factor for a subclass of RCC tumor lines.

In the present study, we demonstrated that RCC produce IL-6 in vitro and possibly in vivo. IL-6 production was significantly increased when stimulated with TNFα at both the transcriptional and translational levels with possible synergism when IFNγ was added. IL-6, in turn, was found to stimulate mildly the growth responses of a small proportion of RCC tumor lines tested. The production of IL-6 has been found in many tumors including various epithelial tumor lines [3, 17]. Indeed the production of IL-6 by tumors may simply be a nonspecific manifestation of the carcinogenic process. Yet, in view of the growth effects IL-6 has on various tumors, the possibility of IL-6 having a specific autocrine function in cancer growth cannot be overlooked. The actions of IL-6 in RCC in this setting are as yet unclear. Experiments are unerway in our laboratory to study the effects of IL-6 on RCC growth in vivo in a nude mouse model.

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