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# Interleukin-6 and TNFα production in human renal cell carcinoma

JEAN GOGUSEV, MICHEL AUGUSTI, YVES CHRÉTIEN, and DOMINIQUE DROZ

*INSERM U90; Clinique Urologique, Pr. Cukier; Clinique Urologique, Pr. Dufour; and Laboratoire de Pathologie Rénale, Hôpital Necker, Paris, France*

**Interleukin-6 and TNFα production in human renal cell carcinoma.** Several clinical and biological features suggest that cytokines implicated in the inflammatory response are produced by renal cell carcinoma (RCC). To define if alterations of tumor necrosis factor alpha (TNFα), interleukin 1 alpha (IL-1α), IL-1β and IL-6 gene expression are present in this malignancy, samples from 19 tumors as well as samples from seven paired normal renal tissue were examined using Northern blot and immunohistochemical analysis. In addition, the expression of these cytokines was evaluated in seven RCC-derived cell cultures using Northern blot or RT-PCR. TNFα and IL-6 proteins were measured in culture supernatants using specific bio- and immunoassays. Consistent levels of IL-6 mRNA were detected in 17 of the 19 tested tumors whereas TNFα specific transcripts were present in seven of eight available RNA samples. TNFα and IL-6 mRNA were also detected in five of the seven paired normal kidneys. By immunolabeling, IL-6 antigen was not detected in RCC cells in any of the 19 studied samples. In contrast, using anti-TNFα antibody a strong labeling of stromal endothelia and macrophage cells was detected in all the 19 cases, and evident TNFα staining of the carcinoma cells themselves was observed in eight cases. Spontaneous IL-6 mRNA expression was detected in five RCC cell cultures and TNFα mRNA in four. The cultured cells exhibited positive TNFα immunolabeling in six of seven cases but were always IL-6 negative. Bioactive IL-6 was detected in all culture supernatants while bioactive TNFα was not detected. However, immunoreactive TNFα protein was measured in two of three supernatants tested. Altogether these data indicate that RCC cells produce TNFα and IL-6 *in vivo* and *in vitro* at the RNA and protein level.

Renal cell carcinomas (RCC) are the most frequent kidney tumors in adults. Recent studies have described cell differentiation in these tumors, suggesting that clear cell type carcinomas differentiate more or less towards the proximal tubule, whereas basophilic cell type carcinomas share similarities with the blastematous tubules [1–6]. It is well known that growing RCC almost always present areas of necrosis and of hemorrhage even when the tumors are of small size, and comprise abundant inflammatory cells in the stroma [7]. Clinically, RCC may be associated with fever, increased acute phase reactant blood proteins in serum and, in some cases, with secondary AA amyloidosis [7]. These features suggest, as has been observed in other human tumors, that RCC may produce increased levels of tumor necrosis factor alpha (TNFα), interleukin-1 and -6 (IL-1 and IL-6) cytokines known to be implicated in the inflammatory response. In this regard it has been reported that IL-6, TNFα, IL-1α and IL-1β genes are expressed in normal renal tissue at the RNA level [8]. More recently, IL-6 expression has been observed in RCC and it has been suggested that IL-6 may act as a growth factor in these tumors [9–11].

The aim of the present study was to determine whether the synthesis of these cytokines (TNFα, IL-1, IL-6) occurs in RCC and to define the cell type involved in that production. Therefore, we examined the expression of the specific mRNA for each cytokine on one hand and their tissular localization on the other hand, in neoplastic and normal kidney specimens. On the basis of the RCC features observed *in vivo*, we studied in a second step, the production of TNFα and IL-6 by tumor-derived cell cultures using Northern blot and RT-PCR, immunocytochemistry and specific assays.

## Methods

### *Tumor samples*

Nineteen renal cell carcinomas obtained from total nephrectomy were examined including 17 cases of clear cell type and two tubulopapillary carcinomas with basophilic cells. Specimens from the tumorous areas were frozen in isopentane precooled by liquid nitrogen. In seven cases a nontumorous area, taken at the opposite pole of the same kidney, was studied using the same procedures.

### *Tumor derived cell cultures*

Cell cultures were obtained from seven tumors [6 clear cell type RCC and one tubulopapillary RCC (case no. 5, Table 1)]. Immediately after surgical removal, a fragment (1 $cm^3$) of the tumoral mass chosen in a non-necrotic area was minced and trypsinized to obtain single cell suspension. These cells were cultured in complete medium consisting of RPMI-1640 medium supplemented with 10% fetal calf serum, 100 U/ml of penicillin, 100 μl/ml of streptomycin and 2 mM L-glutamine. Initial complete growth medium contained insulin 5 μg/ml and 10 ng/ml of EGF. The last two additives were removed from the medium after the second passage. The renal epithelial nature of the RCC cultures was confirmed by immunostaining for cytokeratin, vimentin, and CD24 as previously described [6]. Human skin fibroblasts cultured under the same conditions were used as controls.

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**Table 1.** Immunocytochemical, mRNAs cytokine expression and supernatants bio and immunoassays in 7 RCC derived cell cultures (cases 1–4, 6 and 7 derived from clear cell type RCC and case 5 derived from tubulopapillary RCC)

| | Tumoral cell cultures | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Immunocytochemistry | | | | | mRNA | | Supernatants Bioassays *U/ml* | | ELISA TNFα |
| Case | CytoK | vim | CD24 | IL-6 | TNFα | IL-6 | TNFα | IL-6 | TNFα | *pg/ml* |
| 1 | + | + | + | 0 | + | 0 | 0 | > 1400 | 0 | ND |
| 2 | − | ND | + | 0 | + | + | 0 | > 620 | 0 | ND |
| 3 | + | ND | + | 0 | + | + | 0 | > 500 | 0 | ND |
| 4 | + | + | + | 0 | + | 0 | + | > 1000 | 0 | ND |
| 5 | + | + | + | 0 | + | +[a] | +[a] | > 500 | 0 | 24 |
| 6 | + | + | + | 0 | + | +[a] | +[a] | > 500 | 0 | 74 |
| 7 | + | + | + | 0 | 0 | +[a] | +[a] | > 500 | 0 | 0 |
| Fibroblasts | 0 | + | 0 | 0 | 0 | | | | | |

Abbreviations are: cytoK, cytokeratin; vim, vimentin; ND, not determined.
[a] RT-PCR

*Isolation of RNA, Northern blot and RT-PCR*

Total cellular and tissue RNA were purified by the guanidinium isothyocyanate/cesium chloride method as described by Chirgwin et al [12], followed by precipitation with 3 M sodium acetate and ethanol. Concentration and purity were determined by absorbance at 260 and 280 nm. The integrity of total RNA was evaluated after fractionation of ethidium bromide stained RNA bands on 1% agarose gel electrophoresis containing 2.2 M formaldehyde.

For Northern blot analysis, aliquots (20 μg) of total cellular RNA were electrophoresed through 1% agarose-formaldehyde gels followed by transfer to nylon membranes (Hybond, Amersham, UK) and hybridization with appropriate probes. The following cDNA fragments were used: the 1.1 kb Pst I fragment of PE4 plasmid containing human TNFα cDNA insert [13], the 1.7 kb IL-1α cDNA obtained from Xho I digested pXM plasmid, the 1.3 kb IL-1β insert obtained by Pst I digestion of pSP64 vector, the 1.2 kb IL-6 fragment obtained by EcoRI digestion of pM2T vector, and the chicken β-actin cDNA purified from PA1 plasmid [14]. All probes were labeled with $^{32}P$ dCTP using the multiprime DNA labeling system (Amersham, UK) as described by Feinberg and Vogeslstein [15]. The membranes were prehybridized at 42°C for 12 hours in buffer consisting of 50% formamide, 5 × standard saline citrate (SSC) (0.15 M NaCl; 0.015 M Na3 citrate = 1 × SSC), 0.1% SDS, 5 × Denhardt's solution and 200 μg/ml salmon sperm DNA. The filters were then incubated for an additional 16 hours in the same buffer with $2 \times 10^6$ cmp/ml of labeled probe. After hybridization, the blots were washed twice in 0.1% SDS, 2 × SSC for 60 minutes at room temperature, and then washed two more times with 0.1% SDS, 0.1 × SSC at 55°C. The blots were then dried and exposed to X-ray films for 12 to 24 hours with an intensifying screen at −80°C.

Reverse transcription of 1 μg of total cellular RNA was performed using superscript reverse transcriptase and random primers according to the manufacturer's instructions (Gibco-BRL, Bethesda, Maryland, USA). The obtained cDNA was processed for PCR using TNFα and IL-6 specific primers purchased from Stratagene (La Jolla, California, USA) at a final concentration of 1 μM. PCR reaction was achieved in a final volume of 100 μl (1 cycle at 94°C for 5 min and 5 min at 60°C), followed by 30 cycles of 1.5 minutes at 72°C, 45 seconds at 94°C and 45 seconds at 60°C, with a final extension of 10 minutes at 72°C.

In order to assess specificity of the hybridization signal, RNAs extracted from phorbol ester stimulated hematopoietic DEL cell line, expressing TNFα, IL-1α, IL-1β and IL-6 were used as positive control.

*Immunohistochemistry*

Cryostat sections (2 μ) were fixed in methanol for 10 minutes and processed using an indirect immunoperoxidase technique as described previously [6]. Cell cultures for immunolabeling were plated in microwell chamber slides (LabTek chambers, NUNC Inc., Illinois, USA) at a density of $1 \times 10^4$ cells/ml. Growing cells were arrested at 80 to 90% confluence, washed in PBS, and after fixation in cold methanol (10 min) used for immunocytochemical staining.

The following primary antibodies (ab) were used: monoclonal anti-CD14 (macrophages) from Becton-Dickinson; monoclonal anti-CD35 (CR1), monoclonal anti-CD57 specific for natural killer (NK) cells and monoclonal anti-endothelial F-VIII antigen from Immunotech (Luminy, France). Rabbit polyclonal abs anti-human TNFα (IP-310), anti-human IL-1 (EP-200) and anti-human IL-6 (LP-716) were purchased from Genzyme (Cambridge, Massachusetts, USA). EP-200 ab consisted of anti-IL-1α and anti-IL-1β in approximately a 1:3 mixture. Anti-TNFα ab was used at a dilution of 1/160° and anti-IL-1 and anti-IL-6 at a dilution of 1/20°.

Monoclonal abs were revealed using rabbit anti-mouse and goat anti-rabbit IgG peroxydase conjugated antibodies (Dako, Denmark). For polyclonal ab we used goat anti-rabbit peroxydase conjugated IgG (Dako). Sections incubated with PBS or with normal rabbit IgG and second ab were used in each case as negative controls.

*IL-6 and TNFα assays*

IL-6 bioactivity of RCC culture supernatants was measured using the IL-6 dependent murine hybridoma cell line B9 [16, 17]. Proliferation of the B9 cells was determined by the MTT colorimetric assay as described by Mosmann [18]. The specificity of the B9 response to IL-6 was tested with a polyclonal

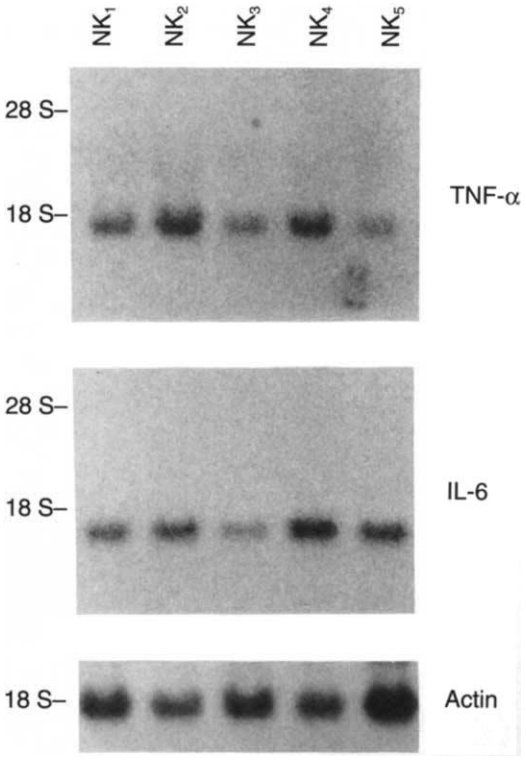


**Fig. 1.** *Expression of TNFα and IL-6 mRNA in 5 normal kidneys (NK).* Total cellular RNA (20 μg) extracted from primary tissue was fractionated on 1% agarose/formaldehyde gels, transferred to nylon membrane and hybridized to 32P-labeled TNFα and IL-6 cDNA probes. The same membrane was rehybridized with β-actin cDNA.

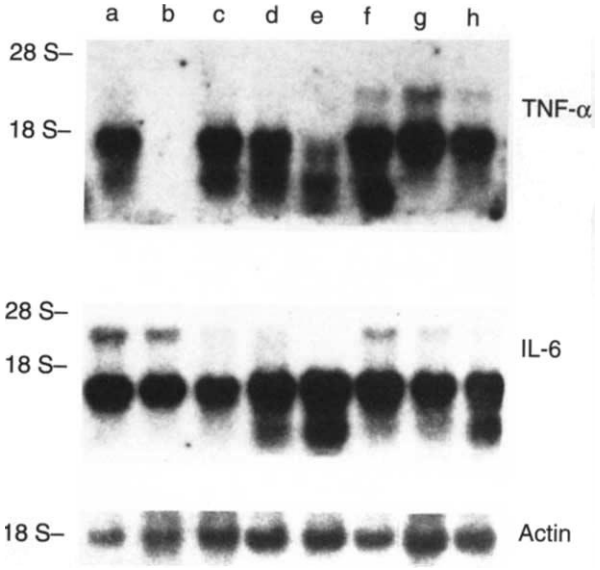


**Fig. 2.** *Northern blot analysis of TNFα and IL-6 mRNA content from 8 primary RCC.* The membrane was hybridized with IL-6, TNFα and β-actin probes. No TNFα transcripts were detected in lane b and there was a weak signal in lane e. Supplementary bands of lower molecular weight are due to nonspecific hybridization signals.

anti-human IL-6 antibody. In the neuralization experiments, supernatants collected five days after passage were incubated during four hours at 37°C with a concentration of 2 μg/ml of the anti-IL-6 ab or murine unrelated immunoglobulin antibody as control. In these conditions we found that this antibody abrogated the biological activity mediated by more then 100 pg/ml of rHu IL-6.

TNFα was evaluated in culture supernatants using bio- and immunoassays. The biological activity of TNFα was determined on actinomycin D-treated murine L929 cells as described [19]. Immunoreactive TNFα protein was measured using specific ELISA kit with detection limit at 15 pg/ml (Medgenics, Fleurus, Belgium).

## Results

### *Cytokine gene expression*

Nondegraded RNA material was obtained from five of the seven normal kidney fragments. After hybridization with specific probes, transcripts corresponding to IL-6 and TNFα were detected in all five cases (Fig. 1). In contrast, lower levels of IL-1β mRNAs were detected in three of five while IL-1α transcripts were not observed (data not shown).

To evaluate the presence of IL-6 mRNA in tumors, total RNAs extracted from nineteen RCC samples were hybridized with IL-6 specific probe. Specific transcript of 1.6 kb was detected in 17 of 19 samples, including 16 of clear cell type and one of basophilic cell type tumors. Eight available RNAs samples were further hybridized with TNFα cDNA fragment and a corresponding transcript of 1.7 kb was detected in seven of them (Fig. 2). All these samples were obtained from clear cell type carcinomas.

A low level of IL-1β mRNA transcripts were detected in the seven tested tumors whereas IL-1α mRNA was not detected (data not shown).

To further investigate if IL-6 and TNFα were expressed in tumor cultured cells, Northern blots or RT-PCR were performed on total cellular RNA extracted from seven RCC derived cell cultures. As shown in Table 1, IL-6 mRNAs were detected in five cases and TNFα mRNAs in four cases. It is of note that all the three cases tested by RT-PCR expressed consistent levels of IL-6 and TNFα specific mRNAs (Fig. 3). Alcaline Southern transfer and probing with $^{32}$P-end-labeled oligonucleotides identified the fragments as TNFα (355 pb) and IL-6 (628 pb), respectively.

### *Immunohistochemistry*

*Normal kidney areas.* Light microscopy examination did not reveal any inflammatory or tumoral changes in these sections. A low number of CD 14 positive interstitial macrophages was present (less than 1 cell per microscopic field at ×40 magnification). CR1 antigen was detected at the membrane of glomerular podocytes and on sparse interstitial cells. NK cells were not detected. A faint staining was obtained with anti-IL-1 ab on the arteriolar endothelial cells. Vascular myocytes and glomerular mesangial cell were positively stained. Anti-TNFα ab stained arteriolar endothelia and smooth muscle cells, as well as glomerular mesangial cells. Endothelial cells from peritubular capillaries and tubular epithelial cells were negative. Anti-IL-6 ab stained smooth muscle cells in arterioles and glomerular mesangial cells. Questionable staining was noted on some glomerular parietal epithelial cells.

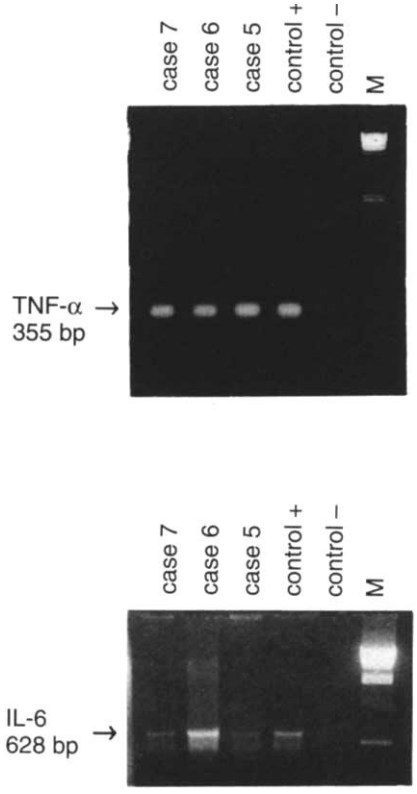


**Fig. 3.** *Detection of TNFα and IL-6 mRNAs in three RCC-derived cell cultures (cases 5, 6 and 7) by RT-PCR.* Total RNA (1 μg) was extracted and reverse-transcribed using random primers. The expected sizes of PCR products for TNFα and IL-6 were 355 and 628 bp, respectively.

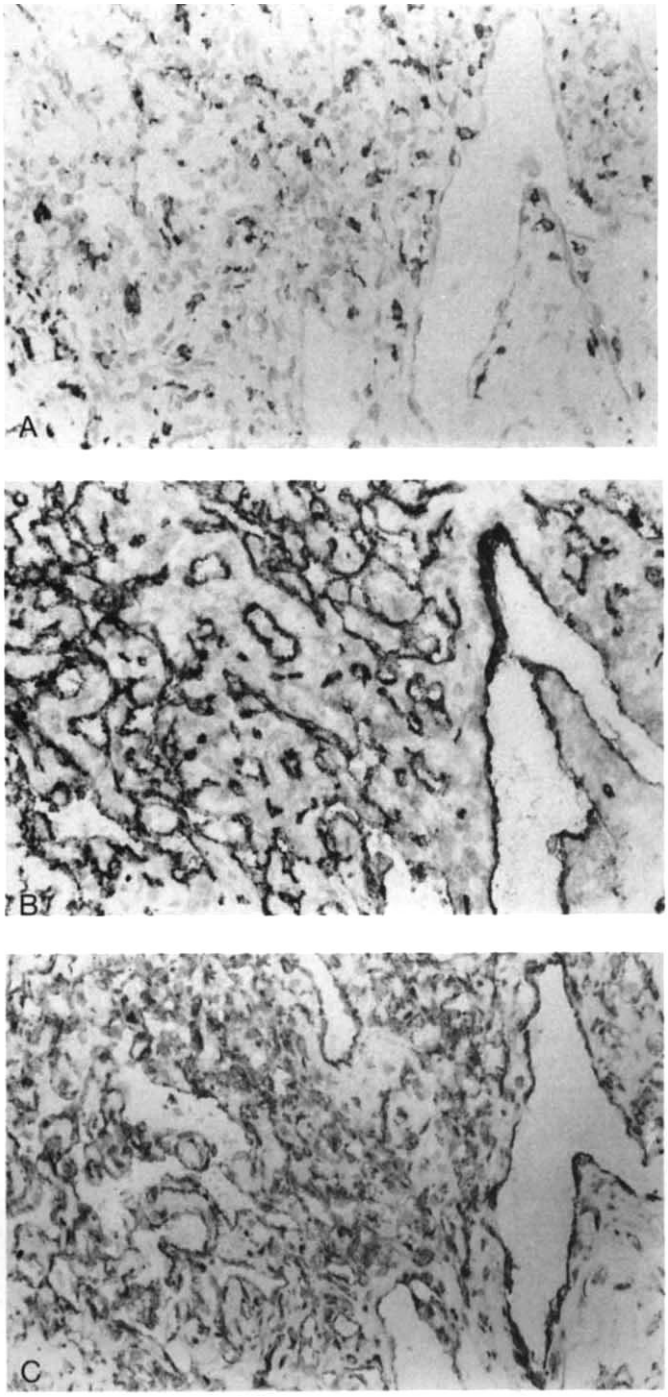


**Fig. 4.** *Frozen sections of RCC immunostained for CD14, FVIII and TNFα antibodies.* Immunoreactivity was examined on serial sections from the same area. **A.** CD14 immunoreactivity (original magnification ×250). Note specific labeling of numerous macrophages present in the supportive stroma. **B.** FVIII immunoreactivity (original magnification ×250). Staining pattern delineating the rich capillary network. **C.** TNFα immunoreactivity (original magnification ×250). Evidence for positive staining of vessels, macrophages and tumor cells.

*Tumors.* Variable numbers of CD14 immunoreactive macrophages were detected in the stroma in all the 19 tumors; in 11 cases they were numerous (1 macrophage/5 to 10 tumoral cells). Some NK cells (less than 6 cells per field at × 40) were present in eight cases. Anti-F VIII antibody staining showed a rich capillary network between the tumoral clumps in all cases (Fig. 4).

Negative staining using anti-IL-1 ab was observed in 12 out of 19 cases. In the other seven cases some faint fixation was observed on the endothelium of stromal blood capillaries; in three of these cases some CD14 immunoreactive interstitial cells were also IL-1 positive. IL-6 immunoreactivity was totally negative in all cases except for the vascular myocytes. In contrast, consistent staining was observed with anti-TNFα ab. In all cases the endothelium of the stromal capillaries was strongly labeled as well as some macrophages or other interstitial cells (Fig. 4). In addition, staining of the tumoral cells themselves was clearly observed in eight cases (2 basophilic cell types and 6 clear cell types) predominantly localized at the basal pole of the cells (Fig. 5). In the 11 remaining cases the topography of anti-TNFα ab staining was difficult to define since it delineated a branching network between the tumoral clumps. In all the 19 cases we noted that the staining exhibited with anti-TNFα ab was more diffuse compared to the specific labeling of macrophages and vessels (Fig. 4c).

*RCC cultures.* The results of the immunochemical analysis summarized in Table 1 show that in all cases the majority of the cells were CD24 positive. Cytokeratine immunoreactivity was observed in six of the seven cell cultures (Fig. 6). IL-6 was not detected, whereas in six cases the cells stained with anti-TNFα

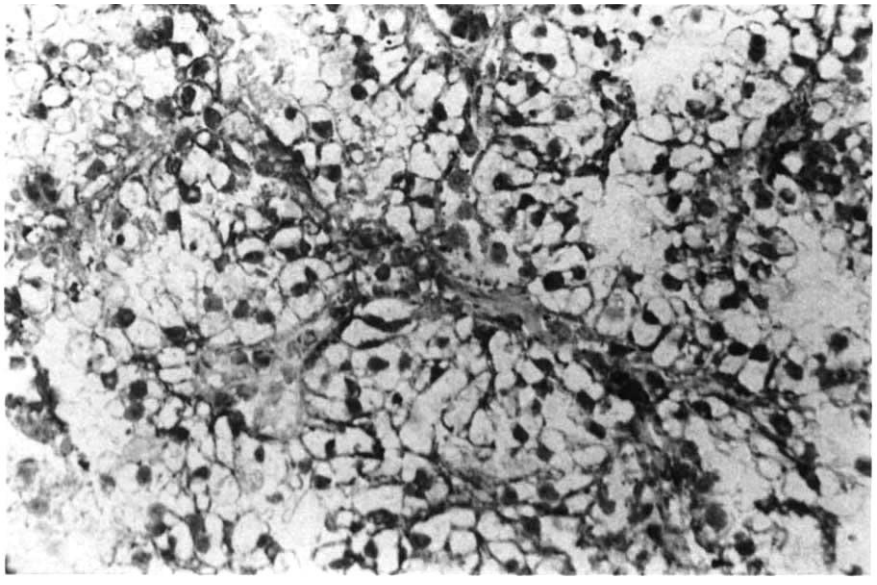

**Fig. 5.** *TNFα immunoreactivity in clear cell type carcinoma (original magnification ×400).* Note the labeling of the tumor cell membrane.

ab (Fig. 6). The cultured skin fibroblasts were vimentin positive and were not stained with anti-IL-6 or anti-TNFα ab.

*Levels of IL-6 and TNFα in culture supernatants*

The seven culture supernatants collected at the second or third passage were monitored for the production of IL-6. The concentrations ranged from 500 to more than 1400 U/ml as detected by the B9 bioassay (Table 1). To ascertain that supernatant-induced B9 cell proliferation was due to IL-6, samples with IL-6 activity were tested in the presence of anti-IL-6 polyclonal antibody. Preincubation of the seven culture supernatants with this anti-IL-6 ab significantly reduced (77 to 85% of inhibition) the proliferation of the B9 cells, giving evidence that the activity detected in supernatants was attributable to IL-6.

Determination of secreted TNFα in culture supernatants by biological activity or immunoreactivity is shown in Table 1. Measurable TNFα cytotoxic activity could not be detected in any of the supernatants tested. However, significant levels of immunoreactive TNFα protein was measured in two of three unconcentrated supernatants (Table 1). Remarkably, TNFα protein was not detected by ELISA and immunocytochemistry in case 7.

## Discussion

The present study demonstrates that the normal human kidney constitutively expresses significant levels of TNFα and IL-6 mRNA. Lower amounts of IL-1β specific mRNA were also detectable. Immunoreactive IL-6, TNFα and IL-1β were observed in the renal parenchyma mainly in the arteriole walls and in the glomerular mesangial cells, but not in the tubular cells. These findings are in agreement with those previously reported concerning IL-6, TNFα and IL-1β expression in normal kidney tissue at the RNA [8, 20] or protein level [21]. In addition, it has recently been shown that normal renal tubular epithelial cells *in vitro* spontaneously express IL-6 mRNA but neither IL-1 nor TNFα transcripts [22].

In RCC, we observed significant levels of TNFα and IL-6 RNA transcripts compared to the paired normal tissue, whereas similar low levels of IL-1β specific transcripts were present in both normal and tumoral tissue.

Although consistent levels of IL-6 RNA were observed in the tumors, immunoreactive IL-6 was not detected in the RCC *in vivo*, except in the normal arterial walls included in the tumor. Likewise, cultured RCC cells were not stained with anti-IL-6 ab, in spite of the presence of IL-6 mRNA and of the measurable amounts of bioactive IL-6 protein in the culture supernatants. These data suggest that RCC cells produce IL-6 protein both *in vivo* and *in vitro*, which is not stored in the producing cells.

Constitutive production of IL-6 has been found in various human tumors, including adenocarcinomas, soft tissue tumors and lymphomas [23], as well as in epidermoid, ovarian and bladder carcinoma cell lines [17, 24, 25]. In studies using cell cultures derived from human RCC, it was found that tumoral cells produce significant amounts of bioactive IL-6 protein *in vitro* [9, 10, 26, 27], which is in agreement with our results. However, these authors reported that variable percentages of the tumoral cells either *in vivo* [26] or *in vitro* [9, 11] were IL-6 immunoreactive, which is in disagreement with our observations. This discrepancy could be due to the different origins or specificities of the anti-IL-6 antibodies. Whatever the reasons for these discordant immunohistochemical results, it can be concluded that RCC cells produce IL-6 *in vivo*. In this regard, the increased levels of serum acute phase reactant proteins frequently observed in patients with RCC [7] could be related to the IL-6 production by the neoplastic cells.

Concerning TNFα expression, our results indicate that the consistent levels of TNFα mRNA detected in RCC are, at least in part, related to the supportive stroma. Indeed, we observed that infiltrating macrophages and the vascular network of the supportive stroma were clearly stained by the anti-TNFα ab. Similarly, TNFα mRNA synthesis has been evidenced by *in situ* hybridization in activated macrophages present in colorectal cancer stroma but not in the neoplastic cells [28]. The strong

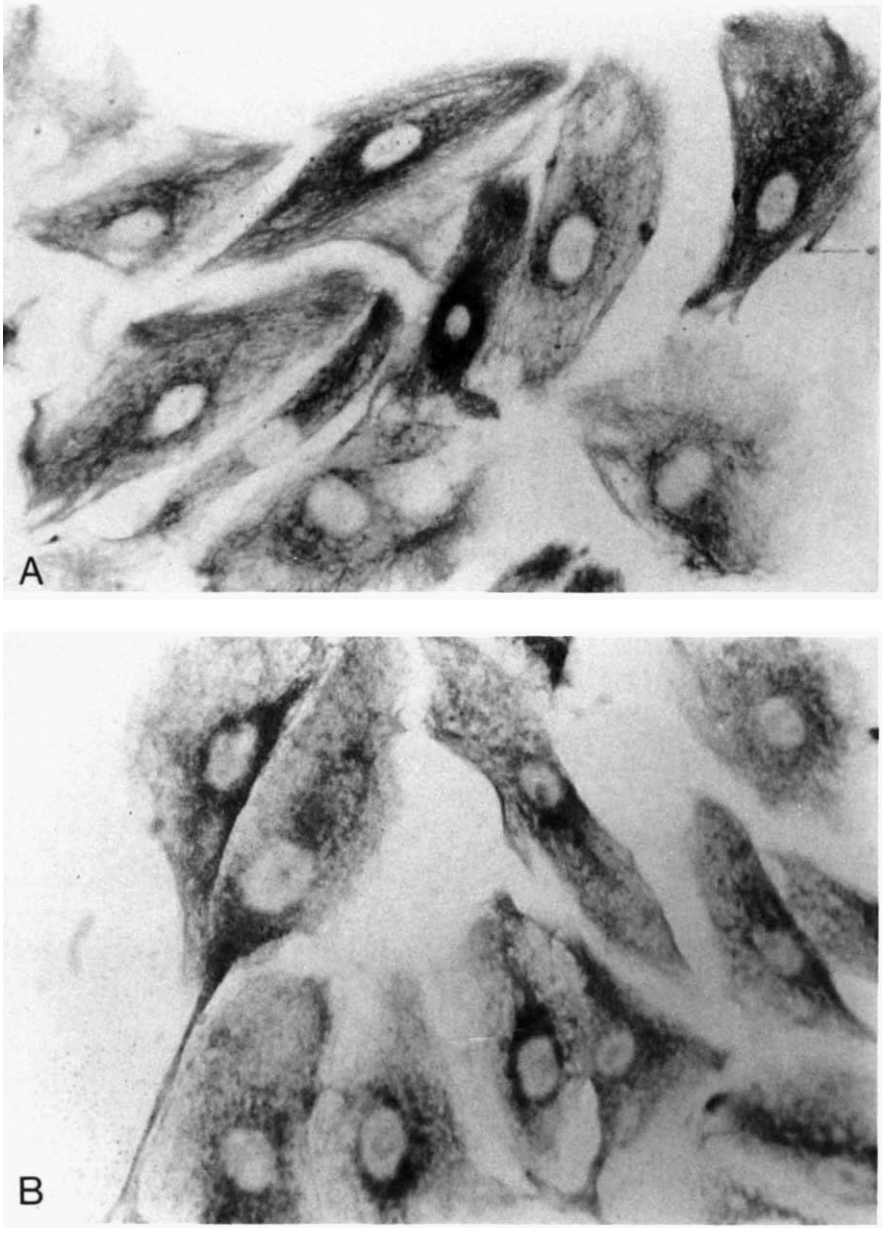


**Fig. 6.** *Cytokeratin and TNFα immunostaining of RCC cultures cells (Table 1, case 1) (original magnification ×400).* **A.** Anticytokeratin immunoreactivity: evidence of filamentous cytoplasmic staining. **B.** TNFα immunoreactivity showing fine granular pattern.

TNFα immunostaining of the stromal capillary vessels observed in RCC is intriguing, since it was not observed in the nontumoral areas of the corresponding kidney. Moreover, using the same antibody and the same technical procedure, TNFα staining of the renal blood capillaries was not observed in inflammatory conditions, such as renal transplant acute rejection crisis or glomerulonephritis, while it was detected in tubular cells (unpublished observations). Therefore, TNFα immunostaining of capillary endothelial cells appears as particular feature of the RCC supportive stroma and could be related to the intense neoangiogenesis present in these tumors. In this respect, it has been reported that TNFα [29, 30] is a potent activator of endothelial cells *in vitro* and plays a major role in angiogenesis *in vivo* [31].

In addition to the observation that TNFα is produced by the supportive stroma our results argue for tumoral cell production of this cytokine. Indeed, in tumors, TNFα positive RCC cells were clearly detected in 40% of the studied cases. Furthermore, TNFα producing cells were directly identified by immunostaining utilizing anti-TNFα ab in six of seven RCC derived cultures, whereas specific TNFα transcripts were detected in four cases. This discrepancy between the TNFα mRNA levels and the presence of TNFα antigen in the cells might be related to the transcriptional and post-transcriptional regulation of this molecule [32]. It is of note that in our material the TNFα production was evidenced in clear cell type tumors as well as in tubulopapillary tumors with basophilic cells.

Within the limits of the L929 assay, TNFα biological activity was not detected in RCC culture supernatants while immunoreactive TNFα could be measured in two cases. These data

suggest that either RCC cells *in vitro* synthesize inactive TNFα or that the released active protein is rapidly inactivated or degraded. It has recently been reported that normal renal tubular epithelial cells, when stimulated by LPS, are able to express TNFα transcripts [33, 34], whereas the levels of bioactive TNFα measured in supernatants remained remarkably low, suggesting that the cells express cell membrane associated and secreted TNFα molecule [34]. Expression of TNFα mRNA has also been documented in several long-term cultured epithelial tumor cell lines, especially in those resistant to TNFα induced cytotoxicity [35, 36]. Interestingly, it was observed that the cell lines resistant to TNFα did not produce detectable amounts of TNFα protein *in vitro* as evaluated by the L929 assay. However, after protein labeling and immunoprecipitation, TNF product was detectable in supernatants and cell lysates from ovarian carcinoma cells [37]. One hypothesis to explain all these data would be that the TNFα molecule produced by the cultured cells is complexed to its soluble receptors, and thus has lost its biological activity [38]. Whether the TNFα protein produced by the RCC cells *in vivo* is bioactive or not remains unsolved. However, in addition to the frequency of tumor necrosis, several facts such as the modulation of the expression of HLA class I antigen [6], EGF-receptor [39] or ICAM-1 and VCAM adhesion molecules (unpublished observations) by the tumor cells, could be related to the presence of bioactive TNFα molecule.

In conclusion, we emphasize the TNFα and IL-6 cytokine gene expression by RCC cells themselves as well as by the supportive stroma. These findings may explain some of the histopathological and clinical features observed in patients with this malignancy, and highlight their involvement in RCC growth and spread.

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*Reprint requests to Dr. D. Droz, Laboratoire de Pathologie Rénale, Département de Néphrologie, Hôpital Necker, 161 rue de Sèvres, 75743 Paris Cédex 15, France.*

## References

1. Holthöfer HA, Miettinen R, Paasivuo R, Lehto VP, Linder E, Alfthan O, Virtanen I: Cellular origin and differentiation of renal carcinomas. A fluorescence microscopic study with kidney specific antibodies, antiintermediate filament antibodies, and lectins. *Lab Invest* 49:317–326, 1983
2. Yoshida SO, Iman A, Olson CA, Taylor CR: Proximal renal tubular surface membrane antigens identified in primary and metastatic renal cell carcinomas. *Arch Pathol Lab Med* 110:825–832, 1986
3. Bander NH: Monoclonal antibodies in urologic oncology. *Cancer* 60:658–667, 1987
4. Cohen C, McCue PA, Derose PB: Histogenesis of renal cell carcinoma and renal oncocytoma. An immunohistochemical study. *Cancer* 62:1946–1951, 1988
5. Medeiros LJ, Michie SA, Johnson DE, Warnke RA, Weiss LM: An immunoperoxidase study of renal cell carcinomas: Correlation with nuclear grade, cell type and histologic pattern. *Hum Pathol* 19:980–987, 1988
6. Droz D, Zachar D, Charbit L, Gogusev J, Chrétien Y, Iris L: Expression of the human nephron differentiation molecules in renal cell carcinomas. *Am J Pathol* 137:895–905, 1990
7. Millan JC: Tumors of the kidney, in *Uropathology* (vol 2), edited by Hill GS, New York, Churchill Livingstone, 1989, pp. 623–701
8. Tovey MG, Content J, Gresser I, Gugenheim J, Blanchard B, Guymarho J, Poupart P, Gigou M, Shan A, Fiers N: Genes for IFN-β2 (IL-6), tumor necrosis factor, and IL-1 are expressed at high levels in the organs of normal individuals. *J Immunol* 141: 3106–3110, 1988
9. Miki S, Iwano M, Miki Y, Yamamoto M, Tang B, Yokokawa K, Sonoda T, Hirano T, Kishimoto T: Interleukin-6 (IL-6) functions as an *in vitro* autocrine growth factor in renal cell carcinomas. *FEBS Lett* 250:607–610, 1989
10. Gruss HJ, Brach MA, Mertelsmann RH, Herrmann F: Interferon-gamma interrupts autocrine growth mediated by endogenous interleukin-6 in renal cell carcinoma. *Int J Cancer* 49:770–773, 1991
11. Koo AS, Armstrong C, Bochner B, Shimabukuro T, Tso CL, de Kernion JB, Belldegrun A: Interleukin-6 and renal cell cancer: Production, regulation and growth effects. *Cancer Immunol Immunother* 35:97–105, 1992
12. Chirgwin J, Przybyla A, McDonald R, Rutter W: Isolation of biologically active ribonucleic acid from sources enriched in ribonucleases. *Biochemistry* 18:5294–5299, 1979
13. Wang AM, Creasey AA, Ladner MB, Lin LS, Stricker J, Van Arsdell JN, Yamamoto R, Mark DF: Molecular cloning of the complementary DNA for human tumor necrosis factor. *Science* 228:149–154, 1985
14. Cleveland DW, Lopata MA, Mac Donald RJ, Cowan NJ, Rutter WJ, Kirshner MW: Number and evolutionary conservation of an alpha and beta tubulin and cytoplasmic beta and alpha actin genes using specific cloned cDNA probes. *Cell* 20:95–105, 1980
15. Feinberg AP, Vogelstein BA: A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal Biochem* 132:6–13, 1983
16. Aarden LA, DeGroot ER, Shaap OL, Lansdorp PM: Production of hybridoma growth factor by human monocytes. *Eur J Immunol* 17:1411–1416, 1987
17. Kirnbauer R, Kock A, Schwartz T, Urbanski A, Krutmann J, Borth W, Damm D, Shipley G, Ansel JC, Luger TA: IFN-beta2, B cell differentiation factor 2, or hybridoma growth factor (IL-6) in expressed and released by human epidermal cells and epidermoid carcinoma cell lines. *J Immunol* 142:1922–1928, 1989
18. Mossmann T: Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxic assays. *J Immunol Meth* 65:55–63, 1983
19. Meager A, Leung H, Wooley J: Assays for tumor necrosis factor and related cytokines. *J Immunol Meth* 116:1–17, 1989
20. Barath P, Fishbein MC, Cao J, Berenson J, Helfant RH, Forrester JS: Tumor necrosis factor gene expression in human vascular intimal smooth muscle cells detected by *in situ* hybridization. *Am J Pathol* 137:503–509, 1990
21. Fukatsu A, Matsuo S, Tamai H, Sakamoto N, Matsuda T, Hirano T: Distribution of interleukin-6 in normal and diseased human kidney. *Lab Invest* 65:61–66, 1991
22. Frank J, Engler-Blum G, Rodemann HP, Müller GA: Human renal tubular cells as a cytokine source: PDGF-β, GM-CSF and IL-6 mRNA expression *in vitro*. *Exp Nephrol* 1:26–35, 1993
23. Tabibzadeh SS, Poubounidis D, May LT, Sehgal PB: Interleukin-6 immunoreactivity in human tumors. *Am J Pathol* 135:427–433, 1989
24. Watson IM, Sensintaffar JL, Berek JS, Martinez-Maza O: Constitutive production of interleukin 6 by ovarian cancer cell lines and by primary ovarian tumor cultures. *Cancer Res* 50:6959–6965, 1989
25. Rawle FC, Shields J, Smith J, Iliescu U, Merkenschlager MP, Beverly CL, Callard RE: B cell growth and differentiation induced by supernatants of transformed epithelial cell lines. *Eur J Immunol* 16:1017–1019, 1986
26. Takenawa J, Kaneko Y, Fukumoto M, Fukatsu A, Hirano T, Fukuyama H, Nakayama H, Fujita J, Yoshida O: Enhanced

expression of interleukin-6 in primary human renal cell carcinomas. *J Natl Cancer Inst* 83:1668–1672, 1991
27. SAKAI A, KAWANO M, KURAMOTO A: Interleukin-6 produced by renal cell carcinoma cells and progression of multiple myeloma. *N Engl J Med* 324:1893–1894, 1991
28. NAYLOR MS, STAMP GWH, BALKWILL FR: Investigation of cytokine gene expression in human colorectal cancer. *Cancer Res* 50:4436–4440, 1990
29. TRACEY KJ, VLASSARA H, CERAMI A: Cachectin/tumor necrosis factor. *Lancet* i:1122–1126, 1989
30. JÄÄTTELÄ M: Biologic activities and mechanisms of action of tumor necrosis factor-α/cachectin. *Lab Invest* 64:724–742, 1991
31. LEIBOVICH SJ, POLVERINI PJ, SHEPARD HM, WISEMAN DM, SHIVELY V, NUSIER N: Macrophage-induced angiogenesis is mediated by tumor necrosis factor α. *Nature* 329:630–632, 1987
32. SARIBAN E, IMAMURA K, LUEBBERS R, KUFE D: Transcriptional and post-transcriptional regulation of tumor necrosis factor gene expression in human monocytes. *J Clin Invest* 81:1506–1510, 1988
33. WUTHRICH RP, GLIMCHER LH, YUI MA, JEVNIKAR AM, DUMAS SE, RUBIN-KELLEY VE: MHC class II, antigen presentation and tumor necrosis factor in renal tubular epithelial cells. *Kidney Int* 37:783–792, 1990
34. JEVNIKAR AM, BRENNAN DC, SINGER GC, HENG JE, MASLINSKI W, WUTHRICH RP, GLIMCHER LH, RUBIN-KELLEY VE: Stimulated kidney tubular epithelial cells express membrane associated and secreted TNFα. *Kidney Int* 40:203–211, 1991
35. SPRIGGS D, IMAMURA K, RODRIGUEZ C, HORIGUCHI I, KUFE DW: Induction of tumor necrosis factor, expression and resistance in a human breast tumor cell line. *Proc Natl Acad Sci USA* 84:6563–6566, 1987
36. KRÖNKE M, HENSEL G, SCHLÜTER C, SCHEURICH P, SCHÜTZE S, PFIZENMAIER K: Tumor necrosis factor and lymphotoxin gene expression in human tumor cell lines. *Cancer Res* 48:5417–5421, 1988
37. SPRIGGS DR, IMAMURA K, RODRIGUEZ C, SARIBAN E, KUFE DW: Tumor necrosis factor expressions in human epithelial tumor cell lines. *J Clin Invest* 81:455–460, 1988
38. LOETSCHER H, GENTZ R, ZULAUF M, LUSTIG A, TABUCHI H, SCHLAEGER J, BROCKHAUS M, GALLATI H, MANNEBERG M, LESSLAUER W: Recombinant 55kDa tumor necrosis factor (TNF) receptor. Stoichiometry of binding to TNFα and TNFβ and inhibition of TNF activity. *J Biol Chem* 266:18324–18329, 1991
39. GOMELLA LG, ANGLARD P, SARGENT ER, ROBERTSON CN, KASID A, LINEHAN WM: Epidermal growth factor receptor gene analysis in renal cell carcinoma. *J Urol* 143:191–193, 1990