

Mechanisms of Apoptosis in T Cells from Patients with Renal Cell Carcinoma¹

Robert G. Uzzo,² Patricia Rayman, Vladimir Kolenko, Peter E. Clark, Tracy Bloom, A. Michelle Ward, Luis Molto, Charles Tannenbaum, Lydia J. Worford, Ronald Bukowski, Raymond Tubbs, Eric D. Hsi, Neil H. Bander, Andrew C. Novick, and James H. Finke

Departments of Immunology [R. G. U., P. R., V. K., P. E. C., T. B., A. M. W., L. M., C. T., L. J. W., J. H. F.], Urology [R. G. U., P. E. C., A. M. W., A. C. N., J. H. F.], Clinical Pathology [R. T., E. D. H.], and Hematology-Oncology [R. B., J. H. F.], The Cleveland Clinic Foundation, Lerner Research Institute, Cleveland, Ohio 44195, and Department of Urology, Division of Urologic Oncology, The New York Hospital-Cornell University Medical Center [N. H. B.], New York, New York 10021

ABSTRACT

Tumors may escape immune recognition and destruction through the induction of apoptosis in activated T lymphocytes. Results from several laboratories suggest that FasL (L/CD95L) expression in tumors may be responsible for this process. In this study of patients with renal cell carcinoma (RCC), we provide evidence for two mechanisms of T-cell apoptosis. One mechanism involves the induction of apoptosis via FasL expression in tumor cells. This is supported by several observations, including the fact that tumor cells *in situ* as well as cultured cell lines expressed FasL mRNA and protein by a variety of techniques. The FasL in RCC is functional because in coculture experiments, FasL⁺ tumors induced apoptosis in Fas-sensitive Jurkat T cells and in activated peripheral blood T cells but not in resting peripheral blood T cells. Most importantly, antibody to FasL partially blocked apoptosis of the activated T cells. Moreover, Fas was expressed by T cells derived from the peripheral blood (53% median) and tumor (44.3% median) of RCC patients. Finally, *in situ* staining for DNA breaks demonstrated apoptosis in a subset of T cells infiltrating renal tumors. These studies also identified a second mechanism of apoptosis in RCC patient peripheral T cells. Whereas these cells did not display DNA breaks when

freshly isolated or after culture for 24 h in medium, peripheral blood T cells from RCC patients underwent activation-induced cell death after stimulation with either phorbol 12-myristate 13-acetate/ionomycin or anti-CD3/CD28 antibodies. Apoptosis mediated by exposure to FasL in tumor cells or through T-cell activation may contribute to the failure of RCC patients to develop an effective T-cell-mediated antitumor response.

INTRODUCTION

Functional T cells are central to an intact antitumor immune response. However, despite infiltration into most solid tumors and the identification of T cells capable of preferentially recognizing tumor antigen (1), effective immunity fails to develop *in vivo* (2–5). Whereas defects in proliferation and effector function are seen in peripheral blood T cells of cancer patients, more pronounced alterations have been reported in T cells infiltrating the tumor (6, 7). Recent evidence suggests that cell-mediated immunity may be down-regulated through apoptotic pathways (8–10). This represents one potential mechanism to account for the observed defects in clonal expansion and cytotoxic function of tumor-reactive T cells.

Interactions between the Fas receptor (Apo-1/CD95) and its ligand, FasL,³ (L/CD95L) have been implicated in a number of normal and pathological processes regulating T-cell function. FasL is used by lymphocytes not only as a cytotoxic effector mechanism to induce apoptosis in Fas-expressing targets (11, 12), but also to diminish the immune response once the targeted antigen has been eliminated (13, 14). Fas/FasL-mediated induction of apoptosis is an effective mechanism of T-cell homeostasis whereby self-reactive clones can be eliminated (15), conditions of tolerance and immune sanctuaries can be achieved (16, 17), and overexuberance of the immune response can be prevented (18). However, tumor cells may take advantage of these mechanisms to escape immune destruction. Malignant cells from an increasing number of solid tumors including melanoma (19), colon carcinoma (8, 20), esophageal carcinoma (21, 22), ovarian carcinoma (23), hepatocellular carcinoma (24), and astrocytoma (9) have been reported to express FasL. T-TILs are potential targets for these FasL-expressing tumor cells (25).

Here we defined Fas and FasL expression on T cells derived from the peripheral blood and tumor of RCC patients.

Received 7/6/98; revised 2/4/99; accepted 2/11/99.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ Supported by USPHS Grant CA56937 and the American Foundation for Urologic Diseases.

² To whom requests for reprints should be addressed, at The Department of Immunology, NN1–24, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195. Phone: (216) 444-5186; Fax: (216) 444-9329.

³ The abbreviations used are: FasL, Fas ligand; RCC, renal cell carcinoma; PMA, phorbol 12-myristate 13-acetate; TIL, tumor-infiltrating lymphocyte; T-TIL, tumor-infiltrating T lymphocyte; AICD, activation-induced cell death; TUNEL, terminal deoxynucleotidyl transferase-mediated nick end labeling; PBL, peripheral blood lymphocyte; T-PBL, peripheral blood T lymphocyte; RT-PCR, reverse transcription-PCR; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; TdT, terminal deoxynucleotidyltransferase; TNF, tumor necrosis factor; IL, interleukin; PHA, phytohemagglutinin; NFκB, nuclear factor κB.

The expression of these molecules was also defined in renal tumor cells *in situ* and in cultured cell lines. Additional experiments determined the susceptibility of patient T cells to apoptosis. Our findings provide support for tumor-induced apoptosis of T cells via the expression of FasL in RCCs. First, FasL⁺ RCC cell lines induced apoptosis in activated T cells but not in resting T cells after coculture. Moreover, antibodies to FasL partially blocked the apoptosis of T cells. Second, a subset of tumor-infiltrating and peripheral blood-derived T cells from RCC patients expressed Fas. Third, *in situ* TUNEL staining of RCC demonstrated apoptosis in a subset of T-TILs. Finally, T cells from the peripheral blood of RCC patients but not those from normal individuals were susceptible to activation-induced apoptosis when stimulated with PMA/ionomycin or antibodies against CD3/CD28.

MATERIALS AND METHODS

Isolation of T Cells from Peripheral Blood and Tumor.

PBLs were obtained from 21 patients with RCC before surgery. Control samples were obtained from a total of 15 healthy volunteer donors. PBLs were isolated using a Ficoll-Hypaque density gradient (LKD Biotech, Piscataway, NJ) and centrifuged at 2000 rpm for 20 min. T cells were purified by negative magnetic selection using microbeads coated with antibodies to CD14 (macrophages), CD16 (natural killer cells), CD19 (B cells), CD56 (natural killer cells), and glycophorin A (erythrocytes; Stem Cell Technologies, Vancouver, Canada). The T-cell isolation procedure yielded cells that were more than 95% positive for CD3 as defined by immunocytometry. Additionally, RCC tumor specimens were obtained, and TILs ($n = 7$) were prepared after a 2-h digestion at 37°C using collagenase type II (3.5 mg/ml; Sigma, St. Louis, MO) and egg white trypsin inhibitor (1 mg/ml; Sigma). TILs were washed in RPMI 1640 (Biowhittaker, Walkerville, MD) and prepared for immunocytometric analysis. Paired T-PBLs and T-TILs were available in four patients.

Establishment of Short-Term RCC Tumor Cultures.

Primary RCC tumor specimens were obtained from eight patients undergoing nephrectomy, and short-term epithelial cell cultures were established in RPMI 1640 supplemented with 10% fetal bovine serum (Hyclone, Logan, UT), L-glutamine (2 mM), sodium pyruvate (1 mM), nonessential amino acids (0.1 mM), and gentamicin (50 µg/ml; Life Technologies, Inc., Grand Island, NY). Monoclonal staining of short-term epithelial cultures was performed using antibodies against URO-2 (Signet, Dedham, MA) and RCC (Monosan, Inc., San Francisco, CA) to confirm the establishment of cultures of renal cell origin. Cultures were maintained at 37°C with 5% CO₂. All cell lines were trypsinized (1000 units/ml; Worthington, Freehold, NJ) upon confluence, and aliquots were frozen at -80°C in 90% fetal bovine serum and 10% DMSO (Sigma). Frozen aliquots were resuspended in supplemented cloning media and maintained in culture for 3–5 days before the measurement of CD95/CD95L expression. All short-term RCC cultures studied were passage 4 or less. Five established and well-characterized RCC cell lines were obtained from Dr. Neil Bander (SK-RCC-7, -26b, -28, -48, and -54; The New York Hospital-Cornell University Medical College). Additionally, a human colon epithelial cancer cell line

(SW-620), which has previously been shown to express FasL (8), was obtained from the American Type Culture Collection (Rockville, MD).

Immunocytometry Analysis of T-Cell and RCC Epithelial-Cell Surface Antigens. T-PBLs, T-TILs, and Jurkat cells were stained with antibody to external epitopes of CD3, CD4, CD8, CD95, and CD95L. Short-term RCC epithelial cultures and freshly isolated RCC epithelial cells were stained with anti-CD95/CD95L only. Monoclonal antibodies were purchased as FITC (anti-CD95; Becton Dickinson, Mountain View, CA) or PerCP (anti-CD3, anti-CD4, and anti-CD8; Becton Dickinson) conjugates. For all experiments, matched isotype controls were used for each particular subclass of immunoglobulin and system used. Cells were washed in HBSS (Biowhittaker), and individual samples were split into two tubes, one for autofluorescence, and the other stained with the appropriate antibodies. In the case of the biotinylated anti-CD95L, (catalogue number 65322X; PharMingen, San Diego, CA), streptavidin-PE (Becton Dickinson) was used as a secondary antibody for fluorescence. Cells were then fixed in 1% paraformaldehyde and analyzed by flow cytometry (FACScalibur; Becton Dickinson; Ref. 26). Live gating of the forward and orthogonal scatter channels was used to exclude debris and to selectively acquire lymphocyte or epithelial cell events. Individual fluorescence data were determined through the use of acquisition and quadrant analysis software (CELLQUEST; Becton Dickinson). Percentages of cells expressing CD95 and CD95L were calculated, and statistical analyses were performed using the Kruskal-Wallis and Wilcoxon's rank-sum tests.

mRNA Analysis by RT-PCR. RNA was isolated by the guanidine isothiocyanate/cesium chloride method, followed by ethanol precipitation and storage at -70°C. cDNA was synthesized using a specific antisense primer and avian myeloblastosis virus reverse transcriptase (Promega, Madison, WI). The undiluted reverse transcription reaction products were used for PCR amplification, using 20 µM sense and antisense primers and Taq polymerase. PCR reactions were conducted in a Perkin-Elmer/Cetus DNA Thermal Cycler for 35 cycles (denaturation, 2 min, 94°C; annealing, 1 min, 60°C; amplification, 3 min, 72°C). The following sense and antisense primers were used: (a) FasL, TGAGCCACAAGGTCTACATGAGG and GGAAAGAATCCAAAGTGCTTCTC; and (b) GAPDH, GAAGGTGAAGGTCGGAGTC and GAAGATGGTGATGGGATTTTC. The PCR products were separated by agarose gel electrophoresis and visualized by Southern hybridization analysis using radiolabeled oligonucleotide probes. The probe sequences used were as follows: (a) FasL, CTCTCTGGTCAATTTTGAGGAATCTCAGACG; and (b) GAPDH, CAAGCTCCCGTTCTCAGCCTTGACGGTG.

Analysis of FasL Expression by Immunohistochemistry. The identification of FasL expressed by RCC was performed on fixed short- and long-term cell line cytopins and embedded tumor sections. For cytopins, cells were fixed in acetone. Paraffin-embedded tumor sections were subjected to microwave antigen retrieval in 10 mM citrate buffer (pH 6.0). In both instances, the primary antibody to FasL (catalogue number F37720; Transduction Laboratories, Lexington, KY) was used at a 1:40 dilution. Immunoperoxidase staining was performed with an automated immunostainer

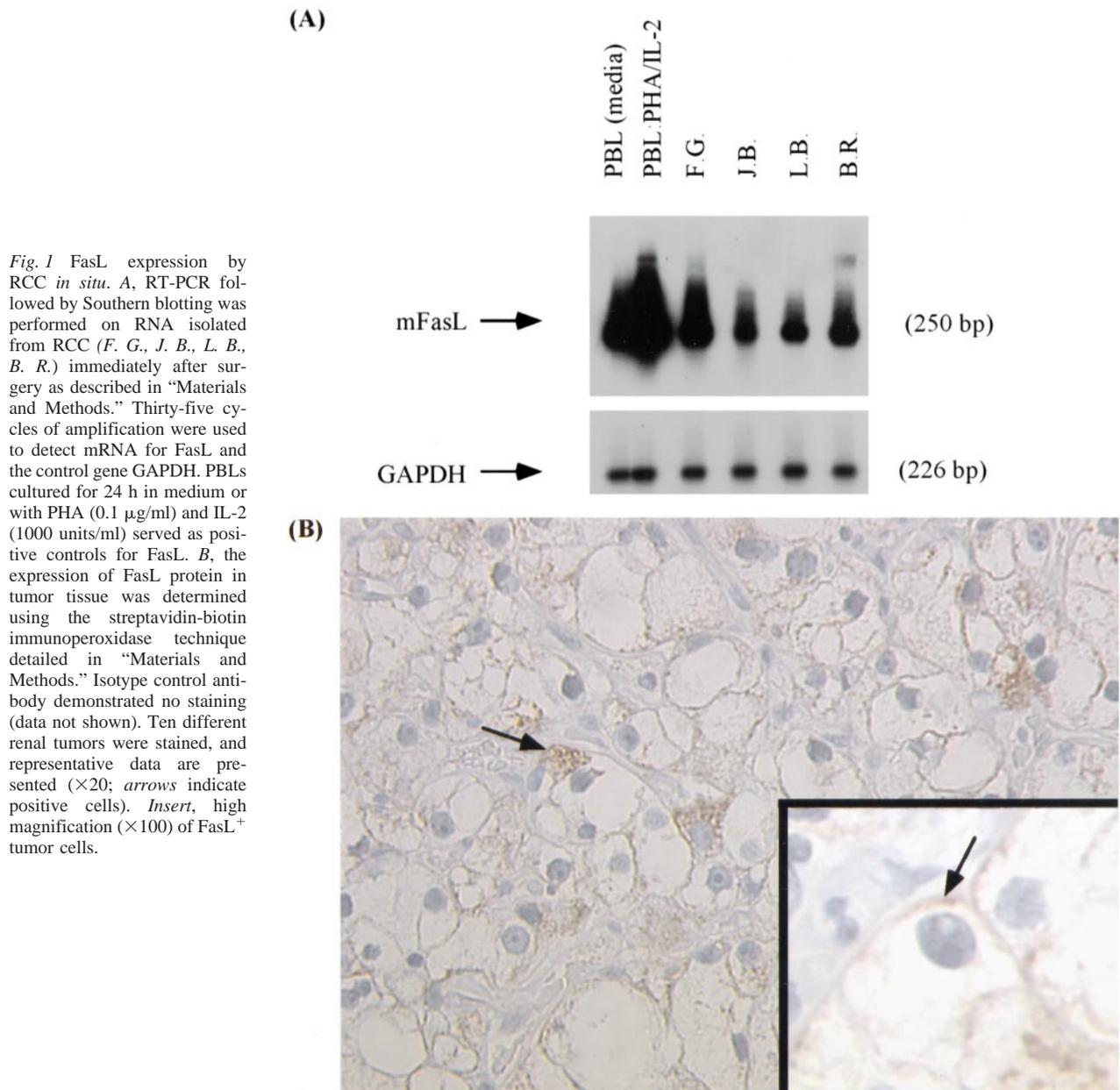


Fig. 1 FasL expression by RCC *in situ*. *A*, RT-PCR followed by Southern blotting was performed on RNA isolated from RCC (F. G., J. B., L. B., B. R.) immediately after surgery as described in "Materials and Methods." Thirty-five cycles of amplification were used to detect mRNA for FasL and the control gene GAPDH. PBLs cultured for 24 h in medium or with PHA (0.1 μ g/ml) and IL-2 (1000 units/ml) served as positive controls for FasL. *B*, the expression of FasL protein in tumor tissue was determined using the streptavidin-biotin immunoperoxidase technique detailed in "Materials and Methods." Isotype control antibody demonstrated no staining (data not shown). Ten different renal tumors were stained, and representative data are presented ($\times 20$; arrows indicate positive cells). *Insert*, high magnification ($\times 100$) of FasL⁺ tumor cells.

(Ventana 320; Ventana Medical Systems). Equivalently concentrated nonimmune isotype-matched mouse IgG was used as a control for nonspecific staining.

Western Blotting. Whole cell lysate protein samples (10–20 μ g) were mixed with an equal amount of 2 \times Laemmli sample buffer, boiled, and resolved by electrophoresis in 10% SDS-polyacrylamide gels. Proteins were transferred to nitrocellulose membranes that were incubated in 5% nonfat dry milk in Tris-buffered saline overnight. Thereafter, membranes were incubated sequentially with anti-FasL antibody (Transduction Laboratories) and then with horseradish peroxidase-conjugated sheep antimouse IgG for 30 min. Membranes were developed with enhanced chemiluminescence (ECL Western Blotting Kit; Amersham).

Coculture of Allogeneic T Cells with FasL-expressing RCC Cell Lines. T-PBLs were preincubated with PMA (10 ng/ml; Sigma) plus ionomycin (0.75 μ g/ml; Sigma) for 3–5 days, which is known to up-regulate surface expression of Fas (CD95; 27). Jurkat T cells were obtained from the American Type Culture Collection and maintained in culture. The sensitivity of Jurkat cells to Fas-mediated killing has been demonstrated (28, 29). Here, T cells were incubated in medium alone or with the transactivating anti-Fas antibody (50 ng/ml; catalogue number 05-201; Upstate Biotechnology, Lake Placid, NY). T-cell preparations were also cocultured with FasL-expressing tumor cells at a T cell:tumor cell ratio of 10:1 for 24–72 h. Neutralization studies were performed using antihuman FasL antibody (catalogue number D041-3; MBL Interna-

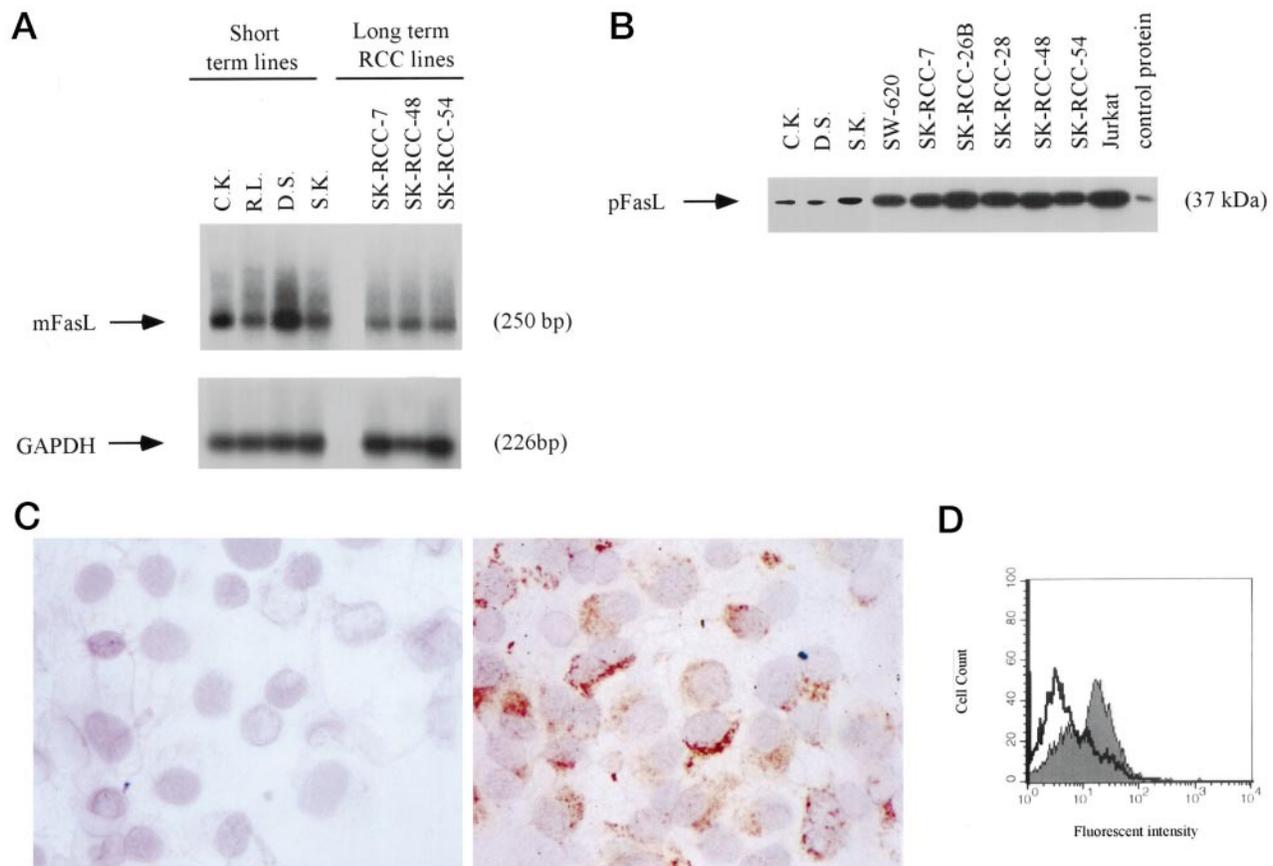


Fig. 2 FasL expression by RCC cultured cell lines. **A**, mRNA expression for FasL and GAPDH was examined in short-term cell lines (passages 2–4) and in long-term established cell lines. mRNA was detected by 35 cycles of RT-PCR and hybridization with a specific oligonucleotide probe (Ransom Hill Biosciences Inc., Ramona, CA). Representative data are presented. **B**, FasL expression in short-term (C. K., D. S., and S. K.) and long-term established cell lines (SK-RCC-7, -26B, -28, -48, and -54) was determined by immunoblotting with antibody to FasL (Transduction Laboratories). Whole cell lysates were prepared from the RCC cell lines, and 10 μ g of protein were used for the assay. Positive controls included lysate from the Jurkat cell line, a colon cancer cell line (SW-620) previously shown to express FasL (8), and a human endothelial FasL control peptide (Transduction Laboratories; right panel). FasL expression was noted in 11 of 12 lysates examined. **C**, FasL protein expression in RCC cell lines ($n = 5$) was detected by immunoperoxidase staining of cytopins using anti-FasL antibody (Transduction Laboratories). Immunostaining with control antibody did not show any positive reaction (left panel). **D**, surface expression of FasL was also examined by immunocytometry using anti-FasL antibody (PharMingen; thin line, shaded area) and an isotype control antibody (thick line, open area). Of the eight RCC cell lines tested, three expressed FasL by flow, although all expressed FasL mRNA and protein (Western blot and immunoperoxidase staining). In this panel, positive FasL expression was demonstrated in the RCC-54 cell line.

tional, Watertown, MA). After incubation, the nonadherent T cells were removed and stained for viability with trypan blue (Sigma) or fixed for the TUNEL assay.

Quantitation of DNA Fragmentation by TUNEL Assay.

Fixed cells (1% paraformaldehyde) were stained and analyzed for apoptosis using the APO-BRDU kit system (Phoenix Flow Systems, San Diego, CA; Ref. 30). Briefly, cells were labeled with 50 μ l of DNA solution containing 10 μ l of TdT reaction buffer (0.75 μ l of TdT enzyme and 8 μ l of Br—dUTP and dH₂O). Cells were rinsed before resuspending in 0.1 ml of solution containing fluorescein PRB-1 antibody. Propidium iodide/RNase A solution (0.5 ml) was added to each sample before incubation at room temperature for 30 min. Flow cytometric analysis was performed within 2 h of sample staining. Apoptotic HL60 promyelocytic leukemia cells induced with camptothecin were used as positive controls, whereas uninduced

cells served as negative controls. The percentages of apoptotic T cells were obtained using quadrant analysis software (LYSIS II; Becton Dickinson).

In situ TUNEL assays were performed on formalin- and B5-fixed RCC tumor tissue from 10 patients undergoing nephrectomy. Paraffin-embedded samples were deparaffinized and rehydrated in xylene for 5 min at room temperature. Samples were immersed and fixed in graded alcohol washes (100% to 70% pure ethanol) and then permeabilized using proteinase K (1 μ l of a 2 mg/ml solution diluted 1:100 in 10 mM Tris). TUNEL assays were performed using portions of a DNA fragmentation detection kit (31; Oncogene Research Products, Cambridge, MA) coupled to automated chromogenic detection on the Ventana Gen II *in situ* hybridization instrument (Ventana Medical Systems). Briefly, the 3'-hydroxyl ends of fragmented DNA were labeled with biotinylated dUTP catalyzed by TdT. Labeled

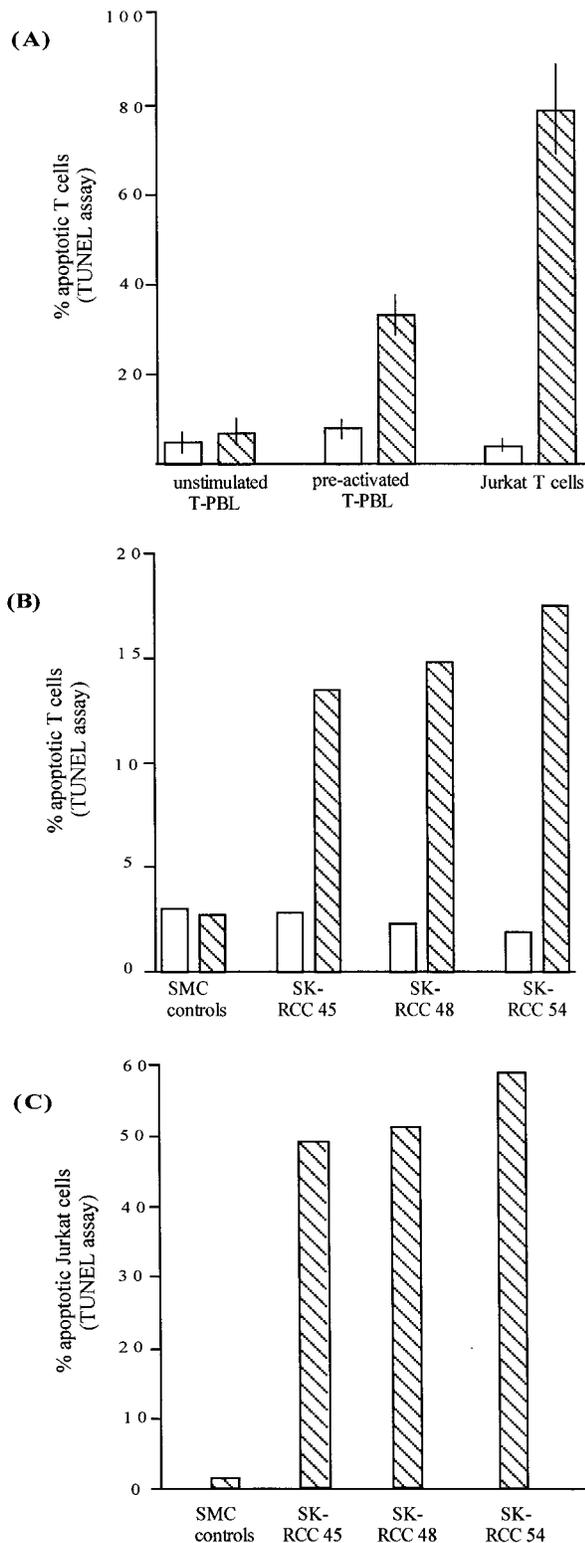


Fig. 3 Sensitivity of Jurkat cells and preactivated T-PBLs to apoptosis. *A*, unstimulated and preactivated T-PBLs from normal donors as well as Jurkat T cells were cultured for 24 h in the absence (□) or presence (▨) of 50 ng/ml anti-Fas monoclonal antibody (Upstate Biotechnologies). TUNEL assay was then performed to determine the level of apoptosis induced. The data presented represent the mean \pm SD of five experi-

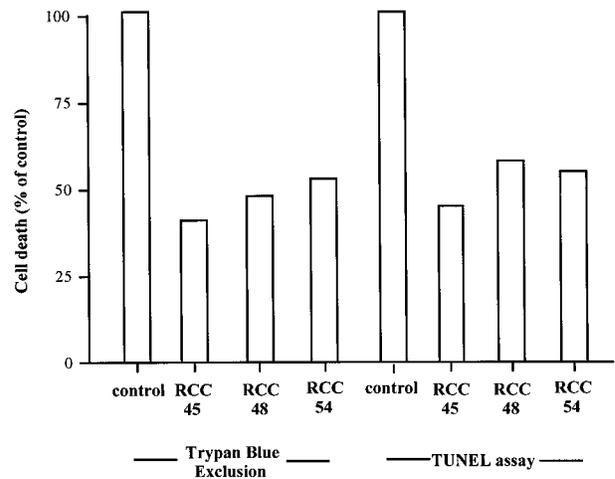


Fig. 4 Apoptosis of T cells induced by RCC cell lines was inhibited by anti-FasL antibody. Jurkat cells were cultured with RCC cell lines ($n = 3$) for 3 days in the presence and absence of anti-FasL antibody (1–5 μ g/ml; clone 4H9; MBL International, Watertown, MA). Thereafter, Jurkat cells were removed from the RCC and assessed for trypan blue dye exclusion and for DNA breaks (TUNEL assay). Representative data from one of four experiments are presented. Similar studies were performed with preactivated T cells and RCC cell lines (data not shown). In both cases, anti-FasL antibody reduced the levels of T-cell apoptosis.

DNA breaks were detected with an alkaline phosphatase-streptavidin conjugate followed by nitroblue tetrazolium and nuclear fast red counterstaining. HL60 cells and HL60 cells incubated with 0.5 μ g/ml actinomycin D for 19 h were used as negative and positive controls, respectively. Specimens were then double-stained with anti-CD3 using the Ventana 320 automated immunostainer. Apoptosis in T-TILs was assessed using conventional light microscopy.

RESULTS

FasL Expression on Human RCCs. Our findings demonstrate that freshly isolated renal tumors express mRNA for FasL. After RT-PCR and Southern blotting, FasL mRNA was detectable in tumor sections from four of four RCC patients evaluated (Fig. 1A). The observation that the tumor cells themselves express FasL is supported by immunostaining of tumor sections with the anti-FasL antibody. Immunostaining demonstrates that FasL protein was expressed in five of five RCC tumors tested, although the level of expression varied among the tumors examined (Fig. 1B). In contrast, isotype control antibody did not stain any tumor cells *in situ* (data not shown).

ments. *B*, unstimulated (□) and 5-day preactivated [PMA (10 ng/ml)/ionomycin (0.75 μ g/ml)] T-PBLs (▨) from healthy volunteers were cocultured with RCC cell lines expressing FasL and a smooth muscle cell line control (SMC). After 24–72 h, T cells were removed and assayed for DNA breaks (TUNEL assay). Representative data with three RCC cell lines are presented. *C*, Jurkat cells were cocultured with RCC cell lines expressing FasL and a smooth muscle cell line control (SMC) for 24–72 h before measuring DNA breaks (TUNEL assay). Representative data with three different RCC lines are presented.

FasL mRNA was also expressed by all short-term RCC cell lines examined (four of four cell lines; passages 2–4) and by all established RCC cell lines tested (five of five cell lines; Fig. 2A). Protein was also detectable by both immunoblotting of whole cell lysates (11 of 12) and immunostaining of cytopins (5 of 5; Fig. 2, B and C). Furthermore, immunocytometry with the α -Fas ligand antibody could also detect surface expression on three of eight RCC cell lines, whereas isotype controls were negative (Fig. 2D).

In the same experiments, fresh RCC tumor samples and cell lines all expressed Fas receptor as measured by immunocytometry (data not shown). However, the level of Fas expression varied considerably among the tumor samples (median, 8.13%; range, 2.7–50.4%; $n = 8$). Additionally, in short-term cultures and in freshly isolated tumors, the percentage of FasL⁺ cells was significantly higher than the percentage of Fas⁺ cells (Fas, median expression = 8.1% and range = 1.75–50.4%; FasL, median expression = 23.3% and range = 4.4–54.6%; $P = 0.016$).

The FasL Expressed on RCC Is Functional and Can Induce Apoptosis in Activated T Lymphocytes. The initial experiments determined the susceptibility of unstimulated and preactivated T cells as well as the Jurkat T cell line to Fas-mediated killing. Median percentages of apoptotic T cells induced by culturing in media with or without transactivating anti-Fas monoclonal antibody are illustrated in Fig. 3A. In the absence of antibody, apoptosis levels were low in all cell populations including preactivated normal cells. In the presence of anti-Fas, both Jurkat and preactivated T cells underwent significantly more apoptosis than unstimulated cells ($P = 0.03$), demonstrating the increased sensitivity of these cell populations to Fas-mediated death.

We next determined whether renal tumor cells expressing FasL could induce apoptosis in T cells and whether the activated population was the most sensitive. Unstimulated, stimulated, and Jurkat T cells expressing Fas were cultured with FasL⁺ renal tumor cultures. As a control, T cells were also cultured with human foreskin fibroblasts or human intestinal smooth muscle cells expressing no FasL. After 24–72 h of culture, T cells were removed from the adherent tumor cells and analyzed for DNA breaks by TUNEL assay. As seen in Fig. 3B, no significant apoptosis was observed in resting T cells exposed to FasL⁺ tumor cells or FasL⁻ control cell lines. In addition, stimulated T cells, which have up-regulated Fas expression, were not killed by FasL⁻ control cultures (Fig. 3B). However, activated T cells and Jurkat cells displayed increased apoptosis when cultured in the presence of FasL⁺ RCC tumors. (Fig. 3, B and C). Blocking experiments with neutralizing anti-FasL antibody demonstrated that the tumor-induced apoptosis of activated T cells was FasL dependent. The inclusion of anti-FasL antibody in the T cell/tumor cultures caused a marked (>45%) reduction in both the percentage of trypan blue-positive T cells and the percentage of T cells with DNA breaks (TUNEL assay; Fig. 4). Isotype-matched control IgG had no effect on reducing apoptosis in T cells (data not shown).

Increased Fas Expression over FasL on T Cells Derived from RCC Patients. Fas expression (APO-1/CD95) was examined on T cells derived from the peripheral blood ($n = 21$) and tumor of RCC patients ($n = 7$) by two-color immunocy-

Table 1 Fas/FasL expression in T-PBLs and T-TILs in patients with RCC

Numbers represent the percentage of CD3⁺, CD4⁺, and CD8⁺ cells expressing Fas (CD95) or Fas-L (CD95-L). Paired PBL and TIL samples were available in four patients. Normal, patients without RCC.

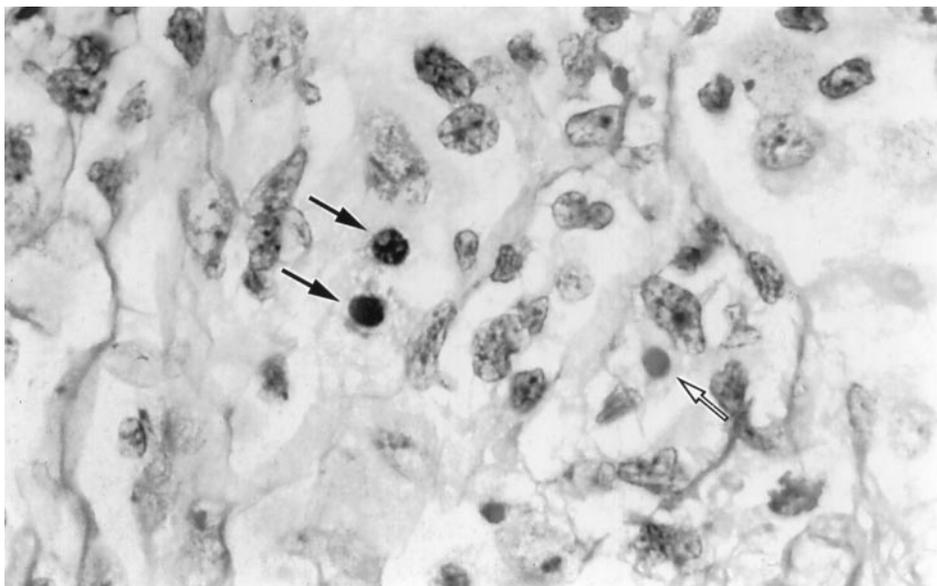
| A. | CD3 ⁺ /CD95 ⁺ | | CD3 ⁺ /CD95L ⁺ | |
|--------------------------|-------------------------------------|-----------|--------------------------------------|----------|
| | Median | Range | Median | Range |
| T-PBL ($n = 21$) | 53.4 | 0.8–78.0 | 11.1 | 0.3–88.1 |
| T-TIL ($n = 7$) | 44.7 | 35.3–85.2 | 15.7 | 2.0–35.6 |
| Normal T-PBL ($n = 7$) | 44.3 | 11.2–60 | 0.8 | 0.4–23.4 |
| B. | CD4 ⁺ /CD95 ⁺ | | CD4 ⁺ /CD95L ⁺ | |
| | Median | Range | Median | Range |
| T-PBL ($n = 4$) | 36.9 | 1.0–51.8 | 2.2 | 0.2–59.4 |
| Normal T-PBL ($n = 6$) | 39.2 | 24.0–51.2 | 1.3 | 0.6–14.1 |
| C. | CD8 ⁺ /CD95 ⁺ | | CD8 ⁺ /CD95L ⁺ | |
| | Median | Range | Median | Range |
| T-PBL ($n = 4$) | 18.8 | 0.1–37.8 | 2.2 | 0.1–30.5 |
| Normal T-PBL ($n = 6$) | 14.1 | 4.0–14.4 | 0.3 | 0.1–10.6 |

tomety. The level of Fas surface expression on patient T cells was compared to that expressed on T cells from healthy volunteers ($n = 7$). There was no statistically significant difference between the percentage of T cells expressing Fas from the peripheral blood (median, 53.4%) or tumor (median, 44.7%) of RCC patients and normal control T cells (median, 44.3%; $P > 0.05$; Table 1). After 24–72 h of stimulation with PMA/ionomycin, Fas expression increased progressively over unstimulated populations (data not shown). Immunocytometry analysis of peripheral blood T cells from both patients and controls demonstrated that a greater percentage of CD4⁺ cells than CD8⁺ cells expressed Fas ($P < 0.001$).

The percentage of T cells expressing FasL was also determined by immunocytometry (Table 1). Very little expression of the ligand was observed on resting T cells from normal individuals (median, 0.8% positive cells), as noted previously by others (32–34). Only a minority of T cells from the blood and tumor of RCC patients were FasL⁺ [median, 11.1% (T-PBLs) and 15.7% (T-TILs)]. These findings also show that a higher percentage of resting CD4⁺ and CD8⁺ T cells from RCC patients and normal individuals express Fas than FasL.

In Situ Detection of Apoptosis in T Cells Infiltrating RCC. Our findings show that FasL⁺ RCCs can induce apoptosis of activated T cells. Furthermore, a subset of T cells from the tumor and blood of RCC patients expresses Fas. These results raise the possibility that TILs may undergo apoptosis in the tumor environment after exposure to FasL⁺ tumor cells, as has been recently demonstrated in esophageal and other solid tumor systems (22). We therefore examined tumor-infiltrating T cells *in situ* for evidence of apoptosis. RCC tumors contain a significant accumulation of CD4⁺ and CD8⁺ T cells (35). The *in situ* TUNEL assay was performed on renal tumors from 10 patients, and representative data are presented in Fig. 5. T-TIL apoptosis was heterogeneously distributed within the tumor bed. In all cases, the patchy pattern of TUNEL-positive T cells was

Fig. 5 Detection of apoptosis in TILs. *In situ* TUNEL assays were performed on tissue sections from 10 different RCCs. Approximately 15–20% of infiltrating lymphocytes were found to undergo apoptosis (closed black arrows). They were confirmed to be T-TILs by immunostaining with anti-CD3 antibody. Nonapoptotic lymphocytes were also identified (open white arrows). The distribution of apoptotic T cells was heterogeneous and was observed throughout the areas of viable tumor.



observed throughout areas of viable tumor, including areas that appeared to be well vascularized. Approximately 15–20% of the total lymphoid population within the tumor bed was found to be apoptotic. Double staining with the anti-CD3 antibody verified that the apoptotic cells were CD3⁺ T lymphocytes.

Peripheral Blood-derived T Cells from RCC Patients Are Susceptible to Activation-induced Apoptosis. Given that a subset of T cells in the tumor bed was apoptotic, we wished to know whether apoptotic T cells were present in the peripheral blood. TUNEL assays did not detect any significant level of DNA breaks in freshly isolated uncultured patient T cells (median, 4.4%; range, 0.9–11.7%; $n = 18$), when compared to T cells from healthy volunteers (median, 2.3%; range, 1.0–3.5%; $n = 5$; $P > 0.05$; Fig. 6). We also examined whether patient T cells would undergo apoptosis after culturing in medium for 24 h. After 24 h, the percentage of patient T cells expressing DNA breaks (median, 7.3%; range, 1.4–21.4%; $n = 12$) was slightly higher than the results observed in normal T cells (median, 4.5%; range, 4.2–7.8%; $n = 7$); however, this difference was not significant ($P > 0.05$; Fig. 6). Because peripheral blood T cells from RCC patients did not display DNA breaks, we determined whether they expressed an earlier marker of apoptosis, the externalization of phosphatidyl-serine from the inner leaflet of the membrane bilayer (36). Freshly isolated purified T cells from patients with RCC and normal controls were stained with annexin V, which has an affinity for phosphatidyl-serine. Our findings ($n = 6$) indicate that the level of annexin V staining was clearly higher in patient (median, 27.31%; range, 18.9–37.3%) than in normal T cells (median, 6.8%; range, 1.8–9.7%; $P < 0.05$; data not shown). These results suggest that although DNA breaks are not evident in peripheral T cells, an apoptotic program has been initiated. More importantly, we have found that DNA breaks can be induced in patient T cells after activation. As shown in Fig. 6B, DNA breaks were observed in peripheral blood T cells of patients stimulated with PMA/ionomycin (median, 20.6%;

range, 10.5–85.0%; $n = 18$). In contrast, no increase in apoptosis was observed in normal donor T cells after a similar stimulation (median, 5.0%; range, 2.6–6.8%; $n = 8$). In T-PBLs from 34% (6 of 18) of the patients examined, stimulation resulted in >40% apoptosis of T cells (range, 42.8–85%). The induction of DNA breaks was not unique to stimulation with PMA/ionomycin because comparable results were observed when T cells were costimulated with antibodies to CD3/CD28 or incubated with TNF- α (Fig. 7). Thus, in a subset of patients, peripheral T cells appear to be susceptible to apoptosis upon activation.

It is generally accepted that resting T cells are largely resistant to AICD; however, upon activation, they become increasingly sensitive to anti-CD3- or PHA-triggered programmed cell death. Here we determined whether AICD in patient T cells coincided with an increased preactivation status of these cells. The level of AICD was compared to the degree of HLA-DR expression on CD3⁺ in the blood of RCC patients, whereas HLA-DR is a marker of activated T cells. However, we did not observe any correlation between the HLA-DR expression levels and AICD (data not shown). Furthermore, we did not detect any correlation between the level of Fas expression on patient T cells and the ability of the cells to undergo AICD (data not shown).

DISCUSSION

Cancer patients, including those with RCC, are known to have impaired antitumor immune responses. Alterations in proliferation, effector function, and intracellular signaling have been demonstrated in T-PBLs, although a more pronounced dysfunction is present in lymphoid cells infiltrating the tumor bed (6, 37, 38). These findings and others suggest that the tumors have developed strategies to evade the immune system (1, 6, 39, 40). Increasing evidence suggests that the induction of apoptosis in T cells may contribute to suppressed antitumor

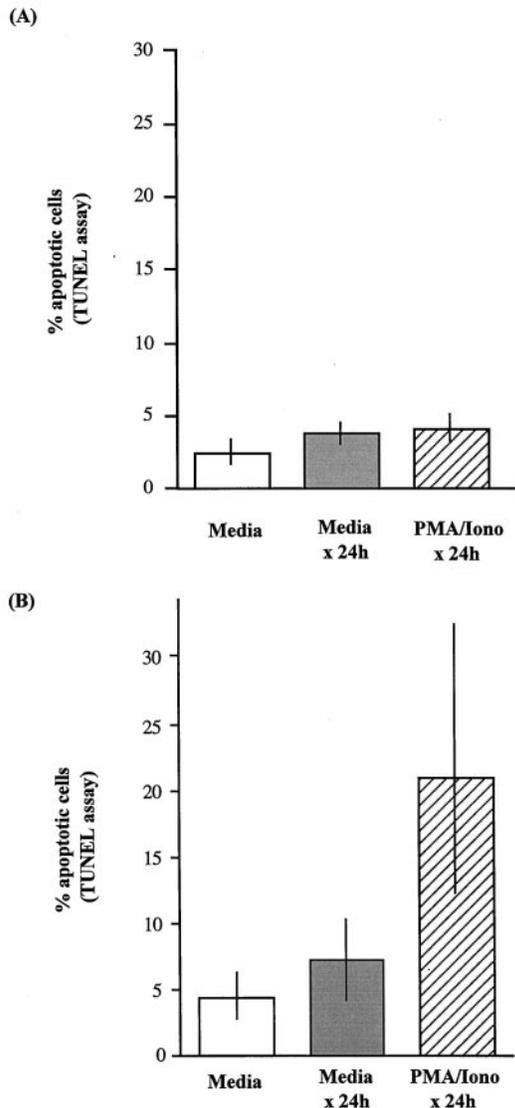


Fig. 6 AICD was detected in peripheral blood T cells from RCC patients. T cells from either normal donors ($n = 8$; A) or RCC patients ($n = 18$; B) were tested for DNA breaks upon isolation (\square). In addition, the same set of T cells was tested for DNA breaks after incubation *in vitro* with medium alone (\blacksquare) or medium supplemented with PMA (10 ng/ml) and ionomycin (0.75 μ g/ml) for 24 h (▨). The results are presented as the median percentage of apoptotic T cells. Neither normal nor patient T cells displayed any significant level of apoptosis (<5%) when freshly isolated. Patient T cells (median, 7.3%; range, 1.4–21.4%) cultured in medium alone for 24 h showed a slightly higher level of apoptosis than did T cells from normal donors (median, 4.5%; range, 4.2–7.8%); however this was not statistically significant. In contrast, patient T cells (median, 20.6%; range, 10.5–85.0%) cultured with PMA/ionomycin demonstrated significantly more apoptosis than did normal T cells (median, 5.0%; range, 2.6–6.8%; $P < 0.05$). In T-PBLs from one-third (6 of 18) of the RCC patients examined, stimulation resulted in >40% apoptotic T cells.

immunity in cancer patients (8–10). Recent data suggest that apoptosis is mediated in part through the Fas/FasL pathway. FasL expression in several types of human tumor cells has been reported to initiate the apoptotic pathway in Fas⁺ T lympho-

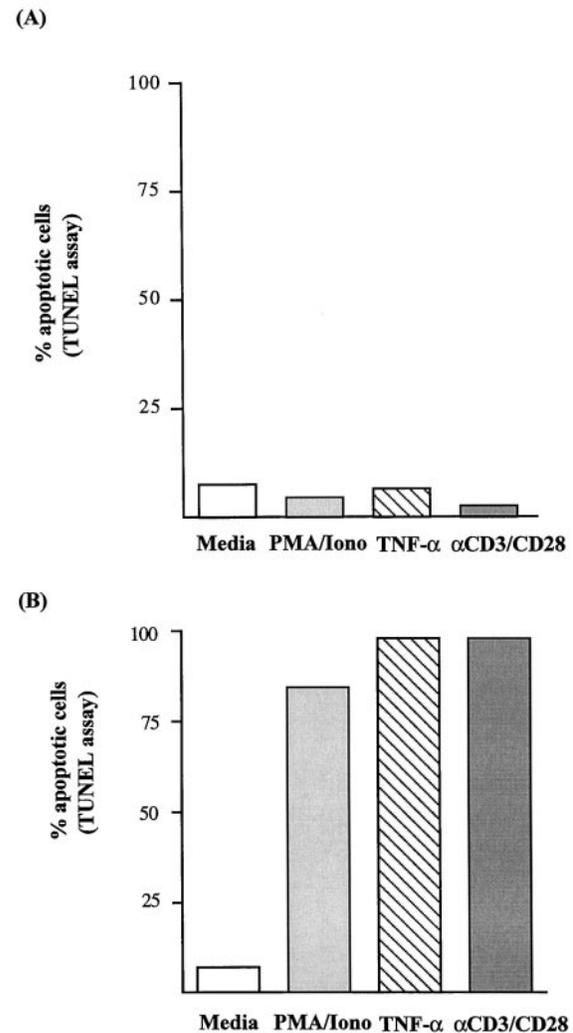


Fig. 7 AICD is induced by multiple stimuli in RCC patient T cells. T cells from a normal donor or a RCC patient were stimulated with one of the following for 24–48 h before performing the TUNEL assay: (a) media [PMA (10 ng/ml)/ionomycin (0.75 μ g/ml)]; (b) cross-linked anti-CD3 (10 μ g/ml; Ortho, Raritan, NJ)/anti-CD28 (10 μ g/ml; BioSource, Camarillo, CA); or (c) TNF- α (10 ng/ml; R&D Systems, Minneapolis, MN).

cytes (19–25). Here we identify a similar mechanism in T cells from patients with RCC. In addition, we also demonstrate that T cells from RCC patients are more susceptible to cell death upon activation, thereby eliminating them as effective immune mediators. In this second mechanism, apoptosis occurs as a consequence of T-cell activation with PMA/ionomycin via the T-cell receptor or after stimulation with TNF- α .

Initial evidence that tumors express FasL came from studies of malignancies of cells that normally express FasL upon activation, such as NK lymphomas and T cell-type large granular lymphocyte leukemias (41). These observations have now been extended to melanomas (19) and solid tumors from several organs including the lung (42), liver (20), esophagus (21, 22), colon (8, 20), and ovary (23). We provide the first evidence that

FasL is expressed on adenocarcinomas of the kidney. Because of the recent controversy over the specificity of certain commercially available anti-FasL antibodies (43), we have documented FasL in RCC using multiple techniques. Freshly isolated RCC tumor as well as all short-term and established RCC cell lines tested expressed FasL mRNA by RT-PCR, and all but one short-term cell line demonstrated FasL protein by Western blotting. Additionally, immunostaining of both cell line cytopins and tumor tissue detected FasL, as did immunocytometry in some cell lines. It was noted that expression of FasL by tumor cells *in situ* was patchy, with some cells expressing high levels, whereas others appeared negative for FasL. The presence of apoptotic T cells in the tumor bed was also patchy, a finding similar to that previously reported in esophageal carcinomas (22). In that study, the heterogeneous expression of FasL on the tumor correlated with increased levels of DNA breaks observed in T cells infiltrating regions of the FasL-expressing tumor. Whether apoptosis correlates to heterogeneous FasL expression in the renal tumor bed is unclear.

Several lines of evidence from *in vitro* coculture experiments suggest that FasL expressed by RCC is functional. First, FasL⁺ RCC induced apoptosis in Jurkat cells that are sensitive to the Fas death pathway. These results are in agreement with other studies in which Jurkat cells became apoptotic after exposure to FasL⁺ colon, ovarian, and brain tumors (8, 9, 23). Tumor-induced apoptosis of Jurkat cells was Fas dependent because Jurkat cells rendered Fas⁻ with Fas-specific antisense oligonucleotides are not lysed by FasL⁺ tumors (8). Second, apoptosis of peripheral blood T cells was FasL⁺ RCC is dependent on the activation status of the lymphocyte population. It is likely that the higher expression level of Fas on activated *versus* resting T cells accounts for the increased sensitivity of the activated T cells to RCC-induced apoptosis. Third and most important was the observation that the apoptosis of Jurkat cells and activated T cells induced by FasL⁺ RCC could be inhibited by the addition of anti-FasL antibody to the cultures. This work shows that RCC can induce apoptosis in activated T cells by virtue of augmented expression of FasL as one possible mechanism of immune escape.

Although it is clear that FasL expression contributes to the immune dysfunction of patient T cells, it is likely that in RCC patients, tumor-induced apoptosis of T cells is not exclusively mediated by FasL. This is supported by the observation that the ability of some RCC cell lines to induce apoptosis in T cells was not blocked by anti-FasL antibody. It is unlikely that the apoptosis induced by these RCC cell lines is due to IL-10 or transforming growth factor β because high molecular weight soluble products from these lines were not active. Moreover, cultured RCCs do not express mRNA for IL-10 (44, 45). It is possible that other RCC-derived products such as gangliosides may contribute to the apoptosis of T cells.⁴

Based on evidence that some tumors have adopted a Fas/

FasL mechanism as a means of affecting immunosuppression, a recent study in RCCs examined Fas receptor expression in TILs and PBLs (46). Cardi *et al.* reported Fas expression on peripheral blood T cells, although at reduced levels relative to expression in T-TILs and peripheral blood T cells from healthy controls. We observed Fas expression on T cells from the blood and tumor of RCC patients that did not differ significantly from the level of Fas on normal T cells. Consistent with a recent report (46), Fas expression in RCC patient T cells was nearly 2-fold higher on CD4⁺ subsets than on their CD8⁺ counterparts. The reason for this observation is unclear.

We also identified a second mechanism by which T-cell deletion may occur in RCC patients. This involves the induction of apoptosis upon T-cell activation (AICD). We demonstrated that peripheral blood T cells from RCC patients were susceptible to activation-induced apoptosis as defined by the presence of DNA breaks. AICD was induced by several different stimuli in patient T cells, but not in those from normal healthy volunteers. Our findings are similar to those recently reported in patients with multiple myeloma where PBLs underwent apoptosis after stimulation with pokeweed mitogen or PHA (47). The mechanism responsible for AICD in RCC patient T cells is not known. It may be that patient T cells are in a chronic but impaired state of preactivation due to long-term exposure to tumor antigen and that subsequent stimulation causes AICD. Activation by recognition of specific antigenic peptides can trigger self-destruction of CD8⁺ or CD4⁺ T cells by AICD (48, 49), a process that contributes to the termination of an ongoing cellular immune response *in vivo*. AICD initiated in this manner may be mediated via induction of FasL expression and subsequent interaction with Fas (32, 33, 34). However, it is clear that expression of Fas alone was not sufficient to induce apoptosis in patient T cells. Although 50% of peripheral blood T cells were Fas⁺, there were no detectable DNA breaks when cells were freshly isolated or after resting *in vitro* for 24 h. We could not link AICD to the expression of the activation marker HLA-DR or to the level of Fas expression. However, it remains possible that AICD susceptibility will be linked to the activation status of the patient T cells through the analysis of other activation markers. We did observe that whereas patient T cells displayed no DNA breaks when initially isolated, they did express one early marker of apoptosis, the externalization of phosphatidyl-serine. Thus, it is possible that patient T cells are in a preapoptotic state. It may be that activation shifts the balance to apoptosis with the induction of DNA breaks. This may occur via activation through the T cell receptor after exposure to antigen, antigen-presenting cells, or tumor or after exposure to TNF- α . Alternatively, patient T cells may be more susceptible to apoptosis because they have impaired activation of the transcription factor NF κ B. We have reported that T cells from approximately 60% of RCC patients are impaired in normal κ B binding activity (37, 50). There is growing evidence that defective activation of NF κ B makes cells more susceptible to apoptosis due to the reduced expression of gene products that protect cells from death (51, 52). Additional studies with patient T cells are under way to further define the contribution that defective NF κ B induction makes to the cell sensitivity to AICD.

⁴ R. G. Uzzo. Suppression of NF κ B activation in T cells by soluble products from renal cell carcinoma is mediated by tumor-derived gangliosides, submitted for publication.

REFERENCES

1. Finke, J. H., Rayman, P., Hart, L., Alexander, J. P., Edinger, M. G., Tubbs, R. R., Klein, E., Tuason, L., and Bukowski, R. M. Characterization of tumor-infiltrating lymphocyte subsets from human renal cell carcinoma: specific reactivity defined by cytotoxicity, interferon- γ secretion, and proliferation. *J. Immunother.*, *15*: 91–104, 1994.
2. Roszman, T., Elliott, L., and Brooks, V. V. Modulation of T-cell function by gliomas. *Immunol. Today*, *12*: 370–374, 1991.
3. Miescher, S., Stoeck, M., Qiao, L., Barras, C., Barrelet, L., and von Flidner, V. Preferential clonogenic deficit of CD8-positive T-lymphocytes infiltrating human solid tumors. *Cancer Res.*, *48*: 6992–6998, 1988.
4. Loeffler, C. M., Smyth, M. J., Longo, D. L., Kopp, W. C., Harvey, L. K., Tribble, H. R., Tase, J. E., Urba, W. J., Leonard, A. S., Young, H. R., and Ochoa, A. C. Immunoregulation in cancer-bearing hosts. Down-regulation of gene expression and cytotoxic function in CD8⁺ T cells. *J. Immunol.*, *149*: 949–956, 1992.
5. Yoshino, I., Yano, T., Murata, M., Ishida, T., Sugimachi, K., Kimura, G., and Nomoto, K. Tumor-reactive T-cells accumulate in lung cancer tissues but fail to respond due to tumor cell-derived factor. *Cancer Res.*, *52*: 775–781, 1992.
6. Alexander, J. P., Kudoh, S., Melsop, K. A., Hamilton, T. A., Edinger, M. G., Tubbs, R. R., Sica, D., Tuason, L., Klein, E., Bukowski, R. M., and Finke, J. T cells infiltrating renal cell carcinoma display a poor proliferative response even though they can produce interleukin 2 and express interleukin 2 receptors. *Cancer Res.*, *53*: 1380–1387, 1993.
7. Miescher, S., Stoeck, M., Qiao, L., Barras, C., Barrelet, L., and von Flidner, V. Proliferative and cytolytic potentials of purified human tumor-infiltrating T lymphocytes. Impaired response to mitogen-driven stimulation despite T-cell receptor expression. *Int. J. Cancer*, *42*: 659–666, 1988.
8. O'Connell, J., O'Sullivan, G. C., Collins, J. K., and Shanahan, F. The Fas counterattack: Fas-mediated T cell killing by colon cancer cells expressing Fas ligand. *J. Exp. Med.*, *184*: 1075–1082, 1996.
9. Saas, P., Walker, P. R., Hahne, M., Quiquerez, A. L., Schnuriger, V., Perrin, G., French, L., Van Meir, E. G., de Tribolet, N., Tschopp, J., and Dietrich, P. Y. Fas ligand expression by astrocytoma *in vivo*: maintaining immune privilege in the brain? *J. Clin. Investig.*, *99*: 1173–1178, 1997.
10. Walker, P. R., Saas, P., and Dietrich, P. Y. Role of Fas ligand (CD95L) in immune escape – the tumor strikes back. *J. Immunol.*, *158*: 4521–4524, 1997.
11. Lowin, B., Hahne, M., Mattmann, C., and Tschopp, J. Cytolytic T-cell cytotoxicity is mediated through perforin and Fas lytic pathways. *Nature (Lond.)*, *370*: 650–652, 1994.
12. Kagi, D., Vignaux, F., Ledermann, B., Burki, K., Depraetere, V., Nagata, S., Hengartner, H., and Goldstein, P. Fas and perforin pathways as major mechanisms of T cell-mediated cytotoxicity. *Science (Washington DC)*, *265*: 528–530, 1994.
13. Alderson, M. R., Tough, T. W., Davis-Smith, T., Braddy, S., Falk, B., Schooley, K. A., Goodwin, R. G., Smith, C. A., Ramsdell, F., and Lynch, D. H. Fas ligand mediates activation-induced cell death in human T lymphocytes. *J. Exp. Med.*, *181*: 71–77, 1995.
14. Daniel, P. T., and Krammer, P. H. Activation induces sensitivity toward APO-1 (CD95)-mediated apoptosis in human B cells. *J. Immunol.*, *152*: 5624–5628, 1994.
15. Castro, J. E., Listman, J. A., Jacobson, B. A., Wang, Y., Lopez, P. A., Ju, S., Finn, P. W., and Perkins, D. L. Fas modulation of apoptosis during negative selection of thymocytes. *Immunity*, *5*: 617–627, 1996.
16. Griffith, T. S., Brunner, T., Fletcher, S. M., Green, D. R., and Ferguson, T. A. Fas ligand-induced apoptosis as a mechanism of immune privilege. *Science (Washington DC)*, *270*: 1189–1191, 1995.
17. French, L. E., Hahne, M., Viard, I., Radlgruber, G., Zanone, R., Becker, K., Muller, C., and Tschopp, J. Fas and Fas ligand in embryos and adult mice: ligand expression in several immune-privileged tissues and coexpression in adult tissues characterized by apoptotic cell turnover. *J. Cell Biol.*, *133*: 335–343, 1996.
18. Nagata, S. Apoptosis by death factor. *Cell*, *88*: 355–365, 1997.
19. Hahne, M., Rimoldi, D., Schroter, M., Romero, P., Schreier, M., French, L. E., Schneider, P., Bornand, T., Fontana, A., Lienard, D., Cerottini, J., and Tschopp, J. Melanoma cell expression of Fas(Apo-1/CD95) ligand: implications for tumor immune escape. *Science (Washington DC)*, *274*: 1363–1366, 1996.
20. Shiraki, K., Tsuji, N., Shioda, T., Isselbacher, K. J., and Takahashi, H. Expression of Fas ligand in liver metastases of human colonic adenocarcinoma. *Proc. Natl. Acad. Sci. USA*, *94*: 6420–6425, 1997.
21. Gratas, C., Tohma, Y., Barnas, C., Taniere, P., Hainaut, P., and Ohgaki, H. Up-regulation of Fas (APO-1/CD95) ligand and down-regulation of fas expression in human esophageal cancer. *Cancer Res.*, *58*: 2057–2062, 1998.
22. Bennett, M. W., O'Connell, J., O'Sullivan, G. C., Brady, C., Roche, D., Collins, J. K., Shanahan, F. The Fas counterattack *in vivo*: apoptotic depletion of tumor-infiltrating lymphocytes associated with fas ligand expression in human esophageal carcinoma. *J. Immunol.*, *160*: 5669–5675, 1998.
23. Rabinowich, H., Reichert, T. E., Kashii, Y., Gastman, B. R., Bell, M. C., and Whiteside, T. L. Lymphocyte apoptosis induced by fas ligand-expressing ovarian carcinoma cells. *J. Clin. Investig.*, *101*: 2579–2588, 1998.
24. Strand, S., Hofmann, W. J., Hug, H., Muller, M., Otto, G., Strand, D., Mariani, S. M., Stremmel, W., Krammer, P. H., and Galle, P. R. Lymphocyte apoptosis induced by CD95 (APO-1/Fas) ligand-expressing tumor cells – a mechanism of immune evasion? *Nat. Med.*, *2*: 1361–1366, 1996.
25. Zeytun, A., Hassuneh, M., Nagarkatti, M., and Nagarkatti, P. S. Fas-Fas ligand-based interactions between tumor cells and tumor-specific cytotoxic T lymphocytes: a lethal two-way street. *Blood*, *90*: 1952–1959, 1997.
26. Lanier, L. L., Allison, P. J., and Phillips, J. H. Correlation of cell surface antigen expression on human thymocytes by multicolor flow cytometric analysis: implications for differentiation. *J. Immunol.*, *137*: 1209–1214, 1986.
27. Genestier, L., Fournel, S., Flacher, M., Assossou, O., Revillard, J. P., and Bonnefoy-Berard, N. Induction of Fas (Apo-1, CD95)-mediated apoptosis of activated lymphocytes by polyclonal antithymocyte globulins. *Blood*, *91*: 2360–2368, 1998.
28. Enari, M., Hug, H., and Nagata, S. Involvement of ICE-like protease in Fas-mediated apoptosis. *Nature (Lond.)*, *375*: 78–81, 1995.
29. Scaffidi, C., Fulda, S., Srinivasan, A., Friesen, C., Feng, L., Tomaselli, K. J., Debatin, K. M., Krammer, P. H., and Peter, M. E. Two CD95 (APO-1/Fas) signaling pathways. *EMBO J.*, *17*: 1675–1687, 1998.
30. Darzynkiewicz, Z., Bruno, S., Del Bino, G., Gorczyca, W., Hotz, M., Lassota, P., and Traganos, F. Features of apoptotic cells measured by flow cytometry. *Cytometry*, *13*: 795–808, 1992.
31. Gavrieli, Y., Sherman, Y., and Ben-Sasson, S. A. Identification of programmed cell death *in situ* via specific labeling of nuclear DNA fragmentation. *J. Cell Biol.*, *119*: 493–501, 1992.
32. Dhein, J., Walczak, H., Baumler, C., Debatin, K. M., and Krammer, P. H. Autocrine T cell suicide mediated by Apo-1/(Fas/CD95). *Nature (Lond.)*, *373*: 438–441, 1995.
33. Brunner, T., Mogil, R. J., LaFace, D., Yoo, N. J., Mahboubi, F., Echeverri, F., Martin, S. J., Force, W. R., Lynch, D. H., Ware, C. F., and Green, D. R. Cell-autonomous Fas (CD95)/Fas ligand interaction mediates activation-induced apoptosis in T cell hybridomas. *Nature (Lond.)*, *373*: 441–444, 1995.
34. Ju, S. T., Panka, D. J., Cui, R., Ettinger, R., El-Khatib, M., Sherr, D. H., Stanger, B. Z., and Marshak-Rothstein, A. Fas (CD95)/Fas-L interactions required for programmed cell death after T cell activation. *Nature (Lond.)*, *373*: 444–448, 1995.
35. Finke, J. H., Tubbs, R., Connelly, B., Pontes, E., and Montie, J. Tumor infiltrating lymphocytes in patients with renal cell carcinoma. *Ann. N. Y. Acad. Sci.*, *532*: 387–394, 1988.
36. Zhuang, J., Ren, Y., Snowden, R. T., Zhu, H., Gogvadze, V., Savill, J. S., and Cohen, G. Dissociation of phagocyte recognition of cells

undergoing apoptosis from other features of the apoptotic program. *J. Biol. Chem.*, 273: 15628–15632, 1998.

37. Li, X., Liu, J., Park, J. K., Hamilton, T. A., Rayman, P., Klein, E., Edinger, M., Tubbs, R., Bukowski, R., and Finke, J. T cells from renal carcinoma patients exhibit an abnormal pattern of κ B-specific DNA binding activity: a preliminary report. *Cancer Res.*, 54: 5424–5429, 1994.
38. Kudoh, S., Redovan, C., Rayman, P., Edinger, M., Tubbs, R. R., Novick, A., Finke, J. H., and Bukowski, R. M. Defective granzyme B gene expression and lytic response in T lymphocytes infiltrating human renal cell carcinoma. *J. Immunol.*, 20: 479–487, 1997.
39. Finke, J. H., Zea, A. H., Stanley, J., Longo, D. L., Mizoguchi, H., Tubbs, R. R., Wiltout, R. H., O'Shea, J. J., Kudoh, S., Klein, E., Bukowski, R., and Ochoa, A. C. Loss of T-cell receptor ζ chain and p56^{lck} in T-cells infiltrating human renal cell carcinoma. *Cancer Res.*, 53: 5613–5616, 1993.
40. Kolenko, V., Wang, Q., Riedy, M. C., O'Shea, J., Ritz, J., Cathcart, M. K., Rayman, P., Tubbs, R., Edinger, M., Novick, A., Bukowski, R., and Finke, J. Tumor-induced suppression of T lymphocytes coincides with inhibition of Jak3 expression and IL2 receptor signaling. *J. Immunol.*, 159: 3057–3067, 1997.
41. Tanaka, M., Suda, T., Haze, N., Nakamura, K., Sato, F., Kimura, K., Motoyoshi, M., Mizuki, S., Tagawa, S., Ohga, S., Hatake, K., Drummond, A. H., and Nagata, S. Fas ligand in human serum. *Nat. Med.*, 2: 317–322, 1996.
42. Niehans, G. A., Brunner, T., Frizelle, S. P., Liston, J. C., Salerno, C. T., Knapp, D. J., Green, D. R., and Kratzke, R. A. Human lung carcinoma express Fas ligand. *Cancer Res.*, 57: 1007–1012, 1997.
43. Smith, D., Sieg, S., and Kaplan, D. Technical note: aberrant detection of cell surface Fas ligand with anti-peptide antibodies. *J. Immunol.*, 160: 4159–4160, 1998.
44. Wang, Q., Redovan, C., Tubbs, R., Olencki, T., Klein, E., Kudoh, S., Finke, J., and Bukowski, R. Selective cytokine gene expression in renal cell carcinoma tumor cells and tumor infiltrating lymphocytes. *Int. J. Cancer*, 61: 780–785, 1995.
45. Pisa, P., Halapi, E., Pisa, E. K., Gerdin, E., Hising, C., Bucht, A., Gerdin, B., and Kiessling, R. Selective expression of interleukin 10, interferon- γ , and granulocyte-macrophage colony stimulating factor in ovarian biopsies. *Proc. Natl. Acad. Sci. USA*, 89: 7708–7712, 1992.
46. Cardi, G., Heaney, J. A., Schned, A. R., and Ernstoff, M. S. Expression of Fas (APO-1/CD95) in tumor-infiltrating and peripheral blood lymphocytes in patients with renal cell carcinoma. *Cancer Res.*, 58: 2078–2080, 1998.
47. Frassanito, M. A., Silvestris, F., Cafforio, P., and Dammacco, F. CD8⁺/CD57⁺ cells and apoptosis suppress T cell functions in multiple myeloma. *Br. J. Haematol.*, 100: 469–477, 1998.
48. Moss, D. J., Burrows, S. R., Baxter, G. D., and Lavin, M. F. T cell-T cell killing is induced by specific epitopes: evidence for an apoptotic mechanism. *J. Exp. Med.*, 173: 681–686, 1991.
49. Kabelitz, D., Oberg, H. H., Pohl, T., and Pechhold, K. Antigen induced death of mature T lymphocytes: analysis by flow cytometry. *Immunol. Rev.*, 142: 157–174, 1994.
50. Uzzo, R. G., Clark, P. E., Rayman, P., Bloom, T., Rybicki, L., Novick, A. C., Bukowski, R. M., and Finke, J. H. Alterations in NF κ B activation in T lymphocytes of patients with RCC. *J. Natl. Cancer Inst.*, in press, 1999.
51. Wang, C., Mayo, M. W., and Baldwin, A. S. TNF and cancer therapy induced apoptosis: potentiation by inhibition of NF- κ B. *Science (Washington DC)*, 274: 784–787, 1996.
52. Beg, A. A., and Baltimore, D. An essential role for NF κ B in preventing TNF- α induced cell death. *Science (Washington DC)*, 274: 782–784, 1996.

Clinical Cancer Research

Mechanisms of Apoptosis in T Cells from Patients with Renal Cell Carcinoma

Robert G. Uzzo, Patricia Rayman, Vladimir Kolenko, et al.

Clin Cancer Res 1999;5:1219-1229.

Updated version Access the most recent version of this article at:
<http://clincancerres.aacrjournals.org/content/5/5/1219>

Cited articles This article cites 50 articles, 30 of which you can access for free at:
<http://clincancerres.aacrjournals.org/content/5/5/1219.full#ref-list-1>

Citing articles This article has been cited by 28 HighWire-hosted articles. Access the articles at:
<http://clincancerres.aacrjournals.org/content/5/5/1219.full#related-urls>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
<http://clincancerres.aacrjournals.org/content/5/5/1219>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.