Host immune response in renal cell cancer: Interleukin-4 (IL-4) and IL-10 mRNA are frequently detected in freshly collected tumor-infiltrating lymphocytes

Abstract Human renal cell cancer (RCC) is clearly responsive to immunotherapy. Clinical responses may be mediated by “non-specific” (e.g. natural killer, NK, cells) or “specific” MHC-class-I-restricted tumor-specific CD8+ T lymphocytes. Typically RCC progresses, however, despite significant infiltration of various lymphoid cells. We examined freshly isolated RCC tumor-infiltrating lymphocytes (TIL) derived from seven RCC patients for cytokine expression by the polymerase chain reaction (PCR). Established RCC tumor cell lines derived from these RCC patients were negative for interleukin-2 (IL-2), IL-4, IL-10, and interferon γ and found to be positive for tumor necrosis factor α (TNFα), IL-6, IL-1β, granulocyte/macrophage-colony-stimulating factor (GM-CSF), and transforming growth factor β1 (TGFβ1) message as detected by PCR. An identical pattern of cytokine mRNA expression was identified in other long-term RCC lines and in normal human kidney cells upon culture, but not in two Wilms tumor cell lines tested. Short-term-, and long-term-established RCC lines, but not Wilms tumor lines, secreted substantial levels of GM-CSF, TNFα, IL-1β, and IL-6 as detected by enzyme-linked immunosorbent assay. Both RCC lines and Wilms tumor lines secreted TGFβ1. In comparison, normal kidney cells secreted IL-6 and GM-CSF, but not IL-1β, or TGFβ1 under identical in vitro culture conditions. We applied PCR-based methods to characterize the cytokine mRNA expression pattern in immune cells infiltrating into renal cell cancer without the need for expansion of such effector cells in vitro. Examining freshly collected RCC TIL by PCR from patients with primary cell cancer, we could demonstrate that such cells, but not lympho-mononuclear cells harvested from normal human kidney tissue, typically exhibit IL-4 and IL-10 mRNA expression.

Key words Renal cell cancer · Tumor-infiltrating lymphocytes (TIL) · Interleukin-10

Introduction

Recent results suggest that the sporadic renal cell cancer (RCC) disease gene (in the case of clear-cell-type RCC) is located on chromosome 3 (for review see [1]) and is identical to the von Hippel Lindau disease gene. Patients with RCC, like those with melanoma, are often responsive to immunotherapy with partial or complete remission in up to 35% of patients [2]. RCC-derived tumor-infiltrating lymphocytes (TIL) can frequently by expanded in vitro in the presence of interleukin-2 (IL-2) and analyzed for anti-tumor-directed reactivity [3, 4]. Cytotoxic T lymphocytes (CTL) exhibiting MHC-restricted lysis of RCC have not been frequently demonstrated among TIL by conventional culture methods. Furthermore, a significant proportion of RCC-derived TIL have been shown to exhibit impaired functions, which might in part be explained by dysfunctional signalling mediated by absent or low expression of the ζ-chain subunit of the T cell receptor (TCR), and/or other molecules involved in T cell signalling (e.g. p56lck) [5, 6]. Recently, however, several groups have identified TIL (CD8+ CTL) exhibiting RCC-specific, MHC-class-I-restricted recognition of common HLA-A2 presenting RCC T cell epitopes using different in vitro culture conditions [7-10]. It is conceivable that such an anti-RCC-directed immune response might be associated with tumor regres-
sion in vivo. In vitro expansion and cloning of TIL reflects the potential efficacy of immune effector cells, but does not reflect the ability of such cells to mediate antitumor effects, which is in part due to the balance of different cytokines present within the tumor stroma in situ and the susceptibility of tumor to recognition by immune effector cells. Such cytokines, potentially secreted by immune cells [e.g. T cells, natural killer (NK) cells, B cells, and macrophages], by tumor cells, or by other cells present in RCC (e.g. mesangial cells), define the local environment in which an anti-RCC-directed immune response has to take place. In order to characterize the cytokines present at the site of primary RCC, without the need for expansion of such cells in vitro, we analyzed mRNA by PCR from freshly isolated RCC TIL, as well as established RCC lines, for the presence of various cytokines. In freshly isolated TIL, we identified mRNA expression for IL-10, a cytokine associated with T helper 2 cells (Th2), has been shown to exert suppressive effects on T cell function [11]. The cytokine mRNA expression pattern observed for RCC lines (TNFα, TGFβ1, IL-6, and GM-CSF) was found to be similar to that of cultured normal kidney cells and may reflect a common induction pattern of renal cells in response to in vitro culture.

Materials and methods

Cellular reagents

Long-term RCC cell lines RC3 and RC4 were provided by Dr. J. Finke (Department of Immunology, Cleveland, Ohio). RC1M was provided by Dr. T. Whiteside (Pittsburgh Cancer Institute, PCI, Pittsburgh, Pa.). NT1257 was provided by Dr. A. Knuht (Krankenhaus Nordwest, Frankfurt, Germany), and the Wilms tumor cell lines G-401 and SK-NP1 were obtained from the American Tissue Type Collection, ATCC (Rockville, Md.). In addition, single-cell suspensions from surgically removed RCC (all clear-cell type) were obtained from seven patients undergoing nephrectomy at the University of Pittsburgh Medical Center. None of these patients had received chemotherapy, radiation therapy, or immunotherapy prior to sample collection. Pathological diagnoses (with immunostaining of the derived RCC lines) are compiled in Table 1. Single-cell suspensions obtained from RCC patients have been enriched for either tumor cells or lymphoid cells by differential density centrifugation at the Pittsburgh Cancer Institute. None of these patients had received chemotherapy, radiation therapy, or immunotherapy prior to sample collection. Pathological diagnoses (with immunostaining of the derived RCC lines) are compiled in Table 1. Single-cell suspensions obtained from RCC patients have been enriched for either tumor cells or lymphoid cells by differential density centrifugation at the Pittsburgh Cancer Institute.

In order to exclude contamination with mononuclear cells and to confirm the renal origin of the short- and long-term RCC cell lines examined, cultured RCC lines were tested after 10 in vitro passages for reactivity with a pooled monoclonal antibody mixture recognizing cells of epithelial origin (anti-keratin Ab AE1/AE3, IgG1) obtained from Boehringer Mannheim, Indianapolis, Ind. The mAb URO-2 (IgG2a), URO-4 (IgG1), and URO-5 (IgG2b) were purchased from Signet laboratories, Dedham, Mass. mAb URO-2 recognizes a glycoprotein present on renal glomeruli, proximal tubules, vessels, and interstitial matrix of the renal medulla [14], and mAb URO-4 binds to the adenosine-deaminase-binding protein, present on proximal tubules and a portion of Henle’s loop expressed in most human RCC lines examined [15]. The URO-5 antigen is expressed in a portion of Henle’s loop, distal tubules and collecting ducts of kidney tissue and is detected in bladder tumors, breast carcinomas and lung epidermoid carcinomas [16]. Briefly, RCC lines cultured in vitro were removed from plastic surfaces by a cell scraper in order to avoid trypsinization, which might have had some influence on staining with the mAb employed for immunohistochemistry. After washing with Hanks balanced salt medium (HBSS, Gibco, Grand Island, N.Y.) cytospins were prepared on silanized slides and stained with the appropriate mAb (AE1/AE3, URO-2, URO-4, URO-5) was performed as previously described in detail [17]. Negative controls included the appropriate IgG isotypes and each slide was evaluated by an blinded fashion and rated as showing weak (+), intermediate (++), and strong (+++) staining with the respective mAb.

Flow cytometry

Monoclonal antibody conjugates CD3-phycocerythrin (PE), CD8-PE (T cell subset, cytotoxic T cells), CD14-PE (marker for a majority of peripheral blood monocytes), CD4 (T helper cell subset), CD19-fluorescein-isothiocyanate (FITC) (binding to a 95-kDa antigen present on a majority of peripheral blood B cells), CD20-FITC (binding to a 35-kDa antigen on the majority of peripheral blood B cells), CD16-PE and CD56-FITC (NK cell markers), were purchased from Becton Dickinson. Freshly collected RCC TIL were thawed, washed once with AIM-V medium (Gibco, Grand Island, N.Y.) containing 3% human serum in order to reduce Ab binding to FcR, and washed twice with HBSS (Gibco) supplemented with 1% bovine serum albumin and 1% sodium azide (FACS medium). Cells were then incubated with optimal concentrations of PE- and FITC-conjugated Ab and incubated at 4°C for 30 min. After two washes, cells were resuspended in FACS medium and analyzed on a Becton Dickinson FACScan flow cytometer (Becton Dickinson, Mountain View, Calif.). Data were collected on 5000 viable cells, as determined by forward light scatter intensity and propidium iodide exclusion, and analyzed using Lysys II software. Results are reported as the percentage of cells staining positive with the appropriate Ab.

Template cDNA preparation

For cytokine expression analysis, total RNA from 5 x 10⁶–10⁷ cells was extracted from TIL, from cultured RCC or normal human kidney cells using RNAzol (Biotex Inc., Houston, Tex.) according to Chomczynski and Sacchi [18]. First-strand cDNA synthesis was performed by heating the following reaction mixture at 37°C for 1 h, followed by 5 min at 95°C using a Perkin-Elmer polymerase chain reaction (PCR) thermal cycler. The 40-μl reaction volume contained 8 μg RNA in 16 μl H2O, 8 μl 5× RB, 4 μl dithiothreitol (final concentration 10 mM), 2 μl dNTP (dATP, dCTP, dGTP, dTTP final concentration 1 mM each), 3 μl RNase inhibitor (final amount 120 U), 1 μl actinomycin D (final amount 2 μg), oligo-dT random primers (4 μl final amount 1 mM), and 2 μl...