Suppression of Human Prostate Carcinoma Metastases in Severe Combined Immunodeficient Mice by Interleukin 2 Immunocytokine Therapy

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ABSTRACT

Immunocytokines are antibody-cytokine fusion proteins that combine the unique targeting ability of antibodies with the multifunctional activities of cytokines to activate effector cells in the tumor microenvironment. Here, we demonstrate the therapeutic efficacy of a tumor-specific immunocytokine, huKS1/4-IL2, which effectively inhibited growth and dissemination of lung and bone marrow metastases of human prostate carcinoma in severe combined immunodeficient mice. This antitumor effect was specific and highly effective, irrespective of reconstitution of these mice with human lymphokine-activated killer cells. Survival times of mice treated with huKS1/4-IL2 were increased 4-fold as compared with animals treated with a mixture of the corresponding antibody and recombinant human interleukin-2 (rhIL2). A persistent antitumor response after treatment with the huKS1/4-IL2 immunocytokine in B, T, and natural killer cell-deficient severe combined immunodeficient-BEIGE mice, depleted of granulocytes, implies a major role for macrophages in this treatment effect. Our data demonstrate that immunocytokine-directed interleukin-2 therapy to tumor sites is an immunotherapeutic approach with potent effects against disseminated metastases of human prostate carcinoma and suggest that this treatment could be effective in an adjuvant setting for patients with minimal residual disease.

INTRODUCTION

Prostate carcinoma is the most frequent cancer in men, with an estimated 184,500 new cases being projected in the United States in 1998 with a death rate exceeding 39,000 (1). The most common therapy used for patients with advanced disease is androgen withdrawal or suppression, which is rarely curative, and most deaths from this malignancy are due to metastases that are resistant to therapy.

There has been increased interest in applying immunologic strategies to treat prostate cancer, particularly in the use of local immunotherapy with immunoregulatory cytokines, which is referred to as cytokine gene therapy. The effectiveness of such an approach for prostate carcinoma was established in studies involving the challenge of the Copenhagen rat with syngeneic Dunning rat prostate carcinoma cells modified to secrete xenotypic murine cytokines, IL-2 (2, 3), IFN-γ (3), and granulocyte macrophage colony-stimulating factor (3, 4), indicating an antiprostate cancer immune response. The role for IL-2 in the induction of this antitumor immune response was further supported by the finding that Dunning rat prostate carcinoma cells, genetically modified ex vivo to express IL-2, produced a local inflammatory response, resulting in the elimination of the injected tumor cells, even when mixed with wild-type parental cells (2). This gene transfer is consistent with the paracrine nature of IL-2 working physiologically at high concentrations within a few cell diameters from its cell of origin. A possible role for T cells was implicated when murine TRAMP-C1 prostate carcinoma cells, that had been transduced with the costimulatory molecule B7.1, were rejected by immunocompetent, syngeneic mice, but not from T cell-deficient athymic nude mice (5). Attempts to translate preclinical results from such gene therapy approaches to the clinic have led to several still ongoing Phase 1 clinical trials (6).

We developed an alternative approach for directing cytokines preferentially to the tumor microenvironment by a simple modus operandi, which complies with the paracrine nature of most cytokines. Thus, we previously established the proof of concept for this approach with several immunocytokines that combine the unique targeting ability of antibodies with the multifunctional activities of cytokines. These immunocytokines achieved sufficient local concentrations of cytokines, such as IL-2, to induce the T cell-mediated eradication of melanoma (7) and colon carcinoma metastases (8) and NK cell-mediated elimi-
ination of neuroblastoma metastases (9, 10) in syngeneic mouse tumor models.

Here, we extend these findings by demonstrating that a humanized immunocytokine, huKS1/4-IL2, directed against an epithelial cell adhesion molecule extensively expressed on biopsy specimens of metastatic human prostate carcinoma and on most epithelial cell-derived tumors (11), effectively suppresses growth and dissemination of lung and bone marrow metastases of human prostate carcinoma in SCID mice. Furthermore, we prove the specificity of this antitumor effect and demonstrate that the survival times of mice treated with the immunocytokine increased 4-fold compared with those treated with a mixture of the corresponding antibody and recombinant human IL-2 (rhIL2). The effector cells involved in this treatment effect are most likely macrophages, because the immunocytokine therapy is effective in granulocyte-depleted SCID-BEIGE mice, which lack functional T, B, and NK cells.

MATERIALS AND METHODS

Animals and Cell Lines. Male C.B.-17 scid/scid and scid/beige mice were obtained from Taconic Farms (Germantown, NY), at 6–8 weeks of age, and housed under specific-pathogen-free conditions. All animal experiments were performed according to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

The PC-3.MM2 cell line was kindly provided by Dr. I. J. Fidler (M. D. Anderson Cancer Center, Houston, TX). It was derived from a liver metastasis produced by the parental PC-3 human prostate carcinoma cell line growing in the spleen of a nude mouse. The cell line was maintained as a monolayer culture in EMEM, supplemented with 10% fetal bovine serum, 1 mM sodium pyruvate, 1 × nonessential amino acids, 2 mM L-glutamine and a 2-fold vitamin solution (BioWhittaker, Inc., Walkersville, MD). Cell cultures were maintained on plastic and incubated in 5% CO2/95% air at 37°C and were free of Mycoplasma TC (Gen-Probe, San Diego, CA).

Antibody, Cytokine, and Fusion Proteins. The generation of the murine KS1/4 mAb, recognizing KSA, a human epithelial cell adhesion molecule, was described previously (12) as was the subsequent construction of the recombinant humanized huKS1/4-IL2 fusion protein (13). Briefly, the humanized V region cDNAs were inserted into an expression vector containing the constant regions of human IgG1 heavy chain and the cytomegalovirus promoter/enhancer for the expression of both chains. The mature sequence of human IL-2 heavy chain, and the cytomegalovirus promoter/enhancer for the expression of both chains. The mature sequence of human IL-2 heavy chain, and the cytomegalovirus promoter/enhancer for the expression of both chains. The mature sequence of human IL-2 heavy chain, and the cytomegalovirus promoter/enhancer for the expression of both chains.

The concentration of IL-2 in both the huKS1/4-IL2 and ch14.18-IL2 immunocytokines was calculated as two molar equivalents of IL-2/mole of immunocytokine. The rhIL2 used in control experiments had a specific activity of 16 × 106 IU/ml (Chiron Corp., Emeryville, CA).

Saturation Binding Assay. Binding assays were performed with radiolabeled huKS1/4 antibody and huKS1/4-IL2 immunocytokine on the human prostate carcinoma cell line, PC-3.MM2. Radiolabeling with 125I was achieved, as described previously (14). Briefly, 1 mg of the respective protein was incubated for 30 min on ice with 1 mCi of 125I (100 mCi, 3.75 GBq; Amersham, Arlington Heights, IL) in polystyrene tubes coated with 100 µg of Iodo-Gen reagent (Pierce Chemical Co., Rockford, IL). Nonincorporated 125I was removed by gel filtration on PD10 columns (Pharmacia Biotech Inc., Piscataway, NJ).

For the assay, 5 × 104 PC-3.MM2 cells were added to 24-well microtiter plates and cultured for 24 h. Saturation binding was then determined by adding the respective 125I-radiolabeled proteins at various concentrations to the cells followed by incubation at 4°C for 2 h. Cells were then washed three times with ice cold PBS containing 1% BSA, and the amount of radiolabel bound was determined in a γ-scintillation counter. Nonspecific binding was determined for both proteins in the presence of a 200-fold excess of unlabeled mAb mKS1/4. The number of binding sites and the binding constants for each construct were determined by Scatchard plot analysis (15).

Experimental Lung and Bone Marrow Metastasis Model. Human prostate carcinoma cells, PC-3.MM2, were harvested with trypsin, washed three times with PBS and used only if their viability exceeded 90%, as determined by trypan blue staining. Experimental metastases were induced by injecting single-cell suspensions of 2 × 106 cells in 200 µl of PBS into the lateral tail vein. After 28 days, lungs were placed into Boin’s fixative and examined under a low magnification microscope for tumor foci on their surface. Metastatic scores were established based on the percentage of lung surface covered with metastases when they were fused and could not be counted as individual foci. For evaluation of bone marrow metastases, the bone cavities of both femurs and tibiae of each animal were flushed with 3 ml of PBS. The cell pellet was used for total RNA isolation and subsequent RT-PCR for the detection of HBA.

For reconstitution of SCID mice with human effector cells, peripheral blood mononuclear cells were isolated from the blood of healthy volunteers by density gradient centrifugation on Ficoll-Paque (Pharmacia Biotech, Inc.). LAK cells were generated by incubation in AIM V serum-free lymphocyte medium (Life Technologies, Inc.-BRL, Grand Island, NY) supplemented with 500 IU of recombinant interleukin 2 (rIL-2) for 3 days at 1 × 106 cells/ml. Animals receiving LAK cells received i.p. injections of 12 × 106 cells, 24 h after tumor cell inoculation. Treatment with the huKS1/4-IL2 immunocytokine was initiated at this time, consisting of daily intraperitoneal injections (×7) at the dose levels indicated.

RNA Isolation, Reverse Transcription, and PCR Amplification. Total cellular RNA was isolated with a silica gel membrane binding procedure, RNeasy (Qiagen, Inc., Chatsworth, CA). RNA content and purity were determined by optical density 260/280 readings. Synthesis of cDNA was accomplished with 1 µg RNA in the presence of Moloney murine leukemia virus reverse transcriptase, SUPERSCRIPT II (Life
The use of the huKSl/4-IL2 immunocytokine for suppression of pulmonary metastases was demonstrated in SCID mice. Because the prostate carcinoma model used here also expressed large amounts of the KSA target antigen (data not shown), saturation binding assays revealed similar binding patterns for huKSl/4-IL2 fusion protein and the huKSl/4 antibody on PC-3.MM2 cells. Thus, calculation of dissociation constants (Kd) and the number of binding sites from saturation binding curves and Scatchard plot analyses indicated an average Kd of 2.6 nm and 7.2 $\times$ 10$^{-3}$ binding sites/cell for both mAb huKSl/4 and the huKSl/4-IL2 fusion protein (Fig. 1).

**Effect of huKSl/4-IL2 Immunocytokine on Growth and Dissemination of Pulmonary Metastases of Human Prostate Carcinoma.** The treatment of tumor-bearing SCID mice with the immunocytokine effectively suppressed growth and dissemination of pulmonary metastases induced by i.v. injection of 2 $\times$ 10$^6$ PC-3.MM2 cells (Table 1). A dose-dependent response by the huKSl/4-IL2 immunocytokine was indicated by the absence of metastases at dose levels ranging from 32--4 $\mu$g/injection and the partial occurrence of these metastases at the 2-$\mu$g dose level. The specificity of the immunocytokine therapy was demonstrated, because a nonspecific immunocytokine of the antiganglioside GD$_2$ antibody and IL-2 (ch14.18-IL2) was unable to suppress pulmonary metastases (Table 2). Importantly, an equivalent mixture of mAb huKSl/4 and rhlL2 was ineffective in suppressing metastases, indicating that targeting of IL-2 into the tumor microenvironment is required for an optimally effective antitumor response.

Because the prostate carcinoma model used here also expressed bone marrow metastases in ~50% of the mice who received injections of PC-3.MM2 tumor cells, the effect of the huKSl/4-IL2 immunocytokine on bone marrow metastases was determined by using a highly sensitive RT-PCR for HBA. The sensitivity of this detection system was established by reconstitution experiments, indicating that one tumor cell could be detected in 10,000 naive bone marrow cells (Fig. 2). Only mice treated with the huKSl/4-IL2 immunocytokine, at two different dose levels, showed complete absence of a HBA RT-PCR signal in the bone marrow, in contrast to untreated control animals (Fig. 2), indicating efficacy of the immunocytokine also against bone marrow metastases of human prostate carcinoma.

**Table 1** Dose-dependent effect of huKSl/4-IL2 Immunocytokine on experimental lung metastases in SCID mice.

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Metastatic scores**</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>2.2,2,2,3,3,3</td>
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<tr>
<td>32 $\mu$g</td>
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<tr>
<td>4 $\mu$g</td>
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</tr>
<tr>
<td>2 $\mu$g</td>
<td>0,1,1,1,1,1</td>
</tr>
</tbody>
</table>

*a* Mice received injections of 2 $\times$ 10$^6$ PC-3. MM2 cells i.v. Treatment was initiated 24 h after inoculation and consisted of seven daily i.v. injections.

*b* Results are given as metastatic score: 0 = no visible foci; 1 = <5% of lung surface covered with foci; 2 = between 5 and 50% of surface covered with foci; 3 = >50% of lung surface covered with foci.

** Differences between treatment groups and PBS control were statistically significant (P < 0.01).

** Immunocytokine dose (*×7*).

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**RESULTS**

**Characterization of the huKSl/4-IL2 Immunocytokine.** The use of the huKSl/4-IL2 immunocytokine for suppression of lung and bone marrow metastases of human prostate carcinoma cells, PC-3.MM2, was validated by fluorescence-activating cell sorting analysis, indicating that more than 99% of these cells expressed large amounts of the KSA target antigen (data not shown). Saturation binding assays revealed similar binding patterns for huKSl/4-IL2 fusion protein and the huKSl/4 antibody on PC-3.MM2 cells. Thus, calculation of dissociation constants (Kd) and the number of binding sites from saturation binding curves and Scatchard plot analyses indicated an average Kd of 2.6 nm and 7.2 $\times$ 10$^{-3}$ binding sites/cell for both mAb huKSl/4 and the huKSl/4-IL2 fusion protein (Fig. 1).

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**Fig. 1** Saturation binding analysis and Scatchard plot. Serial dilutions of $^{125}$I-labeled huKSl/4-IL2 fusion protein were tested in a saturation binding assay using human prostate carcinoma cells, PC-3.MM2, as target cells. The binding constant was calculated from a corresponding Scatchard plot (inset).

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Technologies, Inc.), according to the manufacturer's guidelines. The cDNA was heated at 100°C for 3 min and chilled on ice. A cDNA equivalent of 100 ng was used in a 25-μl PCR reaction mixture, which contained 20 μM Tris-HCl (pH 8.4), 50 μM KCl, 0.2 μM deoxynucleotide triphosphate, 2.5 units of Taq DNA polymerase (Life Technologies, Inc.) and 0.5 μM sense and antisense oligonucleotide primers for amplification of HBA. Amplification was done with sense 5’AGCAGAGTATAGACCGAGTC3’ and antisense 5’ACAAAGCAATGCTATCACC3’ for 35 cycles (15 s 96°C, 30 s 61°C, 90 s 72°C), leading to a 295-bp fragment. If amplification revealed no HBA signal, the cDNA integrity was tested by amplification of murine glyceraldehyde-3-phosphate-dehydrogenase (mGAPDH) with sense 5’CATTGACTCACTATGCG3’ and antisense 5’CACACCCATCACAAACATG3’ for 25 cycles (15 s 96°C, 30 s 55°C, 90 s 72°C), leading to a 295-bp fragment.

**In Vivo Depletion of Granulocytes.** SCID-BEIGE mice were depleted of mature granulocytes with the mAb RB6--8C5 (16, 17). The RB6--8C5 hybridoma was a kind gift from Dr. R. Coffman (DNAX Research Institute, Palo Alto, CA). Granulocyte depletion was accomplished by injecting 0.5 mg RB6--8C5 i.p. into SCID-BEIGE mice on days -1, 3, and 7. Human prostate carcinoma cells, PC-3.MM2 (2 $\times$ 10$^6$), were injected iv. on day 0, followed by the treatment with huKSl/4-IL2 or PBS starting on day 1. Confirmation of granulocyte depletion was determined by differential leukocyte counts of blood smears (0.01 ml) stained with SureStain Wright-Giemsa (Fisher Scientific, Pittsburgh, PA).

**Statistics.** The statistical significance of differential findings between experimental groups of animals was determined by the nonparametric Wilcoxon rank sum test. Findings were regarded as significant if two-tailed Ps ≤0.01.
Targeted IL-2 Suppresses Prostate Cancer Metastases

huKSl/4-1L2 immunocytokine-induced immune response by equally effective in suppressing pulmonary metastases irrespective of T, B, and NK cells, and granulocytes. depletion of granulocytes in T, B, and NK cell-deficient mice (Table 2), indicating that the immune response are the major effector cell population involved in the antitumor effect induced by the huKSl/4-IL2 immunocytokine.

DISCUSSION

We demonstrated here that targeting IL-2 to the tumor microenvironment of human prostate carcinoma cells (PC-3.MM2) with an immunocytokine (huKSl/4-IL2) effectively suppressed growth and dissemination of pulmonary and bone marrow metastases and significantly prolonged the life span of immunodeficient SCID mice. The antitumor effect achieved by the huKSl/4-IL2 immunocytokine was specific, because another immunocytokine, chl4.18-IL2, directed against ganglioside GD3, absent from PC-3.MM2 tumor cells, was ineffective. Targeting IL-2 to the KSA docking site for huKSl/4-IL2 in the tumor microenvironment proved critical for its effectiveness in suppressing dissemination of metastases, because a mixture of rhIL2 and mAb huKSl/4 at equivalent dose levels was ineffective in this regard. The efficacy of huKSl/4-IL2 in eliminating bone marrow metastases was demonstrated by a sensitive RT-PCR assay for HBA, detecting one tumor cell in 10,000 naïve bone marrow cells. All mice treated with huKSl/4-IL2 at two different dose levels revealed an absence of tumor cells. The prevention of disseminated metastases by treatment with the huKSl/4-IL2 immunocytokine was further demonstrated by a 4-fold increase in life span of these mice when compared with animals that received only PBS or a mixture of rhIL2 and mAb huKSl/4 at dose levels equivalent to the immunocytokine.

It is noteworthy that the antitumor effect of the immunocytokine against human prostate carcinoma cells was achieved in SCID mice that are deficient in mature B and T lymphocytes. Because these animals have a normal compartment of NK cells, granulocytes, and macrophages, we attempted to identify the effector cells involved in the antitumor effect of the immunocytokine. Thus, when performing the treatment experiments in SCID-BEIGE mice deficient in NK cells, the immunocytokine still induced a pronounced suppression of pulmonary metastases in all mice compared with animals treated with PBS. Because the prevention of metastases was not complete in four of six mice that still revealed less than 5% of lung surfaces covered with metastases, NK cells are only partially involved in the antitumor effects induced by the immunocytokine. Further evidence for an NK cell-independent treatment effect is provided by the absence of an increased antitumor effect by the immunocytokine following reconstitution of SCID mice with human LAK cells, which are largely composed of NK cells. Granulocyte depletion of NK cell-deficient SCID-BEIGE mice during the period of immunocytokine treatment did not measurably change the antitumor efficacy of the immunocytokine. These findings imply that macrophages are the key effector cells involved in the suppression of disseminated metastases induced by huKSl/4-IL2 in this particular xenograft model of human prostate carcinoma.

Taken together, our preclinical findings are of interest

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Table 2 Effect of huKSl/4-IL2 immunocytokine on experimental lung metastases in SCID and SCID-BEIGE mice depleted of granulocytes

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Treatment</th>
<th>Depletion</th>
<th>Metastatic score</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCID</td>
<td>PBS</td>
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<td>0,1,2,3,3,3</td>
</tr>
<tr>
<td></td>
<td>huKSl/4 + rhIL2</td>
<td>None</td>
<td>1,1,1,1,2,2,2</td>
</tr>
<tr>
<td></td>
<td>chl4.18-IL2</td>
<td>None</td>
<td>1,2,2,2,2,2</td>
</tr>
<tr>
<td></td>
<td>huKSl/4-IL2</td>
<td>None</td>
<td>0,0,0,0,1</td>
</tr>
<tr>
<td>SCID/BEIGE</td>
<td>PBS</td>
<td>None</td>
<td>2,3,3,3,3,3</td>
</tr>
<tr>
<td></td>
<td>huKSl/4-IL2</td>
<td>None</td>
<td>0,0,1,1,1,1</td>
</tr>
<tr>
<td>SCID/BEIGE</td>
<td>PBS</td>
<td>Granulocytes</td>
<td>2,3,3,3,3,3</td>
</tr>
<tr>
<td></td>
<td>huKSl/4-IL2</td>
<td>Granulocytes</td>
<td>0,0,0,0,1,1</td>
</tr>
</tbody>
</table>

a Mice received injections of 2 × 10^6 PC-3.MM2 cells i.v. on day 0. Treatment was initiated 24 h later (day 1) and consisted of daily i.v. administrations of 0.1 ml of PBS, 4 µg of huKSl/4, and 12,000 IU rhIL2 or 4 µg of either the nonspecific immunocytokine, chl4.18-IL2, or the tumor-specific immunocytokine huKSl/4-IL2, as indicated for 7 consecutive days.

b Depletion consisted of three injections of 0.5 mg of RB6-8C5 (antigranulocyte antibody) or PBS, i.p., on days 1, 3, and 7.

Results are given as metastatic score: 0 = no visible foci; 1 = <5% of lung surface covered with foci; 2 = between 5 and 50% of surface covered with foci; 3 = >50% of lung surface covered with foci. Differences between huKSl/4-IL2-treated groups and controls were statistically significant (P < 0.01).
when considering the future clinical application of the huKSI/4-1L2 immunocytokine for the treatment of human prostate carcinoma. Several phase I clinical trials are currently ongoing that evaluate the efficacy of certain cytokine gene therapies (6), as well as the presentation of prostate carcinoma antigens by individual patients’ dendritic cells to induce a T cell-mediated immunity against prostate carcinoma. However, there are also some doubts about the ultimate success of such approaches. One major disadvantage limiting these therapies is their need for autologous tumor or dendritic cells requiring customization of treatment for individual patients. Another growing concern is that effective T-cell-mediated immunity against prostate carcinoma may be difficult to achieve, based on findings that metastatic human prostate carcinoma cells can escape T cell recognition via divergent mechanisms of defective MHC class I assembly, involving down-regulation of TAP-2 and/or MHC class I heavy chain expression (18). For clinical applications of

Fig. 2  Sensitivity of PC-3.MM2 tumor cell detection in naive bone marrow by HBA RT-PCR and effect of huKSI/4-1L2 immunocytokine on experimental bone marrow metastasis. Naive bone marrow cells were reconstituted with PC-3.MM2 tumor cells at reciprocal ratios of $10^7:10^6$. A negative control of naive bone marrow is shown in Lane B (a). Positive controls of PC-3.MM2 tumor cells are depicted in Lane T (a and b). Analyses are shown of bone marrow metastases induced by i.v. injection of PC-3.MM2 into control mice (Lanes 1–6; b); mice treated with 8 µg of huKSI/1-1L2 (Lanes 7–12; b); and 16 µg of huKSI/4-1L2 (Lanes 13–18; b). Treatment was initiated 24 h after tumor cell inoculation and consisted of daily injections of immunocytokine for 7 consecutive days. Murine glyceraldehyde-3-phosphate-dehydrogenase (mGAPDH) was amplified to confirm the integrity of cDNA (c).

Fig. 3  Kaplan-Meier plot depicting survival of SCID mice who received i.v. injections of PC-3.MM2 cells followed after 24 h with daily injections of 0.1 ml of PBS (dotted line, $n = 6$) or a mixture of 32 µg of huKSI/4 and 96,000 IU rhIL2 (dashed line, $n = 5$), or 32 µg of huKSI/4-1L2 immunocytokine (solid line, $n = 4$) over 7 consecutive days. Treatment with immunocytokine significantly increased life span over mixture ($P <0.01$) and PBS controls ($P <0.001$).

Fig. 4  Effect of LAK cells on growth suppression of experimental lung metastases in SCID mice by huKSI/1-1L2 immunocytokine. PBS [0.1 ml (□)] or huKSI/1-1L2 [8 µg (○)] or 16 µg (□□)] were administered i.v. for 7 consecutive days, starting 24 h after tumor inoculation. Mice were reconstituted with $12 \times 10^6$ LAK cells i.p. on the first day of treatment (a) or did not receive LAK cells (b). Data are expressed as metastatic scores: 0 = no visible foci; 1 = <5% of lung surface covered with foci; 2 = between 5 and 50% of surface covered with foci; 3 = >50% of lung surface covered with foci, with group medians. Differences in tumor load were statistically significant between treatment groups and PBS control ($P <0.01$). There was no statistical difference between presence (a) and absence (b) of LAK cells.
Targeted IL-2 Suppresses Prostate Cancer Metastases

Fig. 5 Growth suppression of experimental lung metastases of PC-3-MM2 cells in SCID-BEIGE mice depleted of granulocytes. Lung specimens were obtained 28 days after tumor cell inoculation. Representative lungs are shown from the control groups (top) and the treatment groups (bottom) that received either daily injections of PBS (0.1 ml) or 4 µg of huKS1/4-IL2 immunocytokine over 7 consecutive days, respectively. Lungs are shown from mice depleted of granulocytes by RB6-8C5 mAb (b and d) and from nondepleted mice (a and c).

...immunotherapies for prostate carcinoma, these findings provide a rationale for focusing on strategies than can circumvent sole reliance on MHC-mediated class I tumor cell recognition by cytotoxic T cells.

Immunocytokines containing IL-2 are capable of activating potent T cell-dependent antitumor immune responses (6, 7), but do not solely rely on this type of immune response for cancer therapy. We demonstrated this previously in an immunocompetent mouse neuroblastoma model where NK cells were shown to be the major immune effector cells capable of eradicating established bone marrow and hepatic metastases (10). Here, we demonstrate effective suppression of bone marrow and pulmonary metastases of human prostate carcinoma in SCID mice by immune effector cells other than B, T, or NK cells. Because the same huKS1/4-IL2 immunocytokine used in these experiments is presently entering into Phase I clinical trials of prostate carcinoma, it is certainly of interest that this immunocytokine is capable of inducing suppression of metastases by activating and proliferating various effector cells. In this regard, we also showed previously that the huKS1/4-IL2 immunocytokine is able to induce antitumor effects via CD8+ T cells in a murine colon carcinoma metastases model (8). Clearly, the induction of a T-cell-mediated immune response has the advantage of memory T cells inducing a long-lived protective tumor immunity and thus performing as a tumor vaccine. However, a T-cell-mediated immune response has clearly defined requirements that cannot always be met, particularly by cancer patients who are highly immunosuppressed after extensive chemo- and radiotherapies.

The huKS1/4-IL2 immunocytokine provides an approach for the treatment of human prostate carcinoma that is not limited by customization of treatment to individual patients like cytokine gene therapies and other cell-based immunotherapies. IL-2 immunocytokines have the added advantage of being able to activate and expand a variety of effector cells, thus, providing a broader base for a potentially effective treatment of prostate carcinoma in an adjuvant setting for patients with minimal residual disease.

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