Interleukin 4 Receptor on Human Lung Cancer: A Molecular Target for Cytotoxin Therapy


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ABSTRACT

Previous studies have demonstrated that human lung tumor cell lines express interleukin 4 (IL-4) receptors, and IL-4 can mediate modest to moderate antiproliferative activity in vitro and in vivo in animal models of human lung tumors. On the basis of these studies, IL-4 was tested in clinical trials; however, it showed little antitumor activity in lung cancer patients. In the present study, we examined the expression of IL-4 receptors (IL-4Rs) in lung tumor samples and normal lung tissues and tested whether an IL-4-targeted agent will have better antitumor activity in vitro and in vivo compared with IL-4.

IL-4R expression was tested by immunohistochemistry in 54 lung tumor samples and normal lung tissues in a tissue array, by reverse-transcription PCR and Northern blot analyses in lung tumor cell lines. Cytotoxic activity of IL-4 cytotoxin [IL-4(38-37)-PE38KDEL], composed of a circular permuted IL-4 and a mutated form of Pseudomonas exotoxin (PE38KDEL), was tested by protein synthesis inhibition and clonogenic assays in seven lung tumor cell lines. Antitumor activity of IL-4 cytotoxin was tested in vitro and in immunodeficient animal models of human lung tumors.

We observed that IL-4Rs are expressed at higher levels in situ in lung tumor samples compared with normal lung tissues and IL-4 cytotoxin is highly and specifically cytotoxic to lung tumor cell lines in vitro. Intratumoral and i.p. administration of IL-4 cytotoxin to immunodeficient mice with s.c. established human lung H358 non-small cell lung cancer tumors mediated considerable antitumor activity in a dose-dependent manner with the higher dose producing durable complete responses. On the other hand, H460 non-small cell lung cancer tumors expressing low levels of IL-4R did not respond to IL-4 cytotoxin therapy.

Because IL-4 cytotoxin mediates its antitumor activity through IL-4R, and a variety of lung tumors expressed high levels of IL-4R, we propose testing the safety of this agent in patients with lung cancer.

INTRODUCTION

Lung cancer originates in the bronchus and lung parenchyma and accounts for ~14% of all new cases of cancer and 28% of cancer-related deaths in the United States (1). The 5-year relative survival rate for stage I lung cancer as defined by the American Joint Commission on Cancer is >50%; however, the overall 10-year relative survival rate is 7% (2). The relative survival rate for advanced-stage disease is extremely low. Lung cancer is characterized by the abnormal expression of cell-surface antigens and the secretion of cytokines, which define its various clinicopathological features. Human lung cancer cell lines have been extensively studied and shown to express various cell surface antigens such as epidermal growth factor receptor (3), intercellular adhesion molecule-1 (4), and insulin-like growth factor receptor (5). These antigens have the potential to be tumor markers and can be targeted for cancer therapy.

In previous studies, we have reported that a variety of solid tumor cell lines, including renal cell carcinoma, squamous cell carcinoma of the head and neck, malignant glioma, AIDS-associated Kaposi’s sarcoma, and breast cancer cell lines overexpress high-affinity IL-4Rs (6–10). The ability of IL-4 to up-regulate adhesion molecules (e.g., intercellular adhesion molecule-1), inhibit cell proliferation, and mediate signal transduction through Janus tyrosine kinases and signal transduction and activation of transcription 6 in these cell lines demonstrates that IL-4Rs are functional (6, 10, 12, 13). Human lung tumor cell lines also express IL-4R, and IL-4 has been shown to mediate antiproliferative activity in vitro and in vivo in lung cancer models (14). On the basis of these studies and the fact that IL-4 can cause proliferation of cytotoxic T cells, it was tested in clinical trials in patients with lung and other cancers. Most of these clinical trials were later abandoned because IL-4 showed only minimal antitumor activity in lung cancer patients (15). Subsequent to initial preclinical and clinical studies, receptors for IL-4 were extensively studied in terms of their...
structure, function, and signal transduction in various cell types (16, 17). It has been expressed in immune cells, is composed of IL-4 Ra and IL-2 Ry chains; however, type II IL-4R is composed of IL-4Ra and IL-13Ra1 chains. This type of receptor is predominantly expressed in immune cells (17). Type II IL-4R also forms a productive complex for type II IL-13R system (17). In type III IL-4R, all three chains are present, but the IL-4Ra chain may only couple with the IL-2Ry or IL-13Ra1 chain at a given time for biological response to occur (17, 18). It is not known which types of IL-4R are expressed in human lung tumors.

To target overexpressed IL-4R on cancer cells, we have developed a recombinant IL-4 cytotoxin that is composed of circularly permuted IL-4 and a mutated form of a type II IL-4R, in which amino acids 38–37 with a GGNGG linker and then fused to truncated toxin PE38KDEL, consisting of amino acids 364 and 381–618 of PE followed by KDEL (an endoplasmic retaining sequence), was expressed in a variety of normal hematopoietic and nonhematopoietic cells, such as T cells, B cells, monocytes, eosinophils, basophils, endothelial cells, fibroblasts, and epithelial cells (16, 24, 28). To rule out whether IL-4 cytotoxin will mediate toxicity to normal tissues, we have tested cytotoxicity to these cell types (24, 29). We have reported that resting human hematopoietic cells (e.g., T cells, B cells, monocytes, and resting or activated CD34+ precursor cells) show little sensitivity to IL-4 cytotoxin because these cells expressed low levels of IL-4Ra (24). Nonhematopoietic cells, e.g., fibroblasts and endothelial cells, expressed a high number of IL-4Ra; however, IL-4 cytotoxin did not mediate toxicity compared with human tumor cells, which showed high sensitivity (24). To further determine IL-4R mediated cytotoxicity to normal tissues, we injected i.v. human IL-4 cytotoxin to cynomolgus monkeys whose cells will bind human IL-4 (24). In our preliminary study, we have observed only reversible hepatic toxicity at an extremely high dose of IL-4 cytotoxin (200 μg/kg). No other organ or blood toxicity was observed. These results suggest that IL-4 cytotoxin may be tolerated in patients when injected i.v. Additional pharmacological and toxicity studies must be performed in monkeys, and perhaps a Phase I clinical trial should be undertaken to determine the safety and tolerability of IL-4 cytotoxin when given i.v.

Because of the dismal prognosis of patients with lung cancer coupled with the fact that IL-4 cytotoxin is active in tumors expressing IL-4R, we wanted to determine whether IL-4R can be used as a potential target for lung cancer therapy. To address the issue, we examined the structure of IL-4R on several human lung cancer tumors including both NSCLC and SCLC. Our studies demonstrate that both types of lung tumors express type II IL-4R. We also show that IL-4 cytotoxin is specifically and highly cytotoxic to lung tumor cell lines in vitro. Consistent with its specific cytotoxic activity in vitro, cpIL4-PE has significant antitumor activity in vivo in nude mouse models of human lung tumors. Thus, unlike IL-4, cpIL4-PE may be a highly effective modality for the therapy of advanced lung cancer.

MATERIALS AND METHODS

Recombinant Proteins. Recombinant human IL-4 was expressed and purified in our laboratory as described previously (30). The cpIL4-toxin [IL-4(38-37)-PE38KDEL], containing the circularly permuted IL-4, in which amino acids 38–129 were linked to amino acids 1–37 with a GGNGG linker and then fused to truncated toxin PE38KDEL, consisting of amino acids 253–364 and 381–618 of PE followed by KDEL (an endoplasmic retaining sequence), was expressed in Escherichia coli, and purified by modified procedure as described previously (19). The purified cpIL4-PE was provided by Neurocrine Biosciences Inc. (San Diego, CA).

Cell Lines and Tumor Tissues. Seven human lung cancer cell lines (H226, H358, H322, H460, N417, H526, and H1299) were purchased from the American Type Culture Collection (Manassas, VA). The origin of each cell line is either NSCLC or SCLC as shown in Table 1. Cells were cultured in RPMI 1640 medium containing 10% fetal bovine serum (BioWhittaker, Walkersville, MD), 1 mM HEPES, 1 mM l-glutamine, 100 μg/ml penicillin, and 100 μg/ml streptomycin (BioWhittaker). A lung tumor tissue array (54 lung tumor samples) was supplied by Imegenx (San Diego, CA).

Immunohistochemistry. The tissue array was stained for the expression of IL-4Rs using a polyclonal antibody (C-20, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) generated against a peptide located in the carboxyl terminal region of the human IL-4Ra chain. Fifty-four samples were examined by two pathologists (H. R. and W. M.) and scored 0, 1+, 2+, or 3+ based on the staining intensity.

Northern Blot Analysis. Total RNA was isolated using TRIzol reagent (Life Technologies, Inc., Gaithersburg, MD). An equal amount of total RNA (10 μg) derived from four cell lines

| Table 1 Cytotoxic activities of IL-4(38-37)-PE38KDEL to NSCLC and SCLC cell lines |
|-----------------|-----------------|-----------------|-----------------|
| Cell line       | Origin          | IC50 (ng/ml)a   |
|-----------------|-----------------|-----------------|-----------------|
| NSCLC           |                 |                 |
| H226            | Squamous cell carcinoma | 0.08 |
| H322            | Bronchioloalveolar carcinoma | 2   |
| H358            | Bronchioloalveolar carcinoma | 0.05 |
| H460            | Large cell carcinoma | >1000 |
| H1299           | Large cell carcinoma | ND b    |
| SCLC            |                 |                 |
| N417            | Small cell lung cancer | 35   |
| H526            | Small cell lung cancer | 48   |

a IC50, the concentration of IL-4 cytotoxin at which 50% inhibition of protein synthesis is observed compared with untreated cells. 
b ND, not done.
was electrophoresed through a 0.8% agarose, formaldehyde-denaturing gel, transferred to a nylon membrane (S&S Nytran; S Scheicher and Schuell, Keene, NH) by capillary action, and immobilized by UV cross-linking (Stratagene, La Jolla, CA). The cDNA for the human IL-4Rα chain was labeled with [α-^32P]dCTP (3000 Ci/mmol; Amersham, Arlington Heights, IL). Membrane-bound RNA was prehybridized for 30 min at 68°C and then hybridized with ^32P-labeled cDNA probes for 1 h at 68°C in ExpressHyb hybridization solution (Clontech Laboratories, Inc., Palo Alto, CA). The membranes were washed and subsequently exposed to an X-AR film for 12–72 h at −70°C to obtain an autoradiogram.

**RT-PCR.** To detect the mRNA expression of IL-4R in human lung cancer cell lines by RT-PCR, 2 μg of total mRNA RNA was incubated at 42°C in 20 μl of reaction buffer containing 10 mm Tris-HCl (pH 8.3), 5 mm MgCl2, 50 mm KCl, 1 mm concentrations each of deoxynucleotide triphosphates, 1 unit/μl RNase inhibitor, 2.5 μM oligo d(T)16, and 2.5 μM/μl Moloney murine leukemia virus reverse transcriptase (Perkin-Elmer Life Sciences) for 30 min. Then, a 10-μl aliquot of reverse transcription reaction was amplified in a 50-μl final volume of PCR mixture containing 10 mm Tris-HCl (pH 8.3), 2 mm MgCl2, 50 mm KCl, 1 unit of AmpliTaq Gold DNA polymerase (Perkin-Elmer Life Sciences), and 0.1 μg of specific primer. Specific primers were as follows (31): IL-4Rα (product size, 272 bp), 5′-CCCAAGCTTACTGTGCCAACCTGAG-3′ and 3′-CTCATTCTGGCTGCCCAGATTCCGC-5′; IL-13Rα1 (product size, 309 bp), 5′-CCCAAGCTTACGGAAACTCAGCCACCTG-3′ and 3′-GCAGATTCTTTTATGTTAAGCAGAGGTAC-5′; IL-2Rγ (product size, 256 bp), 5′-CCCAAGCTTACGGAAACTCAGCCACCTG-3′ and 3′-AGCGGTCTCGAC-ACGAAAACGATTCCGC-5′. Glyceraldehyde-3-phosphate dehydrogenase served as an internal control. The PCR product (20 μl) was run on a 1% agarose gel for UV analysis.

**Radioimmunoassay.** Recombinant human IL-4 was labeled with 125I (Amersham), served as an internal control. The PCR product (20 μl) was run on a 1% agarose gel for UV analysis.

**Radioreceptor Binding Assay.** Recombinant human IL-4 was labeled with 125I (Amersham) using IODO-GEN reagent (Pierce, Rockford, IL) as described previously (10). The specific activity of the radiolabeled IL-4 was estimated to be 18.9 μCi/μg protein. For binding experiments, 5 × 10^5 cells in 100 μL of binding buffer (RPMI 1640 containing 0.2% human serum albumin and 10 mm HEPEs) were incubated with 200 pM 125I-IL-4 with or without 40 nM unlabeled IL-4 at 4°C for 2 h. Cell-bound 125I-IL-4 was separated from unbound 125I-IL-4 by centrifugation through a phthalate oil gradient, and radioactivity was determined with a gamma counter (Wallac, Gaithersburg, MD). In some experiments, the number of IL-4Rs and binding affinity were calculated with the LIGAND program as described previously (13).

**Protein Synthesis Inhibition Assay.** The cytotoxic assay of IL-4 cytotoxin was tested as described previously (7). Typically, 10^5 cells were cultured in leucine-free medium with or without various concentrations of IL-4(38-37)-PE38KDEL for 20–22 h at 37°C. Then, 1 μCi of [3H]leucine (NEN Research Products, Boston, MA) was added to each well and incubated for an additional 4 h. Cells were harvested, and radioactivity incorporated into cells was measured by a beta plate counter (Wallac).

**Clonogenic Assay.** The in vitro cytotoxic activities of IL-4 cytotoxin on H358, H322, and H460 cells were also determined by colony-forming assay. The cells were plated in triplicate in 100-mm Petri dishes with 7 ml of medium containing 10% fetal bovine serum and allowed to attach for 20–22 h. The number of cells/plate was chosen such that >100 colonies were obtained in the control group. The cells were exposed to different concentrations of IL-4 cytotoxin (0–100 ng/ml) for 9 days at 37°C in a humidified incubator. The cells were washed, fixed, and stained with crystal violet (0.25% in 25% ethanol). Colonies consisting of >50 cells were scored. The percentage of colony survival was determined from the number of colonies remaining in the treated groups divided by the number of control group colonies.

**Animals and Antitumor Activity in Vivo.** Four- to 5-week-old (20–22 g body weight) male athymic nu/nu mice were obtained from Frederick Cancer Center Animal Facilities (National Cancer Institute, Frederick, MD). The mice were housed in sterilized filter-topped cages and maintained in a pathogen-free animal facility. Animal care was in accordance with the guidelines of the NIH Animal Research Advisory Committee. Human lung cancer cell lines (H358 or H460) were implanted by s.c. injection of 5 × 10^6 cells in 150 μl of PBS plus 0.2% human serum albumin into the dorsal surface of the mice. After 4–5 days, when tumors were established, excipient (0.2% human serum albumin in PBS) or IL-4 cytotoxin was administered either i.p. (500 μl) or i.t. (30 μl) using a 27-gauge needle. Tumor sizes were carefully measured by a Vernier caliper. The tumor size was calculated by multiplying the length and width of the tumor. Each treatment group consisted of a minimum of five mice.

**Statistical Analysis.** The statistical significance of tumor regression was calculated by Student’s t test.

**RESULTS**

**Immunohistochemistry of Human Lung Tumor Tissue Array.** First, we examined the extent of expression of IL-4R in lung tumor samples as determined by immunohistochemical staining. As shown in Fig. 1, lung tumors intensely stained with IL-4R antibody (Fig. 1, C-F) compared with normal lung tissues that showed weak staining (Fig. 1, A and B). Fifty-four lung tumor samples were analyzed. Between 66 and 79% of the samples were positive for IL-4R staining as analyzed by two independent pathologists. The staining intensity was scored as grade 0, 1+, 2+, or 3+. Two to seven percent of the lung tumor samples were grade 3+, 13–28% grade 2+, 44–52% grade 1+, and 20–30% grade 0.

**Expression of IL-4R mRNA in Human Lung Tumor Cell Line.** We next determined the expression of mRNA for putative IL-4R subunits in human NSCLC and SCLC cell lines as shown in Fig. 2. With RT-PCR analysis, we found that mRNA for IL-4Rα and IL-13Rα1 chains was expressed in all of the tumor cell lines examined (Fig. 2A). One NSCLC (H460) and two SCLC (N417 and H526) cell lines showed faint bands for IL-4Rα chain mRNA compared with other cell lines. Similarly, the N417 cell line showed a faint IL-13Rα1 chain mRNA band. In contrast, no lung cancer cell lines showed a detectable band for IL-2Rγ chain mRNA. The PM-RCC renal cell carcinoma cell line served as a positive control for IL-4Rα and IL-13Rα1 mRNA, and the H9 T lymphoma cell line served as
positive control for IL-2Rγ chain mRNA. Northern blot analysis of RNA derived from four NSCLC cell lines confirmed the RT-PCR results, demonstrating that IL-4Rα chain mRNA is strongly expressed in two bronchioalveolar carcinoma cell lines (H322 and H358), whereas two large cell carcinoma cell lines (H1299 and H460) showed either very faint or undetectable bands (Fig. 2B).

**IL-4 Binding to Human Lung Cancer Cell Lines.** To determine the binding affinity of IL-4R for IL-4 in human lung cancer cell lines, cells were incubated with \(^{125}\text{I}-\text{IL-4}\) in the absence or presence of 200-fold molar excess of IL-4. Lung tumor cells bound \(^{125}\text{I}-\text{IL-4}\) to varying degrees and was competitively displaced by an excess of unlabeled IL-4 (data not shown). To further characterize the IL-4R in lung cancer cells,
we performed Scatchard analysis in the H358 cell line (Fig. 3A and B). H358 cells bound IL-4 in a concentration-dependent manner. Assessment of the binding data by Scatchard analysis suggests a single receptor type with a $K_d$ value of 2.4 nM. The number of IL-4R in H358 cell line was calculated at 10,600 IL-4 molecules bound/cell. Data are representative of two independent experiments yielding comparable results.

**Human Lung Cancer Cell Lines Are Sensitive to cplIL4-PE.** To target IL-4R on human lung cancer cell lines, we evaluated cytotoxic activity of IL-4 cytotoxin by protein synthesis inhibition assays (Fig. 4). Table 1 shows the $IC_{50}$ for IL-4 cytotoxin (the protein concentration required for the inhibition of protein synthesis by 50%) in six lung cancer cell lines. Three NSCLC cell lines (H226, H322, and H358) were extremely sensitive to IL-4 cytotoxin in which $IC_{50}$ values were $\leq 2$ ng/mL. The cytotoxic activity of IL-4 cytotoxin was neutralized by adding excess IL-4 to cell line cultures (Fig. 4). SCLC cell lines were moderately sensitive to the cytotoxic activity of IL-4 cytotoxin. Consistent with the fact that the H460 NSCLC cell line lacks an IL-4R chain as confirmed by Northern blot analysis, IL-4 cytotoxin was not cytotoxic to this cell line ($IC_{50}$ was $>1000$ ng/mL).

**Inhibition of Colony Formation of Human Lung Cancer Cell Lines by IL-4 Cytotoxin.** We performed a colony-formation assay using the H358, H322, and H460 cell lines. Five hundred cells were plated in each Petri dish and incubated with various concentrations of IL-4 cytotoxin. After 9–14 days of culture, the percentages of colonies formed in control and IL-4 cytotoxin-incubated groups were compared. As shown in Fig. 5, $A$ and $B$, IL-4 cytotoxin inhibited colony formation in H358 and H322 cell lines in a concentration-dependent manner, whereas IL-4 cytotoxin did not inhibit colony formation in H460 cells. The number of colonies in untreated groups served as the 100% control value. One nanogram/milliliter or $0.1$ ng/mL of IL-4 cytotoxin inhibited colony formation by 50% in H322 and H358 cell lines, respectively. These results were comparable with the dose-dependent kinetics observed in the cytotoxicity assay (Fig. 4).

**IL-4 Cytotoxin Regresses IL-4R Over-expressing Lung Tumors in Vivo.** To determine the specific antitumor activity of IL-4 cytotoxin in vivo, we established s.c. lung tumor xenografts by injection of H358 or H460 cells into immunodeficient mice. The H358 cell line is extremely sensitive to IL-4 cytotoxin in vitro, whereas the H460 cell line is unsuitable. Animals were treated with different doses of IL-4 cytotoxin administered i.p. or i.t. according to specified time schedules. H358-tumor-bearing mice were injected with IL-4 cytotoxin either twice daily with 50 or 100 $\mu$g/kg/dose IL-4 cytotoxin for 5 days (total 10 injections) by i.p. injection or i.t. injection.
on alternate days with 200 or 500 μg/kg/dose for a total of 3 days by i.t. injection. H460-tumor-bearing mice were also treated with IL-4 cytotoxin by i.p. (100 μg/kg) or i.t. (500 μg/kg) injection. As shown in Fig. 6A, IL-4 cytotoxin mediated antitumor activity in a dose-dependent manner when given i.p. On day 10, one of six mice in the 100 μg/kg i.p. treatment group showed complete remission of H358 xenografted tumor. On day 59, this mouse remained in complete remission. Overall, the reduction in tumor size in the 50 and 100 μg/kg dose-treatment group was 51% (110 mm²; \( P < 0.005 \)) and 73% (60 mm²; \( P < 0.0005 \)), respectively, compared with control tumors in vehicle-treated control animals (223 mm²). On the other hand, as shown in Fig. 6B, H460 tumor xenografts were resistant to IL-4 cytotoxin and tumor sizes were not significantly different between the control and i.p. treatment (100 μg/kg) groups. On day 60, these mice were sacrificed because of excessive tumor burden.

We next examined the antitumor effect of IL-4 cytotoxin in both H358 and H460 tumor models by i.t. administration of IL-4 cytotoxin. As shown in Fig. 7A, i.t. administration of IL-4 cytotoxin in H358 tumor xenografted animals caused complete regression of tumors. On day 10, two of five mice in the 200 μg/kg i.t. group had a recurrence of tumor growth, whereas all mice in the 500 μg/kg i.t. group remained tumor free until day 60, the last day of the experiment. The reduction in tumor size on day 59 achieved by 200 and 500 μg/kg doses of IL-4 cytotoxin was 86% (32 mm²; \( P < 0.0005 \)) and 100% (0 mm²; \( P < 0.0005 \)), respectively, compared with control tumors (223 mm²). In contrast, H460 tumor xenografted animals were resistant to IL-4 cytotoxin therapy. Tumor sizes were not significantly different between the control and i.t. treated (500 μg/kg) groups (Fig. 7B).

**DISCUSSION**

The goal of the current study was to determine whether human NSCLC and SCLC express IL-4R in situ and whether...
expression of these receptors could facilitate targeting of IL-4 cytotoxin fusion proteins. As in previous reports (6–10), we demonstrated that each of six selected lung tumor cell lines expressed varying levels of IL-4R. Both IL-4Rα and IL-13Rα1 chain mRNA were detected in these cells. Because none of these cell lines express mRNA for the IL-2Rα chain, which has been shown to make functional complexes with IL-4Rα chain in immune cells, we reasoned that human lung cancer express type II IL-4R (17). These results are similar to our previous studies demonstrating that other varieties of human solid tumor cell lines also express type II IL-4R (17). Consistent with RT-PCR and Northern analyses, IL-4R was expressed on the surface of human lung tumor cell as confirmed by 125I-IL-4 binding studies. Similarly, IL-4R was also detected by immunohistochemistry performed on samples of human lung tumor tissue. Using an uncharacterized IL-4R antibody approximately one-third of evaluated lung tumors has been found to express IL-4R (32). In contrast, we found that 66–79% of the lung tumors examined in our study express IL-4R in situ. Doucet et al. (33) have reported that normal human lung fibroblasts express type II IL-4R complex; differences in structure and receptor number have not been reported for comparisons between cancer cells and normal lung fibroblasts.

We report that lung tumor cell lines expressing IL-4Rα mRNA are highly sensitive to the cytotoxic effect of IL-4 cytotoxin in vitro, whereas one cell line that did not show detectable IL-4Rα mRNA was refractory to IL-4 cytotoxin. In general, the expression level of IL-4Rα chain correlated with the degree of cytotoxic activity observed for IL-4 cytotoxin. Similar to results obtained from in vitro experiments, IL-4 cytotoxin mediated significant antitumor activity in IL-4R positive but not IL-4R negative in vivo tumor models. IL-4 cytotoxin mediated antitumor activity in a dose-dependent manner when administered systemically (i.p.). At a higher dose (100 μg/kg), one of six animals showed complete regression of established tumor. None of the animals bearing IL-4R negative lung tumors showed any response to the IL-4 cytotoxin. The antitumor effect of IL-4 cytotoxin was superior when injected i.t. Two of five animals in a low dose group and all six animals in a high dose group showed complete regression of established tumors. These responses were durable for the entire period of observation. IL-4R negative tumors did not show any regression even when
500 μg/kg IL-4 cytotoxin was administered i.t. These results indicate that IL-4 cytotoxin kills lung tumor cells through an IL-4R-dependent mechanism, not by nonspecific cytotoxic activity.

Because primary lung tumor is predominantly localized in the chest cavity, and a majority of lung tumor specimens express detectable IL-4R by immunohistochemistry, it is possible that localized delivery of IL-4 cytotoxin may mediate potent therapeutic antitumor activity. This mode of therapy may allow follow-up therapy by systemic administration to eradicate metastatic spread; however, before these studies are undertaken, the effect of IL-4 cytotoxin on normal lung and heart must be evaluated. In our preclinical models, IL-4 cytotoxin did not elicit pulmonary or cardiac toxicities in mice and monkeys as assessed by clinical examination (24). Only hepatic toxicity was detected, indicating that IL-4-PE is metabolized in liver (21). Because human IL-4 does not bind to murine IL-4R (21), mice would not be a good model to evaluate specific pulmonary and cardiac toxicities of human IL-4 cytotoxin. Perhaps murine IL-4 cytotoxin needs to be developed and tested in these mouse model systems. Alternatively, as human IL-4 binds to monkey IL-4R, additional monkey studies could be initiated to address these issues.

In conclusion, we have demonstrated that human lung tumor cell lines express type II IL-4R and IL-4R is overexpressed in lung tumor samples. Because IL-4R-targeted cytotoxin is highly active in mediating antitumor activities in IL-4R positive lung tumor cells in vitro and in vivo, it is possible that IL-4 cytotoxin may be effective in the management of advanced lung tumors. Therefore, additional preclinical studies should be performed to explore this possibility.

ACKNOWLEDGMENTS
We thank Drs. Donald Fink and Harold Dickensheets for critical reading of this manuscript.

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