

# Interleukin-4 Receptor Cytotoxin as Therapy for Human Malignant Pleural Mesothelioma Xenografts

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**Background.** Malignant pleural mesothelioma (MPM) is an uncommon but highly fatal neoplasm for which only limited treatment is available.

**Methods.** Immunohistochemical analysis was used to determine the expression of interleukin-4 receptors (IL-4R) on mesothelioma cell lines and resected mesothelioma tumors. Radioreceptor binding assays were used to show that these IL-4R were high-affinity receptors. Previously, we had shown that a chimeric protein composed of a circularly permuted IL-4 molecule fused to a truncated form of *Pseudomonas* exotoxin A, IL-4(38–37)-PE38KDEL, could be used to kill IL-4R-bearing tumor cells in vitro. The toxicity of this molecule to mesothelioma cell lines was tested using a protein synthesis inhibition assay. A human mesothelioma xenograft model was then developed to assess the efficacy of this molecule in vivo.

**Results.** All MPM cell lines tested were found to express high-affinity cell-surface IL-4R. Immunohistochemical analysis of resected mesothelioma tumor specimens from 13 patients revealed that all tumors expressed moderate-to-high levels of IL-4R. Coculture of malignant mesothelioma cell lines with IL-4(38–37)-PE38KDEL resulted in a dose-dependent inhibition of tumor cell protein synthesis through an interaction with cell-surface IL-4R. In a nude mouse xenograft model of human MPM, intratumoral administration of IL-4(38–37)-PE38KDEL mediated a dose-dependent decrease in tumor volume and a dose-dependent increase in survival.

**Conclusions.** The chimeric protein, IL-4(38–37)-PE38KDEL, has potent antitumor effects against MPM both in vitro and in vivo.

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Malignant pleural mesothelioma (MPM) is an uncommon but highly fatal neoplasm afflicting between 2,000 and 4,000 Americans annually [1]. Asbestos exposure remains the only proven cause of MPM. Due to the long latency period between asbestos exposure and the development of mesothelioma, this tumor will remain a substantial problem for many years to come [2]. Although the number of deaths from mesothelioma in the United States has been predicted to begin to gradually decline, in Western Europe, where asbestos was used well into the 1980s, the mortality rate is predicted to increase to almost 9,000 deaths per year by 2018 [3]. Uncontrolled trials and institutional experiences with various approaches utilizing surgery, radiation, chemotherapy, hyperthermia, photodynamic therapy, gene

therapy, as well as other modalities have been reported with some success, but no therapy has been definitively shown to be better than supportive care in a randomized study. Clearly, novel effective therapies are desperately needed for this disease.

Several cellular growth factors, cytokines, and their receptors have been shown to be overexpressed on mesothelioma cells [4–6]. We chose to investigate the expression of interleukin-4 receptors (IL-4R) by mesothelioma cell lines and tumor specimens. Interleukin-4 (IL-4) and its receptor have been well characterized [7]. Interleukin-4 was originally identified as a stimulator of activated B cells, and subsequently has been shown to mediate a host of immunomodulatory activities [8–10]. In addition to its presence on lymphocytes and other hematopoietic cells, the IL-4R has been reported to be highly expressed on some epithelial cell lines, as well as a variety of nonhematologic malignancies including melanoma, breast cancer, colon cancer, renal cancer, ovarian

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Dr Maki discloses that he has a financial relationship with Neurocrine Biosciences.

carcinoma, glioblastoma multiforme, AIDS-associated Kaposi's sarcoma, gastric cancer, and non-small cell lung cancer [11–17]. The significance of expression of IL-4R on cancer cells is unknown; however, these receptors are able to mediate biological responses in cancer cells such as regulation of intercellular adhesion molecule-1 and major histocompatibility complex antigen expression, inhibition of cell growth, and induction of apoptosis.

To target IL-4R on cancer cells, we produced a circularly permuted form of IL-4 toxin—IL-4(38–37)-PE38KDEL [18]. This IL-4 toxin has been shown to have potent cytotoxic activity against various IL-4R-bearing cancer cell lines in vitro and in vivo in animal models of human cancer xenografts [19–21]. To study whether malignant pleural mesothelioma expresses IL-4R in vitro and in vivo, we studied the incidence of IL-4R on several established mesothelioma cell lines as well as on human, malignant, pleural mesothelioma surgical specimens. After establishing that the IL-4R is highly expressed by both MPM cell lines and by fresh tumor specimens, we tested the cytotoxic activity of IL-4(38–37)-PE38KDEL against these mesothelioma cell lines and in vivo against human mesothelioma tumor xenografts. Our data indicated that IL-4(38–37)-PE38KDEL has potent cytotoxic activity against human malignant mesothelioma in vitro and in vivo.

## Material and Methods

### *Fresh Human Malignant Pleural Mesothelioma Specimens and Cell Lines*

Human MPM specimens were collected during 14 planned surgical procedures at the University of California, Los Angeles and San Francisco campuses, under protocols and informed consent approved by each institution's human subjects review boards. Specimens were fixed in formaldehyde and embedded in paraffin or snap frozen in tissue freezing medium (Triangle Biomedical Sciences, Durham, NC) or digested in a triple enzyme solution of 0.1% type IV collagenase, 0.01% type V hyaluronidase, and 0.002% type I deoxyribonuclease (Sigma Chemical Co, St. Louis, MO) at room temperature in Hank's balanced salt solution (HBSS) (Mediatech, Herndon, VA) for up to 12 hours, filtered through 100-gauge nylon mesh (Falcon, Franklin Lakes, NJ), washed at least three times, separated from cellular debris by centrifugation in lymphocyte separation medium (Mediatech, Herndon, VA) for 20 minutes at 2,000 rpm, and resuspended in HBSS at  $1 \times 10^7$  cell/mL for subcutaneous injection. These digested cells were used for injection into nude mice for creation of the xenograft model. Triple enzyme digestion was also used when passaging tumors in mice. Slides were prepared from snap-frozen specimens and stored at  $-70^\circ\text{C}$  until staining with the anti-IL-4R antibody, M57 (kindly provided by Immunex Corporation, Seattle, WA), or anti-IL-4R antibody purchased from R&D (Minneapolis, MN).

Human mesothelioma cell lines, MS-1, and the SV40-transfected human mesothelioma cell line, MeT5A, were

kindly provided by Dr. V. Courtney Broaddus (San Francisco General Hospital and the University of California, San Francisco). MSTO-211H is a human mesothelioma cell line purchased from American Type Culture Collection (Manassas, VA). Cell lines were maintained as monolayers in RPMI 1640 (Biofluid, Rockville, MD) supplemented with 10% heat-inactivated bovine serum (Hyclone, Logan UT), 50 mmol/L HEPES (Biofluid), 10 mmol/L glutamine, and 250 U/mL of penicillin/streptomycin.

### *Reagents*

A circularly permuted IL-4 toxin [IL-4(38–37)-PE38KDEL] or cpIL-4-PE that contains amino acids 38 to 129 of IL-4 fused by a peptide linker to amino acids 1 to 37, which are in turn fused to amino acids 353 to 364 and 381 to 608 of *Pseudomonas* exotoxin, with KDEL at positions 609 to 612, was constructed as described previously [22].  $^3\text{H}$ -Leucine was purchased from Duprel (Boston, MA). Radiolabeled iodine ( $^{125}\text{I}$ ) was purchased from Amersham Research Products (Arlington Heights, IL).

### *Immunohistochemistry*

Detection of IL-4R on surgical MPM specimens was performed by immunohistochemistry using a Vectastain Elite ABC Kit (Vector Laboratories, Burlingame, CA). Briefly, 5  $\mu\text{m}$  fixed frozen sections were first incubated with normal blocking serum, followed by an avidin and biotin blocking step to eliminate any nonspecific binding by endogenous biotin or biotin-binding proteins. Slides were then incubated with 3% hydrogen peroxide to eliminate any endogenous peroxidase activity, followed by washing and incubation with either mouse anti-human IL-4R monoclonal antibody (R&D, Minneapolis, MN) or a mouse immunoglobulin G isotype control. The slides were then washed and incubated with a biotinylated secondary antibody. After another washing step, the slides were incubated with streptavidin-peroxidase complexes, washed again, and developed with 3,3'-diaminobenzidine. Development reactions were quenched with water and the slides were then counterstained.

### *Radioreceptor Binding Assay*

Recombinant human IL-4 was enzymatically labeled with  $^{125}\text{I}$  according to the manufacturer's instructions (Bio-Rad, Richmond, CA). The specific activity was estimated to range from 1.4 to  $4.7 \times 10^{10}/\mu\text{Ci}$  per milligram of protein in numerous labelings. For IL-4R assays, equilibrium binding studies were performed by the method described previously [18, 23]. Briefly,  $1 \times 10^6$  cells in 100  $\mu\text{L}$  binding buffer (RPMI 1640 plus 0.2% human serum albumin and 10 mmol/L HEPES) were incubated for 2 hours with 100 pmol/L  $^{125}\text{I}$ -IL-4 without or with various concentrations of unlabeled IL-4 at  $4^\circ\text{C}$  in polypropylene tubes in a shaking water bath. Various studies have shown that IL-4 and IL-13 receptors share two chains with each other in many tumor cell types. To investigate whether IL-4R in mesothelioma tumor cells also interact with IL-13, binding of radiolabeled IL-4 was also competed by excess of unlabeled IL-13 [24]. Cell-bound  $^{125}\text{I}$ -IL-4 was separated from unbound  $^{125}\text{I}$ -IL-4 by cen-

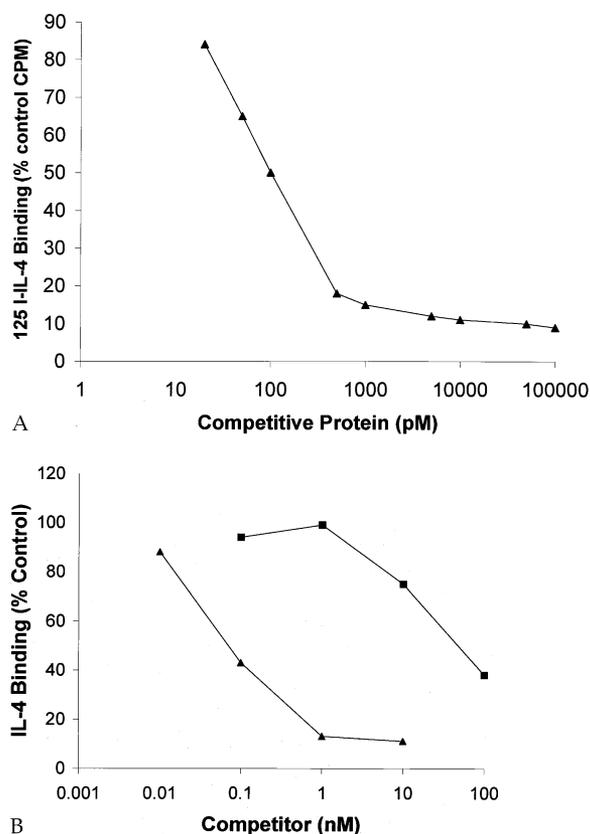


Fig 1. (A) Binding displacement of <sup>125</sup>I-labeled interleukin-4 (IL-4) by unlabeled IL-4. (B) Binding displacement of <sup>125</sup>I-labeled IL-4 by unlabeled IL-4 (▲) or IL-13 (■). (CPM = counts per minute.)

trifugation through a cushion of phthalate oils, which was prepared by mixing 48% Bis(2-ethylhexyl)phthalate (Fisher Scientific, Inc, Fair Lawn, NY) and 52% n-butyl phthalate (Eastman Kodak, Rochester, NY). Cell pellets were counted in a gamma counter (Wallace, Gaithersburg, MD). Data were obtained and analyzed from the average of triplicate wells. The number of receptors and their binding affinities were determined using the Ligand program (Systat, Richmond, CA) [25].

#### Protein Synthesis Inhibition Assay

The cytotoxic activity of cpIL-4-PE on human mesothelioma cell lines was evaluated by measuring inhibition of protein synthesis as described previously [26]. Briefly,  $1 \times 10^4$  tumor cells in 200  $\mu$ L of medium were cultured overnight in 96-well flat-bottom plates. Medium was aspirated and replaced with leucine-free medium with or without various concentrations of cpIL-4-PE. Cells were normally cultured with cytotoxin for 20 hours at 37°C. For the neutralization experiments, cells were preincubated with recombinant human IL-4 or IL-13 for 30 to 45 minutes at 37°C before cpIL-4-PE was added to the tumor cells. Then 1  $\mu$ Ci of <sup>3</sup>H-leucine per well was added and cells were cultured for an additional 4 hours. After freeze-thaw, cells were harvested by a cell harvester

(Cambridge Technology Inc, Cambridge, MA) and radioactivity incorporated into the cells was measured by a beta counter (Beckman, Fullerton, CA). Data were obtained and analyzed from the average of triplicate wells. The concentration of cpIL-4-PE at which 50% inhibition of protein synthesis occurred ( $IC_{50}$ ) was calculated.

#### Human Tumor Xenograft Model

Four-week-old female pathogen-free NCR nude mice were purchased from Taconic Inc (Germantown, NY) and housed in filter-top cages in a laminar flow hood. Human mesothelioma specimens were prepared from freshly harvested and digested human MPM tumors as described above. Tumor cells were then resuspended at  $1 \times 10^7$ /mL and up to  $5 \times 10^6$  cells in 0.5 mL were injected into the flanks of the mice. In this way, a cell line, Meso-1, was generated from a single, fresh, human MPM tumor specimen at the University of California, Los Angeles and passaged serially in nude mice. For all experiments, subcutaneous tumors were induced by injecting  $5 \times 10^6$  cells into the flanks of nude mice. At regular intervals the tumors were carefully measured with fine calipers and the tumor volumes were calculated from the cross-sectional diameters using the following formula: (short diameter)<sup>2</sup>  $\times$  long diameter  $\times$  0.4. In all experiments, treatment was begun on day 5 when tumor volumes ranged from 55 to 75 mm<sup>3</sup>. For the intratumoral dosing experiments, groups of 5 mice were treated with intratumoral injections of Hank's balanced salt solution (vehicle), recombinant human IL-4 control (three divided doses totaling 750  $\mu$ g/kg), low-dose cpIL-4-PE (three divided doses totaling 250  $\mu$ g/kg), or high-dose cpIL-4-PE (three divided doses totaling 750  $\mu$ g/kg) on days 5, 7, and 9. The treatment volume for intratumoral injections was 40  $\mu$ L. For the intraperitoneal dosing experiments, groups of 5 mice with subcutaneous flank tumors were treated with intraperitoneal injection of HBSS, 300  $\mu$ g/kg recombinant human IL-4 (three divided doses totaling 300  $\mu$ g/kg), or cpIL-4-PE (three divided doses totaling 300  $\mu$ g/kg) on days 5, 7, and 9 after tumor inoculation. The treatment volume for intraperitoneal injections was 250  $\mu$ L. For the intratumoral injection experiments, 20 mice were used (four groups of 5 mice each) and for the intraperitoneal injection experiments, 15 mice were used (three groups of 5 mice each). No mice dropped out of any group in either experiment. All mice were sacrificed by cervical dislocation or carbon dioxide asphyxiation. All animals received humane care in accordance with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (National Institutes of Health publication 85-23, revised 1985).

#### Statistical Analysis

Mean tumor volume values were compared using repeated-measures analysis of variance methods (Proc Mixed, SAS, Inc, Cary, NC) in which day (time) was a within-animal factor and treatment group was the between-animal factor. All post hoc *t* tests and their corresponding *p* values were based on this model. Survival of treated mice was compared using the log rank test.

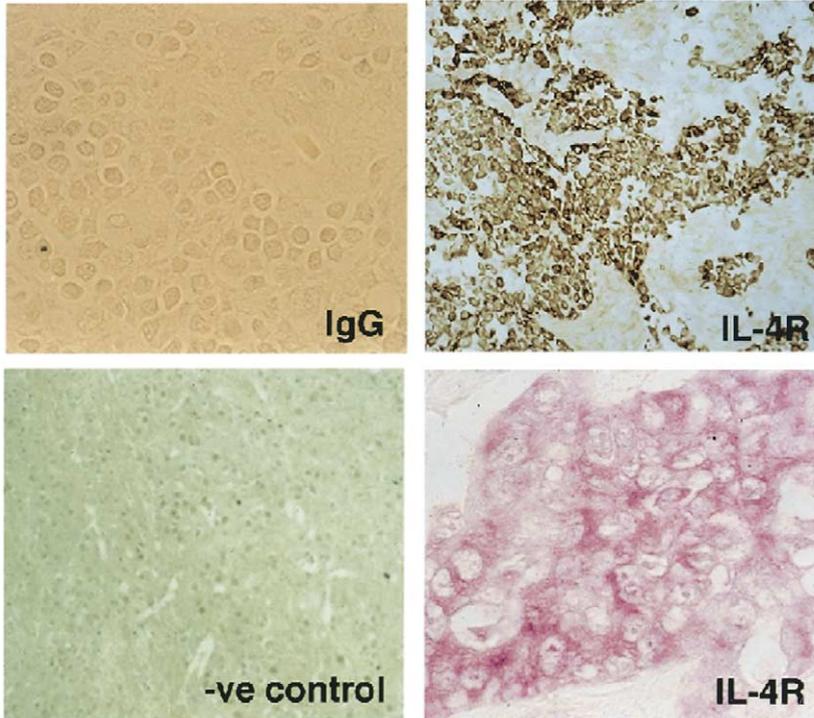


Fig 2. Interleukin-4 receptor (IL-4R) detection in mesothelioma tissues by immunohistochemical analysis. Left upper panel: Mouse immunoglobulin G (IgG) as isotype control (×40). Left lower panel: no secondary antibody (×40). Right upper panel: Anti-IL-4R monoclonal antibody (M-57) (×40). Right lower panel: Anti-IL-4R monoclonal antibody (M-57) and Vector NovaRed stain (×60).

**Results**

*Expression of Interleukin-4 Receptors on Human Malignant Pleural Mesothelioma Cell Lines*

To confirm the expression of IL-4R on human MPM and study its binding affinity, we studied the human mesothelioma cell line, MS-1. Tumor cells were incubated at 4°C with increasing concentrations of <sup>125</sup>I-IL-4 for 2 hours. As demonstrated in Figure 1A, MS-1 cells bound IL-4 in a concentration-dependent manner. Analysis of the binding data by the “Ligand” program indicated that a single class of high-affinity receptors was expressed on MS-1 cells. The calculated receptor density was more than 10,000 sites/cell with K<sub>d</sub> less than 1 pmol/L. We found that IL-13 was also able to compete for the binding of <sup>125</sup>I-IL-4, but IL-4 was superior (EC<sub>50</sub> ~ 0.1 nmol/L) to IL-13 (EC<sub>50</sub> ~ 40 nmol/L) (Fig 1B).

*Expression of Interleukin-4 Receptors on Human Malignant Pleural Mesothelioma Specimens*

We studied 14 specimens from 13 patients undergoing resection of MPM. The mean age of the patients was 65.5 years (range 48 to 79 years) and 85% were male. As shown in Figure 2 and Table 1, MPM tumor specimens exhibited strong overall staining with anti-IL-4R antibody. This was true of both paraffin-embedded MPM tumor cells and tumor cells that had been snap frozen. In 9 of 13 specimens intense (3+) staining was noted, whereas in 4 of 13 specimens moderate (2+) staining was observed. In no specimen was staining weak or absent. In a single patient (patient #9) specimens were obtained both at the time of original treatment and 4 months later

from a distant subcutaneous metastasis. The original specimen stained intensely (3+) whereas the distant subcutaneous metastases stained only slightly less positively (2+). No tumor cell staining was observed on any specimens, however, when using a control mouse immunoglobulin G antibody or when no primary antibody was applied.

Table 1. Immunohistochemistry Results in MPM Specimens

Patient Number	Sex	Age (years)	IL-4R Staining
1	M	65	+++
2	M	73	+++
3	M	77	+++
4	M	69	+++
5	M	71	+++
6	M	60	+++
7	M	48	+++
8	M	79	+++
9	F	56	+++
10	M	76	++
11	M	59	++
12	F	57	++
13	M	53	++

The paraffin-embedded samples were subjected to IHC staining using anti-IL-4 receptor antibody, M-57. The field was viewed under 78X magnification.

+++ = intense staining; ++ = moderate staining.

IHC = immunohistochemistry; IL-4 = interleukin-4; IL-4R = interleukin-4 receptor; MPM = malignant pleural mesothelioma.

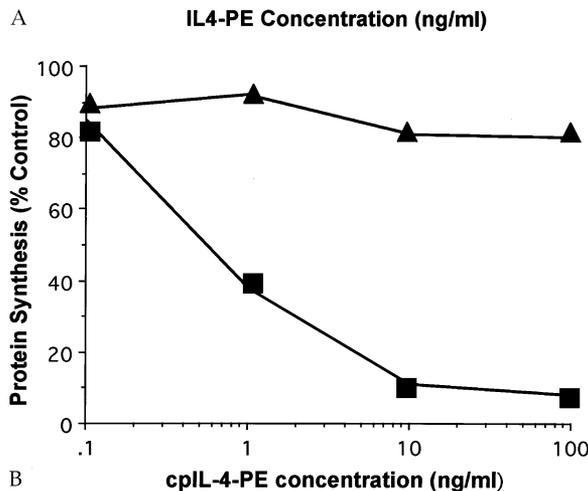
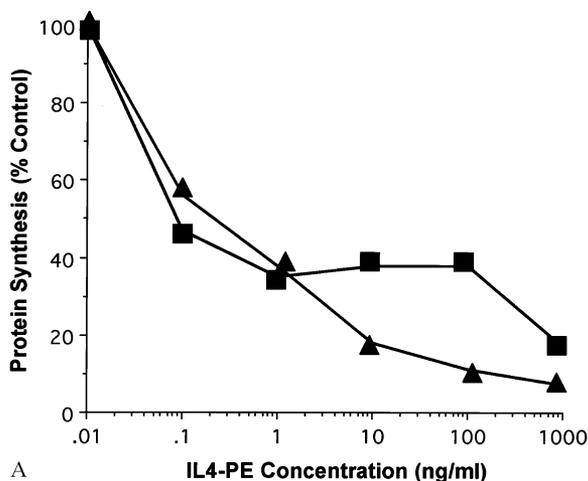


Fig 3. (A) Comparison of protein synthesis inhibition mediated by cpIL-4-PE on mesothelioma cell lines MS-1 (▲) and MSTO-211H (■). Mesothelioma cells were cultured with various concentrations of cpIL-4-PE and protein synthesis was measured by  $^3\text{H}$ -leucine incorporation after 20 hours of culture. The results are presented as the mean percentage of control of untreated cells from triplicate determinations. (B) The mesothelioma cell line MS-1 was cultured with various concentrations of cpIL-4-PE with (■) or without (▲) an excess of IL-4. Protein synthesis was measured after 20 hours of culture by incorporation of  $^3\text{H}$ -leucine. The results are presented as the mean percentage of control of untreated cells from triplicate determinations. (IL = interleukin.)

#### Cytotoxicity of cpIL-4-PE to Human Malignant Pleural Mesothelioma Cell Lines

We examined the ability of cpIL-4-PE to inhibit protein synthesis using two human MPM cell lines. Protein synthesis inhibition was determined by measuring the incorporation of  $^3\text{H}$ -leucine by treated cells. As demonstrated in Figure 3A, both MPM tumor cell lines, MS-1 and MSTO-211H, were extremely sensitive to the inhibitory effects of cpIL-4-PE. The  $\text{IC}_{50}$  was calculated to be 0.09 ng/mL and 0.2 ng/mL in MSTO-211H and MS-1 cells, respectively. The observed protein synthesis inhibition was specific for MPM as no inhibition was observed in a SV40-transformed human mesothelial cell line, MeT5A

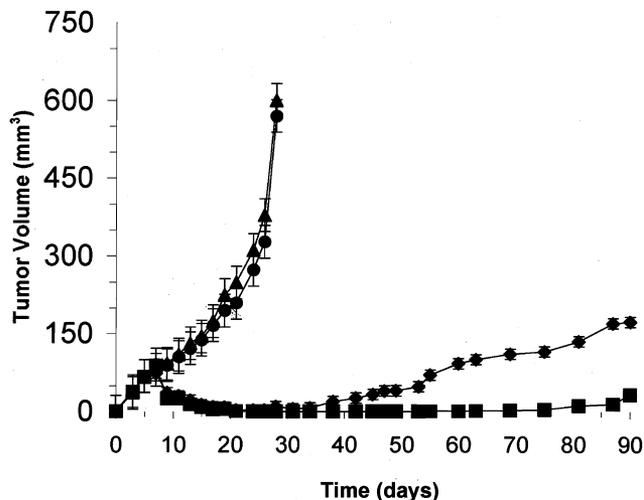


Fig 4. *In vivo* antitumor activity of cpIL-4-PE against mesothelioma tumors in nude mice. Nude mice were inoculated with  $5 \times 10^6$  Meso-1 cells on day 0 and then treated with intratumoral injections of low-dose cpIL-4-PE (◆), high-dose cpIL-4-PE (■), interleukin (IL)-4 control (●), or vehicle control (▲) only. The data presented are the mean  $\pm$  SE of the 5 animals in each group.

(data not shown). To confirm that the cpIL-4-PE-induced inhibition of protein synthesis in human MPM cell lines was manifested through the IL-4R, we cultured the MS-1 mesothelioma cell line in the presence of cpIL-4-PE alone and in combination with an excess of IL-4. As shown in Figure 3B, cpIL-4-PE caused a dose-dependent inhibition of protein synthesis that was completely abrogated by the addition of excess IL-4.

#### cpIL-4-PE Completely Regressed Subcutaneous Malignant Pleural Mesothelioma Tumors

Subcutaneous tumors in NCR nude mice were induced using the Meso-1 cell line as described in Material and Methods. For the intratumoral injection experiments, groups of 5 mice each were treated with intratumoral injection of low-dose cpIL-4-PE, high-dose cpIL-4-PE, recombinant human IL-4 or Hank's balanced salt solution. The results of subsequent tumor measurements in one representative experiment are shown in Figure 4, which demonstrates a dose-dependent decrease in tumor volume in mice treated with the cpIL-4-PE toxin but not in IL-4-treated or control mice. By day 9, after only two intratumoral injections, the tumors in both the high- and low-dose treatment groups began to regress. Tumor volumes in mice treated with 250  $\mu\text{g}/\text{kg}$  of cpIL-4-PE were significantly smaller than those seen in the control and IL-4-treated mice ( $p < 0.0001$ ); by day 21, tumors in 2 of the 5 mice had regressed completely. By day 26, however, tumors were regrowing in 4 of 5 mice, including 1 mouse that had previously appeared tumor-free. The reduction in tumor volume seen in mice treated with 750  $\mu\text{g}/\text{kg}$  of cpIL-4-PE was significantly greater than that seen in the 250- $\mu\text{g}/\text{kg}$  group ( $p < 0.0001$ ). In this low-dose

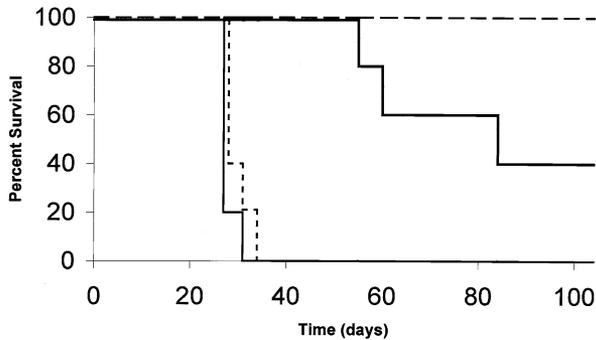


Fig 5. Survival of mice after intratumoral treatment of human mesothelioma xenografts. Groups of 5 mice were inoculated with  $5 \times 10^6$  Meso-1 cells and then treated with intratumoral injections of interleukin-4 control (—), low-dose cpIL-4-PE (—), high-dose toxin (---), or vehicle control (....). The mice were then followed for survival for up to 104 days.

group, all tumors had resolved by day 24 and only one recurred after day 49.

In another experiment, groups of 5 mice with subcutaneous flank tumors were treated with intraperitoneal injection of Hank's balanced salt solution, recombinant human IL-4, or cpIL-4-PE after subcutaneous tumor inoculation. Although intratumoral injection of this dose of cpIL-4-PE produced a significant reduction in tumor volume as compared with control, intraperitoneal dosing did not (data not shown).

#### Effects of cpIL-4-PE on Survival of Nude Mice

Subcutaneous tumors in NCR nude mice were induced as described above using the Meso-1 cell line. Groups of 5 mice each were treated with intratumoral injection of low-dose cpIL-4-PE, high-dose cpIL-4-PE, recombinant human IL-4, or Hank's balanced salt solution. The mice were then followed for survival (Fig 4). The mice were monitored by blinded veterinarian staff, who sacrificed any moribund animals when appropriate in accordance with the NIH "Guide for the Care and Use of Laboratory Animals." The survival curves are represented in Figure 5. The animals in the HBSS and IL-4 control groups all died due to progressive tumor growth by day 34 (median survival of 28 days in both groups). In the group of mice treated with 250  $\mu\text{g}/\text{kg}$  of cpIL-4-PE, 2 animals were alive at 104 days, with a median survival for the entire group of 72 days. This finding is statistically different from both control groups ( $p \leq 0.0016$ ). In the group of mice treated with 750  $\mu\text{g}/\text{kg}$  of cpIL-4-PE, all animals were alive at 104 days (median survival  $> 102$  days) and only 1 mouse had a recurrent tumor. The survival of this group was significantly prolonged from the two control groups ( $p < 0.0001$ ) and from the 250- $\mu\text{g}/\text{kg}$  group ( $p \leq 0.0494$ ). All mice were sacrificed at the end of the 104-day observation period.

#### Comment

Interleukin-4 and its receptor, IL-4R, have been known to exist on lymphoid and hematopoietic cells. The existence

of IL-4R has also been documented on a variety of nonhematopoietic malignancies [27, 28]. Because of the antiproliferative activity of IL-4 on some tumor cell lines, some investigators have proposed its use to inhibit the growth of these tumors. However, no significant antitumor effects have been observed in human IL-4 trials for cancer. Furthermore, in our human tumor xenograft model, administration of human IL-4 did not result in decreased tumor growth or prolonged survival. Therefore, another strategy has been devised to take advantage of the high expression of high-affinity IL-4R on tumor cells. An IL-4-based cytotoxin was created by the fusion of circularly permuted human IL-4 to a mutated form of *Pseudomonas* exotoxin. The resulting cytotoxin can bind to the IL-4R and mediate cell death in vitro and in various xenograft models of human cancer.

We chose human MPM as a tumor model due to its high constant expression of IL-4R. We demonstrated that established mesothelioma cell lines as well as fresh human mesothelioma tumors express significant levels of IL-4R. We then documented the in vitro sensitivity of human mesothelioma cell lines to cpIL-4-PE. Finally, we showed that intratumoral cytotoxin administration in a nude mouse model results in substantial and in some cases complete resolution of rapidly growing subcutaneous mesothelioma tumor nodules. Accomplishing this feat in this difficult tumor model suggests that the activity in vivo in humans may be significant.

Malignant pleural mesothelioma is a difficult human malignancy to treat and does not respond to standard chemotherapy. Radiation is effective only when tumor volumes are small. Despite this difficulty, MPM often remains localized for a prolonged period of time, and patients frequently die with only localized disease. The only effective therapy for reducing tumor volume is surgical debulking. Surgery of any type, however, always leaves microscopic disease that nearly always recurs. Therefore, an active cytotoxin, such as cpIL-4-PE, may afford the single best method of increasing local and possibly systemic control of this resistant tumor. Application directly to the tumor site within the pleural cavity after debulking surgical procedures may produce superior local control rates. This, in turn, may be translated into clinical "cures." Our data showed that intraperitoneal administration did not effectively reduce subcutaneous tumor volume, but we were unable to test doses higher than those given locally due to limited availability of the toxin. Previously, intraperitoneal dosing of cpIL-4-PE has been shown to have remarkable antitumor activity against glioblastoma multiforme, breast cancer, and AIDS-associated Kaposi's tumor [19-21]. Therefore, it is possible that higher intraperitoneal (systemic) doses may demonstrate antitumor activity in mesothelioma, as well.

Previous attempts at treating human tumors with immunotoxins used monoclonal antibodies tagged with various toxins [29]. The large size of the antibodies generally limited their ability to penetrate to sites of disease. The current cytotoxin using a smaller IL-4 protein molecule overcomes this problem. In addition, use of

murine monoclonal antibodies was subject to the development of human anti-mouse antibodies, which limited the effectiveness of early immunotoxin treatments [30]. The current cpIL-4-PE cytotoxin will not be hindered by this problem. Because IL-4 does not cross species barriers, it is not possible to fully assess the toxicity of cpIL-4-PE toxin in murine tumor models. However, in primate studies, the dose-limiting toxicity appears to be reversible liver dysfunction due to hepatic clearance of the *Pseudomonas* toxin. No significant immunologic abnormalities have been documented. Human administration of this toxin, both locally and systemically, is being planned.

In summary, our studies have documented a strong antitumor activity of cpIL-4-PE cytotoxin in vitro and in vivo against mesothelioma tumors. Because most tested mesothelioma tumors appeared to be strongly positive for the IL-4R expression, it is predicted that cpIL-4-PE will have strong antitumor activity against this localized but uniformly fatal tumor. Clinical trials are currently being developed.

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## DISCUSSION

**DR TRACEY L. WEIGEL** (Madison, WI): Doctor Beseth, I have one question. Did you see any difference in IL-4 receptor density in epithelial versus sarcomatous histology in the human specimens tested?

**DR BESETH:** We did not make that distinction. These specimens represented both sarcomatous and epithelial mesotheliomas, which were studied only with immunohistochemistry. The radioreceptor binding assay, which is really the way to accurately figure that out, was only done with the MS-1 cell line. So I cannot answer that question.

**DR W. ROY SMYTHE** (Houston, TX): The possibility certainly exists that IL-4 in a syngeneic animal may enhance the effect of this treatment or it could increase the toxicity.

**DR BESETH:** Right.

**DR SMYTHE:** As you know, IL-4 is a primary modulator of asthma and infiltration of eosinophils, and we know what sort of

damage that can cause in humans with activation and degranulation. Is the human IL-4 sequence utilized in your experiments sufficiently homologous to that of rodents to use this in a syngeneic model, and do you plan to repeat these experiments in syngeneic animals?

**DR BESETH:** That is a very good question. IL-4 does not cross species barriers, so, in effect, when you use a human IL-4-based molecule to treat a human tumor, you cannot expect to see IL-4-based toxicities in mice, whose receptors will not respond to that. You know, I am not sure. I do not even know that mouse IL-4 is available in sufficient quantities to make the toxin. I think that that will not be a future avenue for research. In studies that have been done to date, in terms of primate studies (and I don't know how close primate IL-4 receptor is to human IL-4 receptor), the toxicity that has been seen is a reversible hepatotoxicity based on dose. Even there, however, I am not sure how big the species barrier is between monkeys and humans.