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Advances in Brief

Targeting Interleukin-4 Receptors for Effective Pancreatic Cancer Therapy[1]

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Abstract

We demonstrate that pancreatic cancer tissues express receptors for interleukin (IL)-4 *in situ* at high density. Using the approach of selective receptor targeting, we have tested the efficacy of a recombinant cytotoxin IL4-*Pseudomonas* exotoxin A, which is composed of a targeting moiety (IL-4) and a mutated form of *Pseudomonas* exotoxin. Our results demonstrate that this molecule exerts vigorous antitumor activity against human pancreatic tumors implanted s.c. in immunodeficient animals. Sixty percent of animals treated with intratumoral injections of IL4-*Pseudomonas* exotoxin A experienced complete disappearance of established tumors. Animals with pancreatic tumors implanted orthotopically exhibited prolonged survival that was significantly greater by comparison with untreated animals. Thus, IL-4 receptor-targeted cytotoxin represents a potent agent that may provide an effective therapy for pancreatic cancer.

Introduction

Pancreatic adenocarcinoma is one of the major causes of cancer-related deaths in the United States, with 28,900 patients dying in 1998 (1). Although many approaches are used to treat this malignancy, including surgery and chemotherapy, median survival after diagnosis is 6 months. Treatment-related survival has not improved in the past 30 years (2). An effective new approach against this aggressive disease is urgently needed. We have investigated a novel approach to eradicating pancreatic cancers using an IL-4R[3]-targeted cytotoxin. IL-4Rs have been reported to be expressed in a wide variety of murine and human carcinoma cells *in vitro* and *in vivo* (3, 4). The significance of expression of IL-4Rs on solid tumor cells is not completely understood; however, we and others have observed that solid human tumors respond to IL-4 (4, 5). IL-4 has been shown to have a modest but direct inhibitory effect on the growth of hematopoietic and nonhematopoietic tumor cell lines *in vitro* and *in vivo* (6, 7). Based on these results, IL-4 was tested in the clinic as a treatment for hematopoietic and nonhematopoietic malignancies (3, 4, 8). Clinical results were disappointing, and it appears that further clinical research using IL-4 as an anticancer agent is not being pursued. We have used a different approach, wherein we decided to target overexpressed IL-4R in human solid cancer cells. We therefore produced a recombinant fusion cytotoxic agent that targets IL-4R expressed on tumor cells (4, 9, 10). This molecule is a chimeric protein composed of a circular permuted IL-4 and a truncated form of the bacterial PE [IL4(38-37)-PE38KDEL or IL4-PE]. We have shown that this recombinant toxin is highly cytotoxic to IL-4R-positive tumors in preclinical and Phase I clinical studies (4, 11).

In the current study we report that IL-4Rs are overexpressed on pancreatic cancer cell lines *in vitro* and in tumor specimens derived from patients with pancreatic cancer. On the other hand, IL-4Rs are barely detectable in normal pancreas. Furthermore, overexpressed IL-4Rs on pancreatic cancer cells can be successfully targeted *in vitro* and in orthotopic or s.c. animal models of human pancreatic cancer. IL4-PE induced regression of established pancreatic tumors and enhanced survival of these immunodeficient animals.

Materials and Methods

Recombinant Toxin and Cell Lines. Recombinant IL-4 toxin IL4(38-37)-PE38KDEL, containing the circularly permuted IL-4 mutant in which amino acids 38–129 were linked to amino acids 1–37 via a GGNGG linker and then fused to truncated toxin PE38KDEL, consisting of amino acids 253–364 and 381–608 of PE followed by KDEL, was expressed in *Escherichia coli*, purified by modified procedure as described previously, and provided by Neurocrine Biosciences Inc. (9, 10). Human pancreatic cancer cell lines (PANC-1 and BxPC-3) were purchased from the American Type Culture Collection. Cells were cultured in DMEM (PANC-1) or RPMI 1640 (BxPC-3) containing 10% fetal bovine serum (Biowhittaker Inc., Walkersville, MD), 1 mM HEPES, 1 mM L-glutamine, 100 μg/ml penicillin, and 100 μg/ml streptomycin (Biowhittaker Inc.).

Immunohistochemistry. Immunohistochemistry was performed using the Vector ABC peroxidase kit according to the manufacturer's instructions (Vector Laboratories, Inc., Burlingame, CA). Paraffin-embedded tissue sections were deparaffinized by xylene treatment and washed with alcohol (100% to 50%) and PBS. Sections were incubated with monoclonal antibody against human IL-4R [M57; kindly supplied by Immunex Corp. (Seattle, WA); 20 μg/ml) or isotype control (IgG1) for 18 h at 4°C. Slides were then developed using 3,3′-diaminobenzidine substrate biotinylated peroxidase reagent (Vector Laboratories, Inc.) and counterstained with hematoxylin (Sigma). Immunohistochemical assays were performed two to three times independently with similar results, and slides were assessed by two independent investigators. The percentage of positive fields was counted in a blinded fashion by viewing the entire tumor section under the same magnification.

RT-PCR. Total RNA extracted from the paraffin-embedded tissue sections (30 μm) using a paraffin block RNA isolation kit (Ambion, Inc., Austin, TX) was analyzed for IL-4Rα chain mRNA expression by RT-PCR. Specific primers were used as described previously (12).

Radioreceptor Binding Assays. Cells (1×10^6) in 100 μl of binding buffer (RPMI 1640 containing 0.2% human serum albumin and 10 mM HEPES) were incubated with 200 pM ^{125}I-IL-4 with or without various concentrations (10 pM to 100 nM) of unlabeled IL-4 at 4°C for 2 h. The number of IL-4Rs and binding affinity were calculated using the LIGAND program.

Protein Synthesis Inhibition Assay. The cytotoxic activity of IL4-PE was tested as described previously (10). Typically, 10^4 cells were cultured in leucine-free medium with or without various concentrations of IL4(38-37)-PE38KDEL for 20–22 h at 37°C. Then 1 μCi of [^{3}H]leucine (NEN Research Products, Boston, MA) was added to each well and incubated for an additional 4 h. Cells were harvested, and the radioactivity incorporated into cells was measured by a β plate counter (Wallac).

s.c. Xenografted Pancreatic Tumor Model. Four-week-old athymic nude mice (about 20 g in body weight) were obtained from Frederick Cancer Center Animal Facilities (National Cancer Institute, Frederick, MD). Animal care was

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[3] The abbreviations used are: IL-4R, interleukin-4 receptor; IL, interleukin; RT-PCR, reverse transcription-PCR; PE, *Pseudomonas* exotoxin A.

in accordance with the guidelines of the NIH Animal Research Advisory Committee. Human pancreatic tumor models were established in the nude mice by s.c. injection of PANC-1 or BxPC-3 tumor cells (5×10^6) in 150 μl of PBS plus 0.2% human serum albumin into the flank. Palpable tumors developed within 3–4 days. Tumors were measured by Vernier calipers. In general, five mice were used for each group. To assess the antitumor activity, mice were injected with IL4-PE intratumorally or i.p.

Orthotopically Xenografted Pancreatic Tumor Model. Athymic nude mice were anesthetized with ketamine and xylazine and placed in the supine position. A left lateral abdominal incision was made, and the pancreas was exteriorized. The tumor pieces ($5 \times 5 \times 5$ mm) obtained from s.c. growing tumors were transplanted to the body of pancreas, ligating with absorbable surgical sutures.

Results and Discussion

IL-4R Expression in Normal Pancreas and Pancreatic Cancer Specimens. First we performed immunohistochemical staining using anti-IL-4R antibody (M57) in 12 normal and malignant specimens from human pancreas to assess whether pancreatic cancer tissue specimens express IL-4R *in situ*. Normal pancreas tissues were obtained from the surgical resection of benign pancreatic cyst (sample numbers 19230, 19926, 20416, and 0109c183a) or duodenal polyps (sample number 0110c267b). As shown in Fig. 1*A* and Table 1, one of five normal pancreas tissue samples showed weak to moderate positive staining for IL-4R, whereas four of five samples did not stain

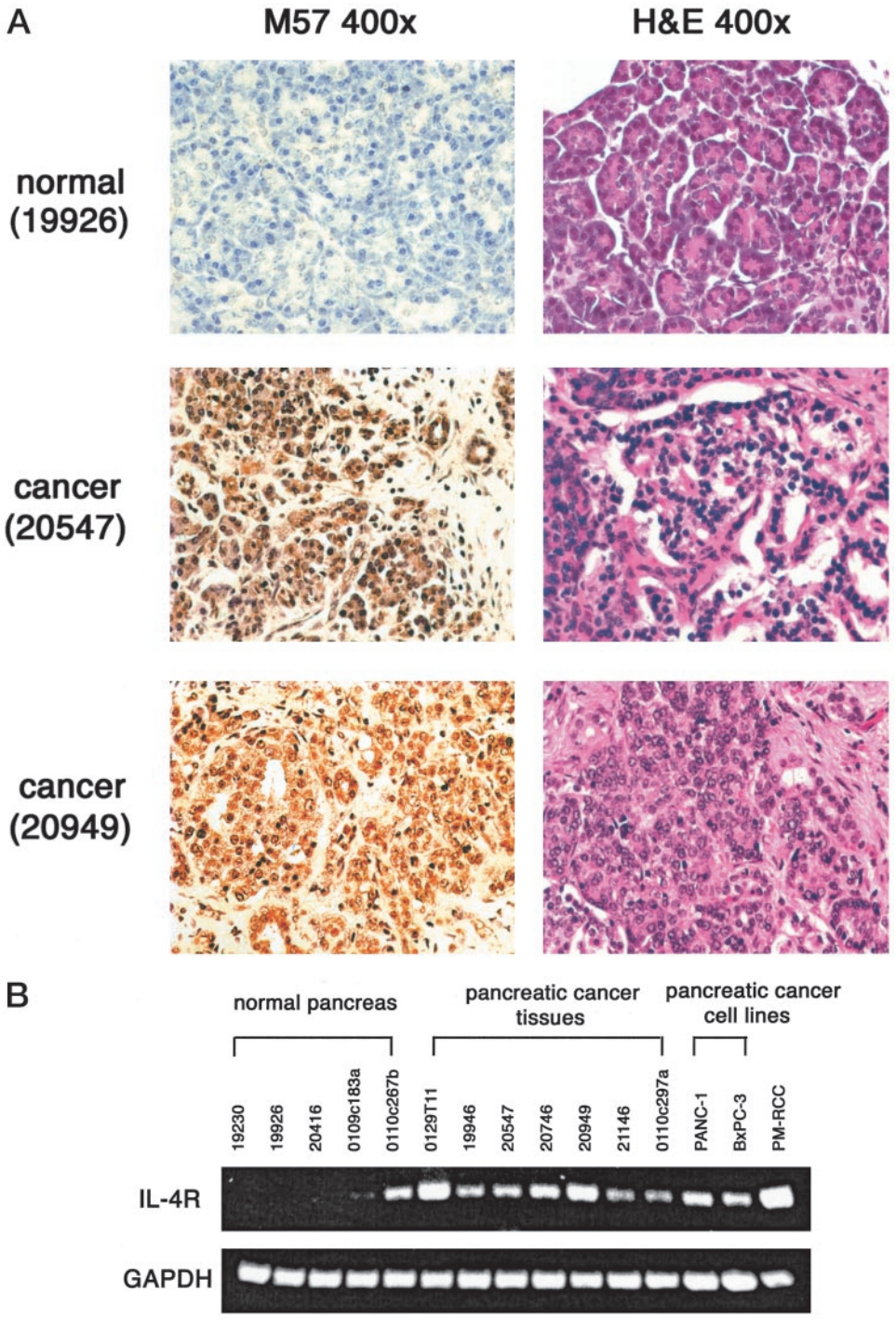

Fig. 1. *In situ* expression of IL-4R in normal pancreas and cancer tissue specimens. Surgically resected tissues were stained with M57 antibody, incubated with biotinylated secondary antibody, and color-developed using diaminobenzidine tetrahydrochloride substrate. Representative results from 12 specimens are presented (*A*). Total RNA extracted from the matching paraffin-embedded tissue sections (30 μm) was analyzed for IL-4R mRNA expression by RT-PCR. Renal cell carcinoma cell line PM-RCC served as a positive control, and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) served as an internal control (*B*).

Table 1 *In situ expression of IL-4R in normal pancreas and pancreatic cancer sections by immunohistochemistry*

	Patient information		Staining	
Sample ID no.[a]	Age/sex	Diagnosis	Intensity	% Fields
19230	49/F	Normal pancreas	−	0
19926	63/F	Normal pancreas	−	0
20416	20/M	Normal pancreas	−	0
0109c183a	40/M	Normal pancreas	+	10
0110c267b	28/M	Normal pancreas	±	5[b]
0129T11	79/F	Islet cell neoplasm	++	90
19946	73/F	Adenocarcinoma	++	90
20547	55/M	Adenocarcinoma	++	40
20746	50/F	Large cell lymphoma[c]	+	30
20949	56/M	Adenosquamous carcinoma	+++	80
21146	74/M	Cystoadenocarcinoma	++	30
0110c297a	47/F	Neuroendocrine carcinoma	++	90

[a] ID, identification.
[b] Sample includes fibroblast regions stained with M57.
[c] Metastasis to pancreas.

with anti IL-4R antibody. In contrast, all seven pancreatic cancer specimens expressed IL-4R, and staining was much more intense when compared with normal pancreas (Fig. 1*A*). Between 30% and 90% of tumor cells were positive for IL-4R in pancreatic cancer specimens.

We also extracted total RNA from paraffin-embedded normal pancreas and pancreatic tumor tissues (the same samples as listed in Table 1) and assessed for IL-4R mRNA expression by RT-PCR analysis (Fig. 1*B*) using specific primer sets for the IL-4Rα chain. Similar to the results obtained for immunohistochemical analysis, RT-PCR results demonstrated that one of five samples of normal pancreas and seven of seven samples of pancreatic cancer were positive for IL-4R mRNA. In addition, two pancreatic cancer cell lines also expressed mRNA for the IL-4Rα chain.

We additionally performed binding studies using ^{125}I-IL-4. Scatchard analysis of results generated by binding assays using the PANC-1 cell line demonstrated that these cells express high numbers of IL-4 binding sites (9200 sites/cell with a K_d value of 370 pM; data not shown). These results suggest that IL-4R is overexpressed in pancreatic cancer when compared with normal pancreas tissue and thus may serve as a unique target for IL-4R-directed cancer therapy.

***In Vitro* Cytotoxicity of IL4-PE to Pancreatic Cancer Cell Lines.** We then examined IL4-PE-mediated cytotoxicity *in vitro* by monitoring inhibition of protein synthesis in the pancreatic cancer cell lines PANC-1 and BxPC-3. The protein synthesis inhibition has been shown to be directly proportional to cell death (13). As shown in Fig. 2*A*, IL4-PE was found to be highly cytotoxic to both pancreatic cancer cell lines. The IC_{50} of IL4-PE was 0.3–0.5 ng/ml in both cell lines. The cytotoxic activity of IL4-PE was neutralized by incubation with

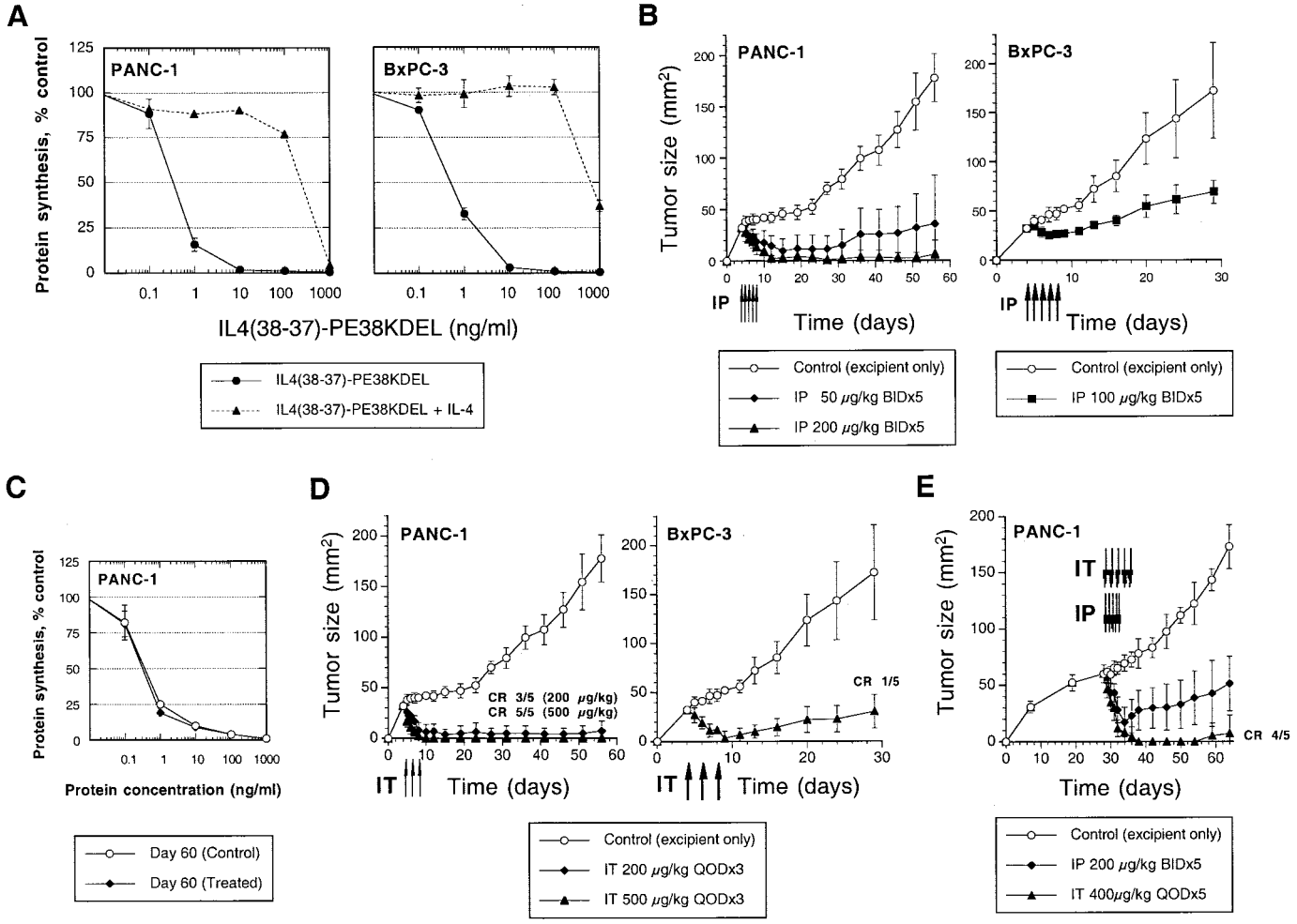

Fig. 2. Antitumor activity of IL4-PE *in vitro* and *in vivo*. *In vitro* cytotoxic activity of IL4-PE (0–1000 ng/ml) to pancreatic cancer cells cultured with or without excess IL-4 (2 μg/ml) was assessed by protein synthesis inhibition assay (*A*). Pancreatic tumors xenografted s.c. in immunodeficient mice were treated with IL4-PE either i.p. (*IP*; *B*) or intratumorally (*IT*; *D*) at the indicated time period. PANC-1 tumors treated i.p. (*B*) were resected at day 60, digested with collagenase and DNase, and cultured. After two passages, the sensitivity of the cells to IL4-PE was confirmed by protein synthesis inhibition assays (*C*). Antitumor activity of IL4-PE in the larger-sized (60.1 ± 8.0 or 211.4 ± 51.4 mm^3) PANC-1 tumors was also assessed by both i.p. and intratumoral routes of administration of the drug at the indicated period (*E*).

an excess amount of IL-4, suggesting specific cytotoxicity due to binding of IL4-PE to IL-4R (13, 14). We also used an irrelevant cytotoxin IL13-PE (IL13-PE38QQR), an IL-13 receptor-specific fusion protein (15), to assess the cytotoxicity to BxPC-3 cells. However, IL13-PE was not cytotoxic to BxPC-3 cells (IC_{50} = 700 ng/ml; data not shown).

***In Vivo* Antitumor Activity of IL4-PE against s.c. Xenografted Pancreatic Cancer in Animals.** Next we examined the *in vivo* antitumor activity of IL4-PE against pancreatic tumor xenografts implanted s.c. in immunodeficient mice. Control PANC-1 and BxPC-3 tumor-bearing mice were injected with excipient control, which showed progressive and linear growth of s.c. tumors. In mice treated with i.p. IL4-PE injections (50–200 μg/kg/day, twice a day $\times$ 5 days from day 4 to day 8), tumor growth was dramatically suppressed (Fig. 2*B*). Although tumor growth resumed gradually in PANC-1 tumor-bearing animals after a long period, two of five animals in the 50 μg/kg dose group and four of five animals in the 200 μg/kg dose group remained tumor free until the last day of the experiment (day 56). In BxPC-3 tumor-bearing mice, tumors were also significantly reduced by i.p. injections of IL4-PE (100 μg/kg/day) on day 29 ($P < 0.03$ compared with untreated control tumors).

To determine whether tumor regrowth was due to selection and growth of IL-4R-negative tumor cells or development of drug resistance, PANC-1 tumors were resected on day 60 from animals either untreated or treated with IL4-PE (100 μg/kg/day) and placed into culture as dispersed cells. After couple of passages, when cell debris and infiltrating cells had disappeared, tumor cells were tested for their sensitivity to IL4-PE. Tumor cells from control and IL4-PE-treated animals maintained their equal sensitivity to IL4-PE as demonstrated by the protein synthesis inhibition assay (Fig. 2*C*). These results demonstrate that neither selection of IL-4R-negative tumor cells nor *in vivo* drug resistance has developed. The recurrence of tumors or partial regression of tumor could be due to inadequate IL4-PE levels at the s.c. tumor site.

To maximize the availability of IL4-PE at the tumor site, s.c. implanted pancreatic cancer xenografts were also treated with IL4-PE by intratumoral administration [200 or 500 μg/kg/day, three times (day 4, 6, and 8)]. Tumors begun regressing immediately upon initiation of intratumoral treatment with IL4-PE in both PANC-1 and BxPC-3 tumor-bearing animals (Fig. 2*D*). Three injections of IL4-PE were enough to eliminate tumors in 9 of 15 animals. PANC-1 tumors were more sensitive to intratumoral injections as indicated by the complete disappearance of established tumors in three of five animals treated with the 200 μg/kg dose and in five of five animals treated with the 500 μg/kg dose of IL4-PE when evaluated on day 59. Although four of five BxPC-3 tumors completely regressed by day 11, tumors recurred in three of these mice, with only one mouse remaining tumor free at day 29. Nonetheless, mean tumor size (30 mm^2) was significantly smaller when compared with that of untreated tumors (172 mm^2; $P < 0.008$). Thus, higher IL4-PE concentration at the tumor site was responsible for better tumor response.

We also assessed antitumor activity of IL4-PE in animals bearing larger, established PANC-1 tumors. Twenty-eight days after the tumor implantation, when tumor size had reached to 60.1 $\pm$ 8.0 mm^2 (211.4 $\pm$ 51.4 mm^3), mice received IL4-PE by either i.p. (200 μg/kg/day, twice a day $\times$ 5 days from day 28 to day 32) or intratumoral (400 μg/kg/day $\times$ 5 at day 28, 30, 32, 34, and 36) routes. During the IL4-PE treatment period, tumors began to rapidly regress by both routes of administration (Fig. 2*E*). Remarkably, intratumoral administration of IL4-PE was able to eradicate tumors in all animals at the end of the treatment. This dramatic tumor response was stable because only one mouse showed a very small tumor on day 58. Complete responder animals remained complete responders for as long as we maintained these animals. Although no tumors disappeared completely by i.p. treatment, mean tumor size (51 mm^2) at day 64 was significantly smaller compared with that of untreated control tumors (172 mm^2; $P < 0.0005$). These results indicate that IL4-PE has significant antitumor activity in two models of s.c. implanted pancreatic tumors.

***In Vivo* Antitumor Activity of IL4-PE against Orthotopically Implanted Pancreatic Cancer in Immunodeficient Mice.** To mimic the clinical aggressiveness of pancreatic cancer, we developed an orthotopic tumor model involving surgical implantation of tumor pieces in immunodeficient mice. This mode of tumor implantation produced consistent results, and 100% of animals showed aggressive tumor growth invading pancreas and spleen. Animals bearing PANC-1 tumors were injected with either excipient only (control) or IL4-PE (100 μg/kg/day, twice a day $\times$ 5 days from day 4 to day 8 after the implantation) i.p. In the control group, animals developed large tumors that were visible transcutaneously and palpable at 3–4 weeks. In contrast, tumors in IL4-PE-treated animals remained nonpalpable and nonvisible until termination. Animals were sacrificed at day 90, and tumor volumes and histology of the pancreas were evaluated. In the control group, animals developed large tumors attached to the pancreas (Fig. 3*A*), with a mean tumor volume of 2557 $\pm$ 716 mm^3 (data not shown). Tumors were invasive to both the spleen (Fig. 3*C*) and pancreas (Fig. 3, *D* and *E*). To our surprise, all animals that received IL4-PE remained completely tumor free and without any evidence of cancer detected in the pancreas (Fig. 3*F*) or other organs.

Next we determined overall survival of animals harboring human pancreatic tumors. Nude mice orthotopically implanted with BxPC-3 tumor were i.p. treated with IL4-PE (200 μg/kg/day, twice a day $\times$ 5 days) from day 4 to day 8. As shown in Fig. 3*G*, the survival of these animals was significantly prolonged. Median survival time was 54 days (range, 36–68 days; $n = 7$) in control animals and 112 days (range, 58–149 days; $n = 7$) in treated animals (Fig. 3*G*). The prolonged survival in treated animals represented a 207% increase compared with control animals. We also determined the effect of IL4-PE on the survival of animals harboring large pancreatic tumors. These mice were allowed to grow pancreatic tumors for 14 days after surgical implantation in pancreas. Mice harboring PANC-1 tumors were treated i.p. with IL4-PE (100 μg/kg/day, twice a day $\times$ 5 days from day 14 to day 18), and overall survival was monitored. As shown in Fig. 3*H*, median survival time of animals was 99 days (range, 72–127 days; $n = 6$) in control animals harboring PANC-1 tumors, whereas the median survival was significantly increased ($P < 0.05$) to 167 days (range, 106–310 days; $n = 6$) in IL4-PE-treated animals. Prolonged survival rate in treated animals was 169% compared with control animals. These results suggest that IL4-PE treatment reduces tumor size and significantly prolongs survival of animals bearing large pancreatic tumors. In addition, we did not observe any toxicity in organs (heart, liver, lung, kidney, and spleen) of IL4-PE-injected animals assessed by histological examination (data not shown).

There have been only a few reports demonstrating the use of effective targeted strategies involving monoclonal antibodies or immunotoxins to treat pancreatic cancer (16, 17). These strategies proved limited because expression of the target antigens was inadequate to support killing of all cancer cells (18). In addition, few agents have been shown to cause regression of advanced pancreatic cancer in humans (2). Because we found here that abundant IL-4R expression *in situ* facilitates efficient targeting of IL4-PE, IL-4R-directed cancer therapy could be beneficial to patients with pancreatic cancer.

Preclinical pharmacological and toxicity studies with IL4(38-37)-PE38KDEL have been performed in various animal models (4). In mice, IL4-PE was very well tolerated, and the LD_{50} was determined

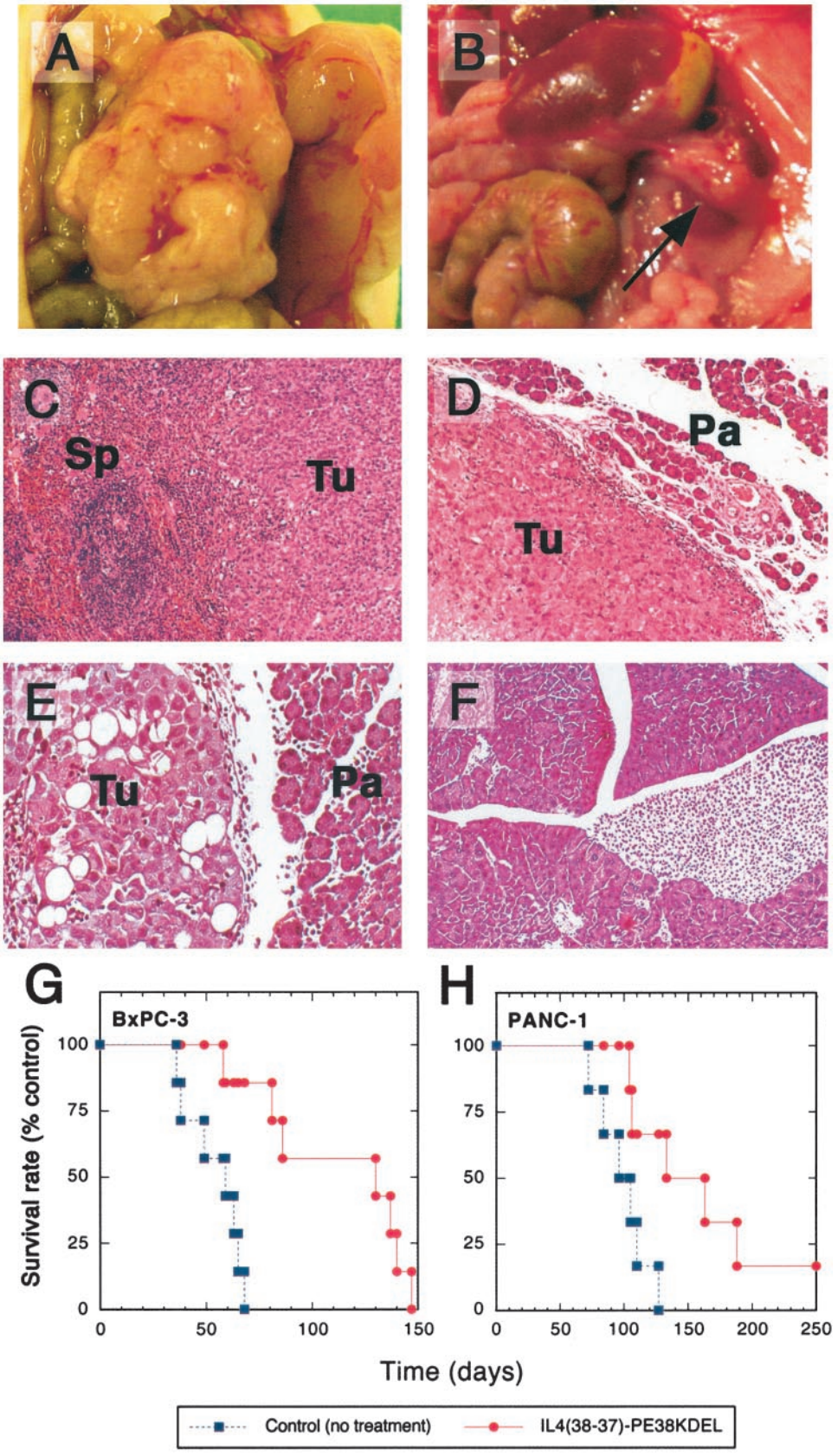

Fig. 3. IL4-PE shows potent antitumor activity in a surgically implanted orthotopic model of pancreatic tumors in immunodeficient mice. Orthotopic pancreatic tumors (PANC-1) were treated i.p. with excipient only (control) or IL4-PE (100 μg/kg/day, twice a day $\times$ 5 days from day 4 to day 8 after the implantation). On day 90, mice were sacrificed, and tumors and surrounding tissues were evaluated. Control mice demonstrated large tumors (*A*) invading the spleen (*C*) and/or pancreas (*D* and *E*; magnification: *D*, $\times$100; *E*, $\times$200). Tumors treated with IL4-PE completely disappeared in all of the mice ($n = 4$; *B* and *F* demonstrate intact pancreas). Orthotopic pancreatic tumors were treated with i.p. administration of IL4-PE (BxPC-3 tumors treated with 200 μg/kg/day, twice a day during day 4–8, *G*; PANC-1 tumors treated with 100 μg/kg/day, twice a day during day 14–18, *H*), and survival was monitored. *Sp*, spleen; *Tu*, tumor; *Pa*, pancreas.

to be 475 μg/kg when given i.v. every other day for three injections (19). In addition, in the present study, IL4-PE doses of up to 200 μg/kg given twice a day i.p. for 5 days and up to 500 μg/kg given intratumorally every other day for three injections were extremely well tolerated without any organ toxicity or mortality. In monkeys, i.v. administration of IL4-PE at doses of 50 and 200 μg/kg was also tolerated with reversible hepatic toxicity (20).[4] Because human IL-4 binds monkey cells, our studies suggest that IL4-PE can be safely administrated in the clinic in the doses of up to 200 μg/kg. Because pancreatic cancer is a localized disease with early metastasis to the peritoneal organs, IL4-PE may be administered intratumorally or by i.p. pumps as an effective therapy. Therefore, additional studies should be performed, and additional primary specimens should be examined for the expression of IL-4R *in situ*. IL4-PE is being tested in a Phase I clinical trial for recurrent malignant glioma (11), advanced renal cell carcinoma, and breast carcinoma. Perhaps a new Phase I clinical trial be initiated in patients with advanced pancreatic cancer to determine safety and tolerability of IL4-PE when given by intratumoral or i.p. routes.

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References

1. Landis, S. H., Murray, T., Bolden, S., and Wingo, P. A. Cancer statistics, 1998. CA Cancer J. Clin., *48:* 6–29, 1998.
2. Lillemoe, K. D. Current management of pancreatic carcinoma. Ann. Surg., *221:* 133–148, 1995.
3. Puri, R. K. Structure and function of interleukin-4 and its receptor. *In:* R. Kurzrock (ed.), Cytokines: Interleukins and Their Receptors, pp. 143–185. Norwell, MA: Kluwer Academic, 1995.
4. Kawakami, K., Kawakami, M., and Puri, R. K. Overexpressed cell surface interleukin-4 receptor molecules can be successfully targeted for antitumor cytotoxin therapy. Crit. Rev. Immunol., *21:* 299–310, 2001.
5. Hoon, D. S. B., Okun, E., Banez, M., Irie, R. F., and Morton, D. L. Interleukin 4 alone and with γ-interferon or tumor necrosis factor inhibits cell growth and modulates cell surface antigens on human renal cell carcinomas. Cancer Res., *51:* 5687–5693, 1991.
6. Paul, W. E. Interleukin-4: a prototypic immunoregulatory lymphokine. Blood, *77:* 1859–1870, 1991.
7. Volpert, O. V., Fong, T., Koch, A. E., Peterson, J. D., Waltenbaugh, C., Tepper, R. I., and Bouck, N. P. Inhibition of angiogenesis by interleukin 4. J. Exp. Med., *188:* 1039–1046, 1998.
8. Atkins, M. B., Vachino, G., Tilg, H., Karp, H. J., Robert, N. J., Kappler, K., and Mier, J. W. Phase I evaluation of thrice daily intravenous bolus interleukin-4 in patients with refractory malignancy. J. Clin. Oncol., *10:* 1802–1809, 1992.
9. Kreitman, R. J., Puri, R. K., and Pastan, I. A circularly permuted recombinant interleukin 4 toxin with increased activity. Proc. Natl. Acad. Sci. USA, *91:* 6889–6893, 1994.
10. Puri, R. K., Leland, P., Obiri, N. I., Husain, S. R., Mule, J., Pastan, I., and Kreitman, R. J. An improved circularly permuted interleukin 4-toxin is highly cytotoxic to human renal cell carcinoma cells. Cell. Immunol., *171:* 80–86, 1996.
11. Rand, R. W., Kreitman, R. J., Patronas, N., Varricchio, F., Pastan, I., and Puri, R. K. Intratumoral administration of recombinant circularly permuted interleukin-4-*Pseudomonas* exotoxin in patients with high-grade glioma. Clin. Cancer Res., *6:* 2157–2165, 2000.
12. Murata, T., Obiri, N. I., Debinski, W., and Puri, R. K. Structure of IL-13 receptor: analysis of composition in cancer and immune cells. Biochem. Biophys. Res. Commun., *238:* 90–94, 1997.
13. Kawakami, K., Leland, P., and Puri, R. K. Structure, function, and targeting of interleukin 4 receptors on human head and neck cancer cells. Cancer Res., *60:* 2981–2987, 2000.
14. Obiri, N. I., Murata, T., Debinski, W., and Puri, R. K. Modulation of interleukin (IL)-13 binding and signaling by the γ_c chain of the IL-2 receptor. J. Biol. Chem., *272:* 20251–20258, 1997.
15. Debinski, W., Obiri, N. I., Pastan, I., and Puri, R. K. A novel chimeric protein composed of interleukin 13 and *Pseudomonas* exotoxin is highly cytotoxic to human carcinoma cells expressing receptors for interleukin 13 and interleukin 4. J. Biol. Chem., *270:* 16775–16780, 1995.
16. Gold, D. V., Alisauskas, R., and Sharkey, R. M. Targeting of xenografted pancreatic cancer with a new monoclonal antibody, PAM4. Cancer Res., *55:* 1105–1110, 1995.
17. Giantonio, B. J., Alpaugh, R. K., Schultz, J., McAleer, C., Newton, D. W., Shannon, B., Guedez, Y., Kotb, M., Vitek, L., Persson, R., Gunnarsson, P. O., Kalland, T., Dohlsten, M., Persson, B., and Weiner, L. M. Superantigen-based immunotherapy: a Phase I trial of PNU-214565, a monoclonal antibody-staphylococcal enterotoxin A recombinant fusion protein, in advanced pancreatic and colorectal cancer. J. Clin. Oncol., *15:* 1994–2007, 1997.
18. Kreitman, R. J. Immunotoxins in cancer therapy. Curr. Opin. Immunol., *11:* 570–578, 1999.
19. Kreitman, R. J., Puri, R. K., and Pastan, I. Increased antitumor activity of a circularly permuted interleukin 4-toxin in mice with interleukin 4 receptor-bearing human carcinoma. Cancer Res., *55:* 3357–3363, 1995.
20. Puri, R. K., Hoon, D. S., Leland, P., Snoy, P., Rand, R. W., Pastan, I., and Kreitman, R. J. Preclinical development of a recombinant toxin containing circularly permuted interleukin 4 and truncated *Pseudomonas* exotoxin for therapy of malignant astrocytoma. Cancer Res., *56:* 5631–5637, 1996.

[4] R. J. Kreitman, R. K. Puri, and I. Pastan, unpublished results.

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