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14. Peritoneal macrophages from normal BALB/c mice were obtained after thioglycolate stimulation and plated at a concentration of 1×10^6 per well in 24-well plates. After nonadherent cells were washed, they were infected with 3×10^6 stationary-phase *L. amazonensis* (isolates MHOM/BR/85/BA-32, MHOM/BR/87/BA-125, or MHOM/BR/89/BA-276) promastigotes per well for 3 hours. Extracellular parasites were removed and the cells were cultured for another 72 hours in a humid atmosphere of 5% CO_2 at 35°C. TGF-β was assayed with the use of the CCL64 cell line (*13*).
15. Footpads were fixed in buffered formaldehyde and embedded in paraffin. Sections were treated with hyaluronidase (1 mg/ml; Sigma) for 30 min at room temperature, blocked with 10% normal goat serum in phosphate-buffered saline (PBS) for 30 min, and incubated with biotinylated MAb 1D11.16 (150 μg/ml) or mouse immunoglobulin G overnight at 4°C. After incubation with avidin–alkaline phosphatase (Biogenex, San Ramon, CA; 1:10; 20 min), the substrate [Fast-Red (1 mg/ml; Sigma) in tris-HCl buffer (pH 8.2) with 0.02% naphtol AS-MX phosphate (Sigma)] was applied for 20 min. Slides were counterstained with Mayer's hematoxylin.
16. BALB/c or C57BL/6 mice (five per group) were infected subcutaneously in the left hind footpad with 5×10^6 stationary-phase promastigotes of *L. amazonensis* (BA-125) or *L. braziliensis* (MHOM/BR/87/BA-92). The course of infection was followed by serial measurements of both footpads with a dial gauge caliper (Starret, Athol, MA) and was expressed in millimeters (mean ± SEM) as the difference between the infected and the contralateral footpad. For histological studies, footpads were harvested 5 weeks after infection, fixed in buffered formalin, and processed for hematoxylin-eosin stain.
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An Eosinophil-Dependent Mechanism for the Antitumor Effect of Interleukin-4

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Murine interleukin-4 (IL-4) exhibits potent antitumor activity when present at the site of tumor cell challenge. Associated with tumor cell death is the appearance of an inflammatory infiltrate comprised predominantly of eosinophils and macrophages, but with few lymphocytes. Antibodies that specifically block the accumulation of granulocytes at the site of inflammation were injected in vivo to define the cell type responsible for the antitumor action of IL-4. These studies implicate eosinophils in IL-4–mediated tumor cytotoxicity. The lymphoid-independent nature of IL-4 action is supported by the analysis of mutant mouse strains with defined lymphocyte immunodeficiencies. The observed regression of established tumor masses by localized IL-4 action provides a rationale for exploring IL-4–mediated tumor killing as a potential therapy for human malignant disorders.

In a previous report (*1*), tumor cells engineered to produce IL-4 by transfection had reduced or absent tumorigenicity when reintroduced into animals. The antitumor activity of IL-4 was also observed on untransfected tumor cells when they were mixed with IL-4–producing cells before host inoculation. Using these transplantation assays, we showed that the antitumor activity of IL-4 acted in a dose-dependent manner on a wide variety of tumor cell types in vivo but not in vitro. Histologic analyses of five distinct tumor types inhibited by IL-4 (plasmacytomas, melanomas, B cell lymphomas, mammary adenocarcinomas, and glioblastomas) have revealed a characteristic host inflammatory reaction associated with tumor cell killing, which consists of the early influx (within 18 hours of tumor cell inoculation) of large numbers of eosinophils, and then macrophage infiltration and, subsequently, fibroblastic scarring.

Given the invariant histologic finding of eosinophil infiltration associated with tumor cell death, we sought to investigate whether IL-4 antitumor activity required the action of eosinophils. To accomplish this, we depleted animals of mature granulocytes (neutrophils and eosinophils) with the monoclonal antibody (MAb) RB6-8C5, which binds to a surface antigen present on mature murine granulocytes. This MAb is specifically cytotoxic for granulocytes and does not bind cells of the monocyte-macrophage or lymphocyte lineages (*2*). Eosinophil depletion with RB6-8C5 was first demonstrated by measurng blood eosinophil concentrations from mice parasitized with the helminth *Nippostrongylus brasiliensis* in the presence or absence of antibody (Table 1). The marked eosinophilia associated with this infection could be reversed within 48 hours by the administration of a single intraperitoneal dose (0.25 mg or more) of the MAb. Antibody treatment also depleted neutrophils and could be demonstrated by the reduction of blood neutrophils after administering comparable doses of RB6-8C5 to normal mice (>95% reduction within 48 hours).

To study the effect of host granulocyte depletion on the antitumor activity of IL-4, we studied two distinct tumor types, the murine BALB/c-derived plasmacytoma J558L and the murine melanoma B16, a spontaneously arising tumor from a C57BL/6 mouse. Both of these cell lines reproducibly form subcutaneous tumors in syngeneic and immunodeficient hosts (*1*). The localized expression of IL-4 at the site of tumor cell transplantation, either by direct tumor cell transfection with an activated IL-4 gene (*3*) or by mixing tumor cells with IL-4–producing cells, eradicates

Table 1. Effect of RB6-8C5 antibody administration on eosinophilia in *Nippostronglyus* (*Nb*)–infected BALB/c mice. A single intraperitoneal dosage of the antibody was administered on day 0, 12 days after the subcutaneous injection of 750 third-stage *Nb* larvae. Blood was obtained by tail bleeds on the days indicated and eosinophils were counted in Discombe's fluid with a hemacytomer (*12*). Data represent the mean eosinophil counts per milliliter in groups of five mice.

Dosage (mg)	Blood eosinophils per milliliter ($\times 10^{-4}$)		
	Day 0	Day 2	Day 4
2	440	92	1
1	548	26	0
0.5	383	13	1
0.25	271	4	3
0	440	249	123

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the tumorigenicity of these cell lines (*1*). The IL-4–producing transfectants of J558L studied were clone I2B1, which secreted 4400 units of IL-4 per 10^6 cells per 48 hours as assayed in vitro on the IL-4–sensitive HT2 T cell line (*4*), and the clone I3L6, which produced approximately five times the amount of IL-4 that I2B1 produced. For the B16 melanoma line, clone BIL4-3, which produced 10,000 U of IL-4 per 10^6 cells per 48 hours, was studied (Table 2). Pretreatment of syngeneic BALB/c mice with the MAb to IL-4, 11B11, restored tumorigenicity of subcutaneously injected I2B1 cells in a dose-dependent fashion, with the highest dose resulting in tumor sizes comparable to that obtained with the J558L parental tumor that does not secrete IL-4. Histologically, there was an absence of eosinophil and macrophage infiltrates in animals so treated. Restoration of tumorigenicity was not obtained with the isotype-matched control MAb, GL113, specific for β-galactosidase.

As with the MAb to IL-4, pretreatment of mice with MAb RB6-8C5 resulted in rapid restoration of tumorigenicity of IL-4–producing I2B1 cells. The kinetics of tumor formation (nodules detectable by 7 days) was similar to that observed with the parental J558L tumor cell line. Histologic analysis of I2B1 injection sites in an animal pretreated with either MAb GL113 or MAb RB6-8C5 was performed (Fig. 1). No tumor was seen at the I2B1 injection site in the control-treated animal; histologically, the characteristic infiltration with eosinophils was observed (Fig. 1A). Much of the tumor was necrotic. In contrast, the I2B1 injection site in a MAb RB6-8C5–treated mouse was characterized by gross tumor formation; histologically confirmed tumor viability with numerous mitoses is seen, with a >95% reduction in the number of infiltrating eosinophils ($P < 0.001$) (Fig. 1B and Table 2) (*5*). Thus, despite the continued expression of IL-4 by the I2B1 tumor cells (confirmed by HT-2 bioassay), inhibition of the localized accumulation of eosinophils restored tumorigencity. Thus, eosinophils are important for the antitumor activity induced by IL-4. Although MAb RB6-8C5 treatment depletes host neutrophils, as well as eosinophils, neutrophils did not infiltrate tumors as a result of IL-4 expression and are therefore unlikely to contribute to the tumoricidal activity. Macrophages were observed in tissue sections of IL-4–producing tumors in animals that had been pretreated with the MAb RB6-8C5, consistent with the specificity of the antibody for mature myeloid cells (*6*).

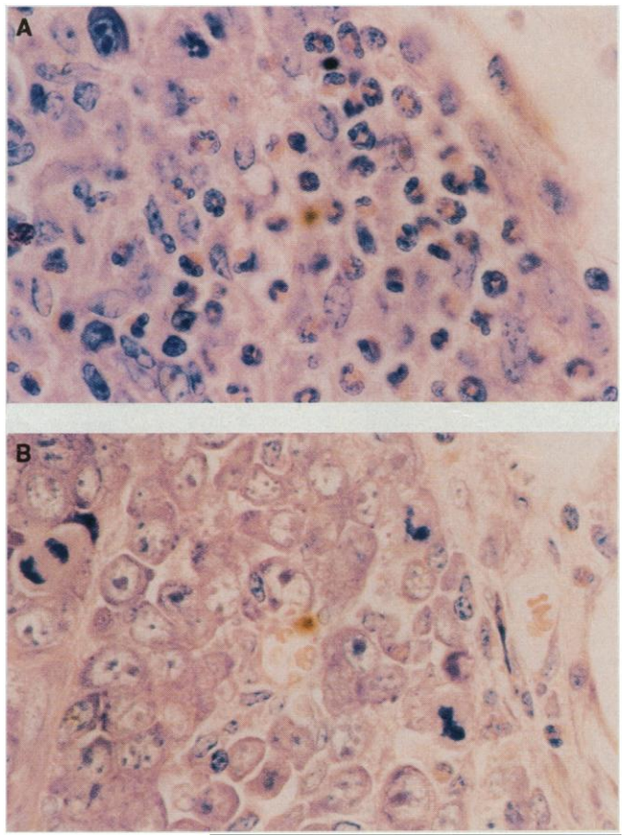

Fig. 1. Host granulocyte depletion restores the tumorigenicity of IL-4–producing tumor cells. Syngeneic BALB/c mice were injected subcutaneously in the left lower abdominal quadrant with 2×10^6 IL-4–producing I2B1 plasmacytoma cells 1 day after treatment with GL113 control antibody or RB6-8C5 antigranulocyte antibody. Antibody administration was repeated 10 days after the initial treatment. Dosages are given in Table 2. (**A**) Tissue section from the site of tumor cell inoculation for the GL113 antibody–treated animal (×1000). The extensive cellular infiltrate consists predominantly of eosinophils, distinguishable by their segmented nucleus and intensely staining pink cytoplasmic granules. A few remaining tumor cells are evident. (**B**) Tissue section from the site of tumor cell inoculation for the RB6-8C5 antibody–treated animal (×1000). The section consists almost exclusively of viable tumor cells with several mitoses evident. A few residual eosinophils remain. Tissue in (A) and (B) was obtained 18 days after tumor cell injection, fixed in 10% formalin, paraffin-embedded, and stained with Giemsa.

Pretreatment of animals with TRFK-5, a neutralizing MAb to murine interleukin-5 (IL-5), was also partially effective in restoring the tumorigencity of IL-4–producing I2B1 cells (Table 2). IL-5 participates in the differentiation of eosinophils from myeloid precursors in the bone marrow. The amount of antibody used was that previously demonstrated to completely inhibit the development of eosinophilia associated with *Nippostrongylus* infection in BALB/c mice (*7*). Tumor-infiltrating eosinophils were still present 96 hours after anti–IL-5 treatment, although they were significantly reduced in comparison with GL113 control–treated animals ($P < 0.01$; Table 2). The weak effect of anti–IL-5, in comparison to antigranulocyte treatment, in restoring tumorigencity of I2B1 cells probably relates to the inability of the former to deplete animals of mature eosinophils. The action of anti–IL-5, albeit weak, further supports an eosinophil-mediated antitumor mechanism of IL-4 action.

In addition to the transfected plasmacytoma line producing moderate amounts of IL-4 (I2B1), the effects of eosinophil depletion on I3L6 cells, another IL-4–transfected J558L plasmacytoma clone with a higher level of IL-4 activity, and an IL-4 transfectant (BIL4-3) of a different tumor type, the B16 melanoma were studied (Table 2). In the case of the high IL-4 producer, I3L6, RB6-8C5 alone failed to restore tumorigencity of this line, but synergy was observed with the addition of anti–IL-4. With the BIL4-3 melanoma tumor, although the antigranulocyte MAb alone was able to restore tumor formation, it again synergized with anti–IL-4. In animals treated with either antigranulocyte MAb or anti–IL-4 MAb, eosinophil infiltration at the tumor site was reduced compared with control treated animals but in each case was significantly greater than that observed in animals treated with the combination of MAbs ($P < 0.001$) (Table 2). The ability to restore tumorigenicity therefore again correlated with the inhibition of localized eosinophil infiltration. These results suggest that the localized expression of IL-4 provides, directly or indirectly, a potent stimulus for the accumulation of eosinophils. Even under conditions (antigranulocyte treatment) in which total body eosinophil counts are significantly reduced, an IL-4 signal of sufficient intensity will promote a rapid accumulation of residual eosinophils to the site of IL-4 production.

Despite a demonstrated role for eosinophils in the mediation of IL-4–induced tumor killing, a role for additional effector cell types, in particular various lymphocyte populations, could not be excluded by the antibody studies. For example, it is possible that helper T lymphocyte populations were operative in providing additional cytokine

signals necessary to elicit the inflammatory reaction observed or that specific or nonspecific cytolytic lymphocytes acted to augment the cytotoxicity associated with the eosinophil reaction. The absence of lymphocytic infiltration and the ability to demonstrate the antitumor action of IL-4 in *nu/nu* mice (*1*) provided evidence that T cell immunity was not required for IL-4–induced tumor cytotoxicity. To extend these findings, we undertook a more extensive analysis of immunodeficient mouse strains (Table 3). IL-4–transfected clones of the J558L plasmacytoma and B16 melanoma tumor cell lines were again studied. With these transplantable lines, the antitumor effect of IL-4 operated not only in syngeneic and *nu/nu* hosts but also in *bg/nu/xid, scid, bg/bg*, and w/w^v mice, providing further evidence that T cells, as well as B cells, natural killer (NK) cells, and mast cells, are not required for IL-4–mediated tumor killing. Although macrophages infiltrated at the tumor site as a result of IL-4 action, the peak of this response was late (more than 48 hours) with respect to the observed accumulation of eosinophils. Most of the tumor cells were killed, particularly in tumor lines that expressed high IL-4, when the eosinophils predominated. Tumor formation was restored despite the presence of macrophages in eosinophil-depleted animals that received IL-4–producing tumor cell lines. Macrophage-mediated tumor killing is not therefore likely to play a predominant role in the tumoricidal effect initiated by IL-4, but cooperative killing by macrophages cannot be excluded on the basis of the data presented.

As noted, IL-4–producing tumors could be generated from the I3L6 line by the pretreatment of animals with a combination of anti–IL-4 and antigranulocyte MAbs (Table 2). Within 2 weeks after cessation of antibody administration, such tumors completely regressed (Fig. 2A). This regression was accompanied by the characteristic eosinophil invasion and tumor cell necrosis, followed by macrophage infiltration and subsequent fibroblastic scarring. This observation led us to test whether IL-4 could mediate the regression of established tumor masses. The direct intratumor injection of a single high dose (1×10^6 U) of recombinant IL-4 (Fig. 2B) or a single intratumor injection of IL-4–producing cells (Fig. 2C) markedly inhibited the growth of established tumors, as demonstrated for the J558L plasmacytoma line. In three of six animals receiving recombinant IL-4, regression of the tumors, in one case complete, occurred within 1 week after injection. The complete regression was maintained over a 60-day observation period. These data provide further support for the ability of IL-4, when present at a sufficiently high concentration locally, to promote the rapid accumulation of host inflammatory cells resulting in tumor cell death. Eosinophil infiltration after but not before IL-4 administration was again observed.

Although IL-4 appears to be particularly potent in mediating tumor cell death locally, we have not observed activity of this cytokine with the J558L and B16 tumor models either in the inhibition of tumor at sites distant from localized IL-4 administration or in stimulating immunogenicity capable of protecting animals on tumor rechallenge (*8*). We have also been unable to detect an augmentation of cytotoxic T lymphocyte (CTL) memory responses by IL-4–producing tumor cells compared with inactivated parental tumor cells (*9*). There is one report (*10*) in which an IL-4–transfected renal cell carcinoma line (RENCA) induced a CTL response after tumor cell immunization. Although animals so immunized demonstrated some degree of specific immunity against subsequent challenge with the parental tumor, no information is provided as to the immunogenicity of inactivated (by irradiation or mitomycin C) RENCA cells alone. It has been demonstrated that growth inactivation of a wide variety of tumor cells may itself stimulate T cell immunity, in the absence of cytokines or other immunomodulators (*11*). It is therefore possible that the CTL response stimulated by IL-4–transfected RENCA cells may have resulted from the inherent immunogenicity of the cell line, as we have observed with J558L (*8*). A generalized role for IL-4 in the induction or augmentation of tumor immunity therefore remains unclear.

The ability, however, of IL-4 to rapidly elicit a localized inflammatory infiltrate ca-

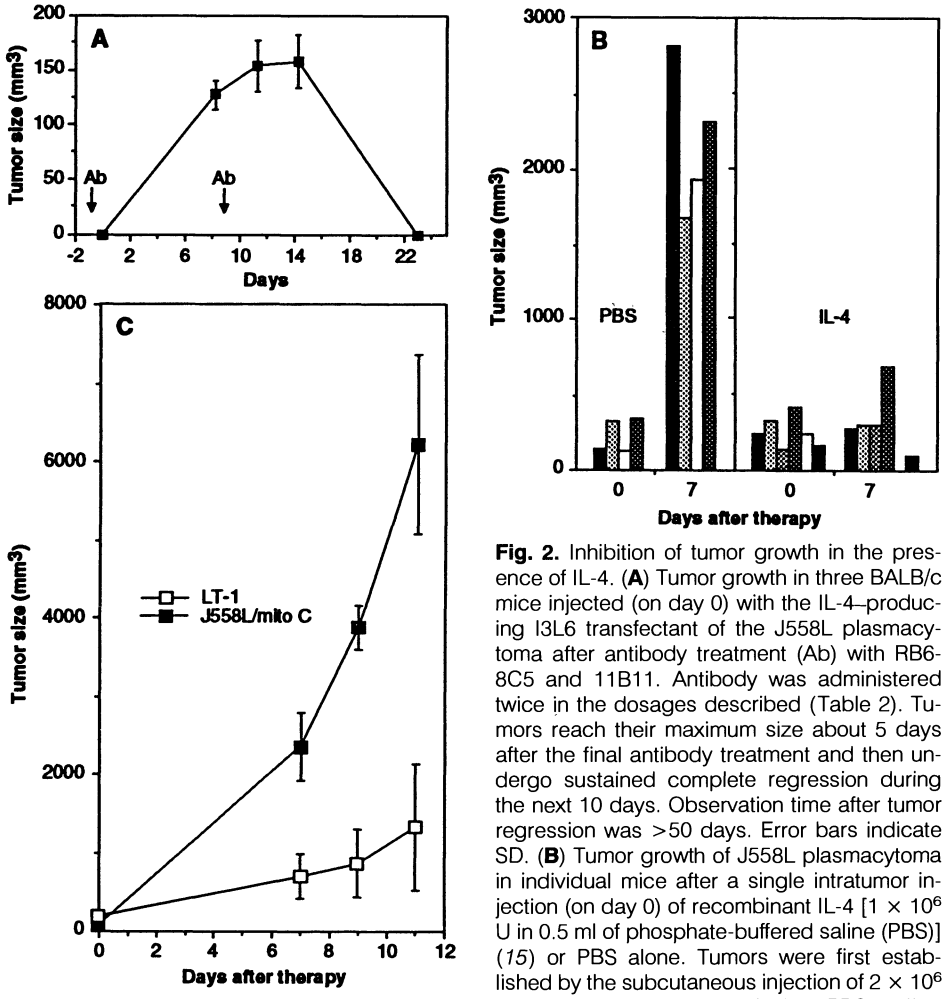

Fig. 2. Inhibition of tumor growth in the presence of IL-4. (**A**) Tumor growth in three BALB/c mice injected (on day 0) with the IL-4–producing I3L6 transfectant of the J558L plasmacytoma after antibody treatment (Ab) with RB6-8C5 and 11B11. Antibody was administered twice in the dosages described (Table 2). Tumors reach their maximum size about 5 days after the final antibody treatment and then undergo sustained complete regression during the next 10 days. Observation time after tumor regression was >50 days. Error bars indicate SD. (**B**) Tumor growth of J558L plasmacytoma in individual mice after a single intratumor injection (on day 0) of recombinant IL-4 [1×10^6 U in 0.5 ml of phosphate-buffered saline (PBS)] (*15*) or PBS alone. Tumors were first established by the subcutaneous injection of 2×10^6 J558L cells 7 to 10 days before PBS or IL-4 therapy. The pretreatment (day 0) size in cubic millimeters (mean ± SD) was 231 ± 98 for the PBS control group and 239 ± 93 for the IL-4 group. (**C**) Tumor growth of J558L plasmacytoma after a single intratumor injection (on day 0) of 10^8 IL-4–producing LT-1 cells or, as a control, 10^8 mitomycin C–inactivated J558L tumor cells. LT-1 is an IL-4 transfectant of J558L, producing 50,000 U per 10^6 cells per 48 hours of IL-4 activity in the in vitro HT2 assay (*1*). Tumors were established 5 days before intratumor injection as described in (B). The pretreatment size in cubic millimeters (mean ± SD) was 98 ± 33 for the J558L/mitomycin C–treated group and 186 ± 99 for the LT-1–treated group. Error bars indicate the SD.

Table 2. Effects of in vivo antibody administration on the antitumor activity of IL-4. Six- to eight-week-old syngeneic BALB/c (for J558L-derived I2B1, I3L6 cells) or C57BL/6 (for B16-derived BIL4-3 cells) mice received the indicated antibodies intraperitoneally 1 day before and (with the exception of 12B1/11B11 experiments) again 10 days after the subcutaneous inoculation of 2×10^6 IL-4–producing tumor cells. Antibodies injected were anti–IL-4 (11B11) (5 mg per mouse, or where indicated, a 1:50 (0.1 mg) or 1:400 dilution (0.013 mg) of the preparation), anti–IL-5 (TRFK-5) (2 mg per mouse), antigranulocyte (RB6-8C5) (0.5 mg per mouse), or an IgG1 control antibody, isotype-matched to 11B11 and TRFK-5 (GL113, against β-galactosidase) (2 mg per mouse) (*13*). IL-4 activity of the tumor cell lines was determined by bioassay with the HT2 indicator T cell clone as described (*4*). Mean tumor growth was assessed at 14 days; volume measurements were estimated as the product of tridimensional caliper measurements (surface length, width, and tumor thickness). Range of tumor volumes is provided in parentheses. Non-IL-4–producing parental tumor lines (J558L plasmacytoma, B16 melanoma) formed tumors with a 100% incidence (Table 3) with the same cell number and route of administration. The numbers of tumor-infiltrating eosinophils were determined on Giemsa-stained tissue sections of tumor sites (prepared as described in Fig. 1 legend) harvested 96 hours after inoculation of cells. Ten to twenty high-power fields (hpf; ×1000) were examined per group (*14*). Numbers represent the mean ± SD.

Tumor cell line	IL-4 activity (U/10^6 cells/ 48 hours)	Antibody	Tumors [ratio (%)]	Tumor volume 14 days after cell inoculation (mm^3)	Tumor-infiltrating eosinophils (per hpf)
I2B1	4,400	GL113	1/9 (11)	60*	23.3 ± 6.1
		RB6-8C5	8/9 (89)	526 (180–720)	1.0 ± 1.2
		TRFK-5	4/9 (44)	180 (60–288)	15.2 ± 4.6
		11B11	4/4 (100)	1035 (720–1344)	2.5 ± 2.2
		11B11 (1:50)	3/3 (100)	690 (480–960)	ND†
		11B11 (1:400)	0/4 (0)	—	ND
I3L6	20,000	GL113	0/7 (0)	—	41.3 ± 10.6
		RB6-8C5	0/5 (0)	—	ND
		11B11	2/8 (25)	48 (40–56)	17.9 ± 5.3
		RB6-8C5+11B11	5/6 (83)	113 (40–192)	12.6 ± 3.7
BIL4-3	10,000	GL113	0/5 (0)	—	27.6 ± 9.5
		RB6-8C5	4/5 (80)	87 (54–111)	3.4 ± 2.2
		11B11	0/3 (0)	—	8.4 ± 3.8
		RB6-8C5+11B11	3/3 (100)	308 (300–315)	0.3 ± 0.5

*Tumor nodule regressed within 5 days after measurement. †ND = not determined.

pable of mediating the regression of established tumor masses suggests a potential clinical utility of this cytokine in the treatment of certain human malignant disorders. On the basis of our findings in animal models, IL-4–based therapy may be useful to test clinically for relatively confined, but surgically inaccessible tumors, for example, certain brain tumors and intraperitoneal malignancies. The broad-spectrum activity of IL-4 against a wide variety of tumor cell types is particularly attractive with regard to exploring its efficacy as a human antitumor agent.

Table 3. Assessment of the antitumor activity of IL-4 in immune-deficient mice. Six- to eight-week-old mice of the genetic strains indicated were inoculated subcutaneously with either 2×10^6 parental (J558L, B16) or IL-4–transfected (12B1, I3L6, BIL4-3) tumor cells. Tumor formation was assessed 14 days after inoculation.

Tumor cell type	Mouse strain	Cellular deficiency	Tumor incidence at 14 days
J558L	BALB/c	None	20/20
I3L6			0/30
I2B1			0/20
J558L	*nu/nu*	T	15/15
I3L6			0/15
I2B1			0/6
J558L	*bg/nu/xid*	B, T, NK	3/3
I3L6			0/3
J558L	*scid*	B, T	4/4
I3L6			0/4
B16	C57/B6	None	20/20
BIL4-3			0/20
B16	*bg/bg*	NK	4/4
BIL4-3			0/8
B16	*w/w^v*	Mast	4/4
BIL4-3			0/10

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3. For the generation of IL-4–producing tumor cells, a genomic fragment of the IL-4 gene was placed under the control of heterologous enhancer-promoter elements [immunoglobulin (IgG) heavy chain enhancer and promoter for the J558L plasmacytoma transfectants I2B1 and I3L6, and the Moloney murine leukemia virus long terminal repeat for the J558L transfectant LT-1 and the B16 melanoma transfectant BIL 4-3]. Gene transfection was performed by electroporation. The details of DNA constructions are provided in (*1*).
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5. The independent-samples *t* test (two-tailed) was used as the test of significance for the determination of *P* values in this report.
6. Comparable numbers of macrophages, demonstrated by immunostaining with MAC-1 and F4/80 MAbs, were observed at 96 hours after tumor cell inoculation in tissue sections from GL113 control and RB6-8C5 granulocyte-depleted animals.
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8. In three independent experiments, BALB/c mice were immunized subcutaneously (sc) with either 2×10^6 IL-4–producing plasmacytoma cells (I3L6) or mitomycin C–inactivated parental cells (J558L/mito), then rechallenged 14 days later with 10^6 viable J558L cells at a different sc site. The total number of animals without detectable tumors by day 30 was 9/13 (69%) for the J558L/mito–immunized group and 9/16 (56%) for the I3L6-immunized group. With an analogous protocol, no protection (100% of animals with tumors) could be shown for C57BL/6 mice challenged with B16 melanoma cells after immunization with mitomycin C–inactivated parental or IL-4–producing (BIL4-3) B16 cells.
9. BALB/c mice (three per group) were immunized with either IL-4–producing plasmacytoma cells (I3L6) or mitomycin C–inactivated parental cells (J558L/mito) as described (*8*). After 10 days, pooled splenocytes from each group were removed and were cultured for 5 days with mitomycin C–treated J558L cells in the presence of IL-2 (30 U/ml). Live cells were then mixed with ^{51}Cr-labeled J558L targets at different effector-to-target (E:T) ratios in a 4-hour ^{51}Cr release assay. No significant differences in specific lysis at any E:T ratio were observed between the groups (J558L/mito–immunized group: Specific lysis was 17%, 9%, and 2% for E:T ratios of 100:1, 50:1, and 10:1, respectively; I3L6-immunized group: Specific lysis was 16%, 7%, and 2% for the same E:T ratios).
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13. Antibodies were prepared either from concentrated supernatants of serum-free cultures (GL113, TRFK-5, RB6-8C5; >95% pure) or from ammonium sulfate–purified ascites obtained by the intraperitoneal growth of hybridoma cells in Pristane-primed *nu/nu* mice (11B11). Antibodies were dialyzed against PBS concentrated to 10 to 20 mg/ml and filter-sterilized. Endotoxin levels measured for the antibody preparations of RB6-8C5, TRFK-5, and GL113 were 15, <5, and <5 endotoxin units (EU)/mg, respectively.
14. In all tumor specimens examined histologically, neutrophils (distinguishable from eosinophils by their neutral cytoplasmic staining with Giemsa) represented <1% of the total number of infiltrating granulocytes.
15. We prepared recombinant IL-4 by immunoaffinity chromatography of I3L6 plasmacytoma supernatant on a Affigel-10 (Bio-Rad) column to which purified 11B11 (anti–IL-4) antibody was bound. Glycine buffer (pH 2.5) was used for elution. Samples were immediately neutralized with sodium phosphate buffer (pH 8.0) and were lyophilized. The specific activity of the product was 1.4×10^8 U per milligram of protein. Purity of the product was demonstrated by a characteristic doublet peak at a molecular weight of 19,100 to 19,600 with reversed-phase high-performance liquid chromatography and the correct NH_2-terminal sequence of the first 20 amino acids. E. I. Dupont de Nemours and Company, Inc. prepared the recombinant IL-4.
16. Care of animals used in the experiments described in this report was in accordance with the institutional guidelines of the Harvard Medical School, the Massachusetts General Hospital, and the DNAX Research Institute of Molecular and Cellular Biology, Inc.

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