Interleukin-10 Inhibits Tumor Metastasis Through an NK Cell–dependent Mechanism

By L.M. Zheng,* D.M. Ojcius,‡ F. Garaud, C. Roth,§ E. Maxwell,* Z. Li,* H. Rong,* J. Chen,* X.Y. Wang,* J.J. Catino,* and I. King*

From *The Tumor Biology Department, Schering-Plough Research Institute, Kenilworth, New Jersey 07033; and ‡Unité de Biologie des Interactions Cellulaires and §Unité de Biologie Moléculaire du Gène, Institut Pasteur, Paris, France

Summary

Interleukin-10 (IL-10) is a recently described pleiotropic cytokine secreted mainly by type 2 helper T cells. Previous studies have shown that IL-10 suppresses cytokine expression by natural killer (NK) and type 1 T cells, thus down-regulating cell-mediated immunity and stimulating humoral responses. We here report that injected IL-10 protein is an efficient inhibitor of tumor metastasis in experimental (B16-F10) and spontaneous (M27 and Lox human melanoma) metastasis models in vivo at doses that do not have toxic effects on normal or cancer cells. Histological characterization after IL-10 treatment confirmed the absence of CD8+ and CD4+ T cells and macrophages at the sites of tumor growth, but abundant NK cells were localized at these sites. This unexpected finding was confirmed by showing that IL-10 inhibits most B16-F10 and Lox metastases in mice deficient in T or B cells (SCID and nu/nu mice), but not in those deficient in NK cells (beige mice or NK cell–depleted mice). However, IL-10 downregulation of pro-inflammatory cytokine production and/or recruitment of additional effector cells may also be involved in the anti-tumor effect at higher local concentrations of IL-10, since transfected B16 tumor cells expressing high amounts of IL-10 were rejected by normal, nu/nu, or SCID mice at the primary tumor stage, and there was still a 33% inhibition of tumor metastasis in beige mice.

A number of cytokines have been used with variable success in attempts to control tumor growth and metastasis (1–8). Many of these cytokines, such as IL-2, IFNγ, TNFα, and GM-CSF, also have direct toxic or stimulatory effects on the tumor cells, and may in some cases lead to malignancy of otherwise normal cells (4, 9), thus complicating their clinical usefulness. We have therefore screened a number of cytokines for their ability to inhibit tumor metastases, and found that one of them, IL-10, is an efficient inhibitor of tumor metastasis in vivo at doses that do not have a direct effect on normal or cancer cells. IL-10 acts on different cell types, stimulating differentiation of B cells into antibody-secreting cells, and inhibiting the capacity of monocytes/macrophages to present antigens (10–12). In addition, IL-10 exerts an indirect effect on the immune system, by inhibiting the secretion of proinflammatory cytokines (IL-1, IL-6, IL-8, GM-CSF, and TNFα) from type 1 helper T cells, monocytes/macrophages, and polymorphonuclear leukocytes, and by inhibiting the secretion of IFNγ by NK cells (12–15). Recent studies have shown that IL-10 downregulates cell-mediated responses and favors the humoral arm of immunity by decreasing production of

cytokines involved in cell–mediated responses (12), and that IL-10 increases the susceptibility to bacterial and parasitic infections (16, 17). We therefore also investigated the mechanism by which IL-10 inhibits tumor metastasis. We find that NK cells are the main effector cells recruited at the nontoxic concentrations of IL-10 used here, although other effector cells and/or mechanisms may also be involved at higher IL-10 concentrations.

Materials and Methods

Tumor Cells and Experimental Animals. For all the experiments described here, 6–8-wk-old female C57/BL6 and BALB/C mice were purchased from Charles River Labs. (Wilmington, MA). The B16-F10 murine melanoma was provided by Dr. I. Fidler and M27 murine lung carcinoma by Dr. P. Brodt. Human IL-10 (hIL-10) was obtained from Schering-Plough Corp. (Union, NJ), and its specific activity was 3.1 × 10⁷ U/mg. Athymic nu/nu mice, beige mice, and SCID mice were from Harlen Spraque Dawley.

Tumor Measurements. Experimental lung metastases were obtained after intravenous injection of B16-F10 murine melanoma cells in C57/BL6 (H-2ᵇ) mice, athymic nu/nu mice, and beige

mice. Spontaneous lung metastases were obtained by subcutaneous injection of M27 Lewis lung carcinoma cells in C57/BL6 mice, and Lox human melanoma cells in SCID mice. hIL-10 (up to 150 μg/kg) was injected intraperitoneally. Tumor diameters were measured with calipers, groups of 10 mice were used for each condition, and each experiment was repeated at least twice. The number of lung metastases was determined in double-blind measurements. Statistical analysis was performed using the non-parametric one tailed Mann-Whitney U test.

For experiments with mice depleted of NK cells, mice were injected intravenously with 100 μg of a mAb against NK1.1 (PharMingen, San Diego, CA) on day −1, day 0 and day 1 after injection of tumor cells (1 × 10⁵ cells/animal). Animals received IgG2a isotype antibodies as a control for the specificity of the anti-NK1.1 mAb. Treatment with hIL-10 started on day 0, day 1 and day 2 after tumor cell injection. Lung metastases were counted on day 14.

For experiments with tumor cells transfected with the IL-10 gene, cells were co-transfected with pSV₂ neo and a plasmid containing the human IL-10 gene. G418-resistant colonies were isolated, and IL-10 protein secretion was measured by ELISA.

Immunohistological Analysis. Sections for immunohistochemistry were prepared and labeled with antibodies as previously described (18). Monoclonal antibodies against CD3 (500 A2), CD4 (L3T4), CD8 (Lyt2), CD90 (Thy-1), NK-1.1, and Mac-3 were from PharMingen; against LGL-1 (4D11), from Dr. L. Mason; against asialo-GM₁, from Wako Chemicals US (Richmond, VA); against Mac-1 and Mac-2, from Boehringer Mannheim Corp. (Indianapolis, IN); and mAb BMA-BM8 against resident tissue macrophages, from Accurate Chemical & Scientific Corp. (Westbury, NY)

Results

Direct Effect of IL-10 on Tumor Cells In Vitro and In Vivo. In preliminary experiments, it was established that hIL-10 does not have direct toxic or stimulatory effects on the tumor cells when tumor cell lines are treated in vitro with up to 1 μg/ml (corresponding to 10⁵ U/ml) of hIL-10 (not shown). Likewise, no discernible toxic effect was observed in mice when hIL-10 doses as high as 700 μg/kg were used, although primary tumor growth in animals was partially affected by treatment with higher concentrations of hIL-10. Thus, murine monocytic (nu/nu or BALB/c mice) and OVCAR 5 human ovarian (nu/nu mice) primary tumors were not affected by hIL-10 doses from 5 to 250 μg/kg daily, while higher doses inhibited slightly the growth of B16-F10 murine melanoma (nu/nu or C57/BL6) and U937 (nu/nu) tumors (≤40% inhibition) (not shown).

*Effect of IL-10 on Tumor Metastasis.* In order to measure the effects of IL-10 on tumor metastasis in animals, hIL-10 (up to 150 μg/kg) was injected intraperitoneally following various doses and schedules. Daily injection of hIL-10 reduced significantly the number of lung metastases in both spontaneous and experimental models. The inhibition was dose-dependent, as shown in Fig. 1 for the experimental metastatic model (B16 cells) and the spontaneous metastatic model (M27 cells). Inhibition in C57/BL6 mice reached 68.4% for B16-F10 tumors at 150 μg/kg, and 90.2% for
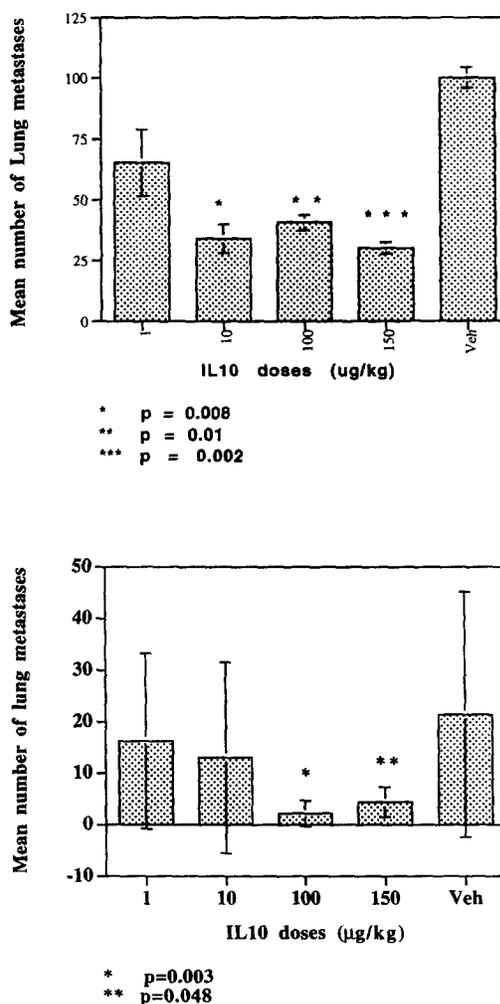
Figure 1. Inhibitory effect of hIL-10 on lung metastasis in C57/BL6 mice of (*A*) B16 and (*B*) M27 tumors. Mice were injected intravenously with 1 × 10⁵ B16 or subcutaneously with 2 × 10⁵ M27 cells on day 0, and hIL-10 treatment was initiated on day 1. Mice treated with hIL-10 exhibited a 64.3% (*P* = 0.008) inhibition of metastasis at a dose of 10 μg/kg IL-10, 58.8% (*P* = 0.01) at a dose of 100 μg/kg, and 68.4% (*P* = 0.002) at a dose of 150 μg/kg for B16 tumors on day 9 after injection; and mice treated with 100 μg/kg and 150 μg/kg of hIL-10 exhibited a 90.2% (*P* = 0.003) and 80.4% (*P* = 0.048) inhibition of metastasis, respectively, for M27 tumors on day 28, compared with the vehicle control groups (Veh).

M27 tumors. Moreover, treatment does not need to begin at the same time as tumor implantation, since administration of hIL-10 for 3 d starting on the day after tumor cell inoculation results in a 66.1% inhibition of metastases of B16 tumors (not shown).

It has recently been shown that Chinese hamster ovary (19) or mammary adenocarcinoma cells transfected (20) with the IL-10 gene were less effective at establishing primary tumors than untransfected cells. We therefore injected animals with B16-F10 tumor cells transfected with the gene coding for hIL-10 and secreting different amounts of hIL-10 to ascertain whether they displayed metastatic behavior. Some of the transfected tumor cells were not able to form solid tumors, and metastases were also effectively
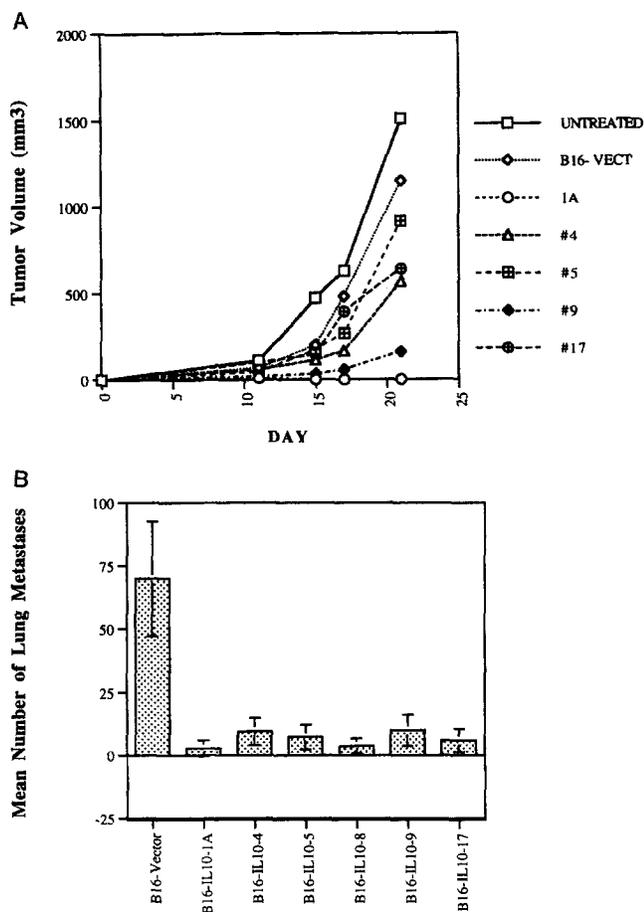
Figure 2. Effects of IL-10-gene transfected B16 tumors. (*A*) Inhibition of solid tumor growth (tumor size given as a function of time, in days). C57/BL6 mice were injected subcutaneously with 1 × 10⁶ IL-10–transfected B16 clones secreting varying amounts of IL-10. Compared to the B16 cell line transfected with the plasmid control (*B16-Vector*), there was 100% inhibition of primary tumor growth for B16 clone #1A, and over 50% inhibition for clones #4 and #9. Groups of ten mice were used for each clone, and each experiment was repeated at least twice. (*B*) Inhibition of B16 metastases. Mice were injected intravenously with 1 × 10⁵ B16 cells transfected with the IL-10 gene. Mice were killed on day 9, and the lung metastases were counted. Lung metastasis inhibition for each clone was as follows: #1A, 100%; #4, 86.4%; #5, 89.6%; #8, 94.7%; #9, 85.8%; #17, 92%. IL-10 secretion (in ng/million cells/48 h) for each clone was as follows: clone #1A, 400-500; #4, 11.8; #5, 3.5; #8, 6.3; #9, 5.9; #17, 1.4.

inhibited. Fig. 2 *A* shows the growth in C57/BL6 mice of B16 tumor cells transfected with the hIL-10 gene. No detectable tumor growth was observed with a clone secreting 400–500 ng IL-10/48 h/1 million transfected cells, while significant growth inhibition was also measured for clones expressing lower IL-10 levels (1.4 to 11.8 ng/million cells/ 48 h). Similarly, hIL-10 secretion by these transfected cells inhibited metastasis of all the B16 clones, with inhibition ranging from 85.8 to 100% (Fig. 2 *B*). Inhibition of metastasis was also observed with two hIL-10 gene–transfected M27 clones, #G1 (secreting 50 ng/million cells/48 h) and #G3 (28 ng/million cells/48 h), for which inhibition on

day 28 after subcutaneous injection of 2 × 10⁵ transfected cells was 82 and 84.3%, respectively (not shown).

Identification of Effector Cells Involved in Metastasis Inhibition. Previous studies have demonstrated that macrophages (2, 3, 21), helper T cells (22, 23), cytotoxic T cells (1, 7, 8), and NK cells (7, 24) may contribute to the rejection of tumors treated with different cytokines or transfected with genes coding for those cytokines. In order to determine the nature of the effector cells responsible for IL-10-mediated inhibition of tumor metastases, the phenotype of immune cells invading the tissues was studied by immunostaining. Cells expressing markers of helper (CD4⁺), cytotoxic T (CD8⁺) lymphocytes, or macrophages (Mac-1⁺, Mac-2⁺, Mac-3⁺, and the macrophage epitope BMA BM8) were rarely if ever observed (not shown). Few cells expressing NK cell markers were found in normal tissues (not shown) or tumors of untreated mice (Fig. 3 *A*), but abundant NK cells were localized in B16 subcutaneous tumors of mice treated with hIL-10 (Fig. 3 *B*) and in subcutaneous tumors of mice injected with IL-10 gene–transfected B16 cells (Fig. 3 *C*). When double immunostaining was performed on the cells expressing the NK cell asialo-GM₁ antigen (Fig. 3 *D*), these were found to also express the LGL-1 marker typical of activated large granular lymphocytes (Fig. 3 *E*).

Metastasis Inhibition in Various Mouse Models. The involvement of NK cells was confirmed by comparing inhibition of tumor metastases in normal C57/BL6 mice, athymic nu/nu mice, SCID mice, beige mice, and mice that had been depleted of NK cells (Table 1). While hIL-10 inhibited most metastases in nu/nu and SCID mice (65.6 and 64.3% inhibition, respectively), there was little inhibition in beige mice or NK cell–depleted mice (14 and 19%, respectively). These effects were paralleled in experiments with IL-10 gene–transfected cells. There was complete metastasis inhibition in nu/nu and SCID mice when transfected B16-F10 cells were inoculated into the animals, whereas there was only 33% inhibition when the transfected cells were introduced into beige mice. Thus, with both treatments, inhibition was much lower in NK cell–deficient mice (beige mice and NK cell–depleted mice) than in SCID or nu/nu mice.

Discussion

We used a number of murine and human tumor cell lines to study the effects of recombinant human IL-10 (hIL-10) protein and of tumor cells transfected with the IL-10 gene on tumor metastasis. Experimental lung metastases were obtained after intravenous injection of B16-F10 murine melanoma cells in normal mice, athymic nu/nu mice, beige mice, and mice depleted of NK cells by antibody treatment; and spontaneous lung metastases were obtained by subcutaneous injection of M27 Lewis lung carcinoma cells in C57/BL6 mice, and Lox human melanoma cells in SCID mice. We found that soluble IL-10 is able to inhibit metastasis of both experimental and spontaneous lung models at IL-10 concentrations that do not have direct in-
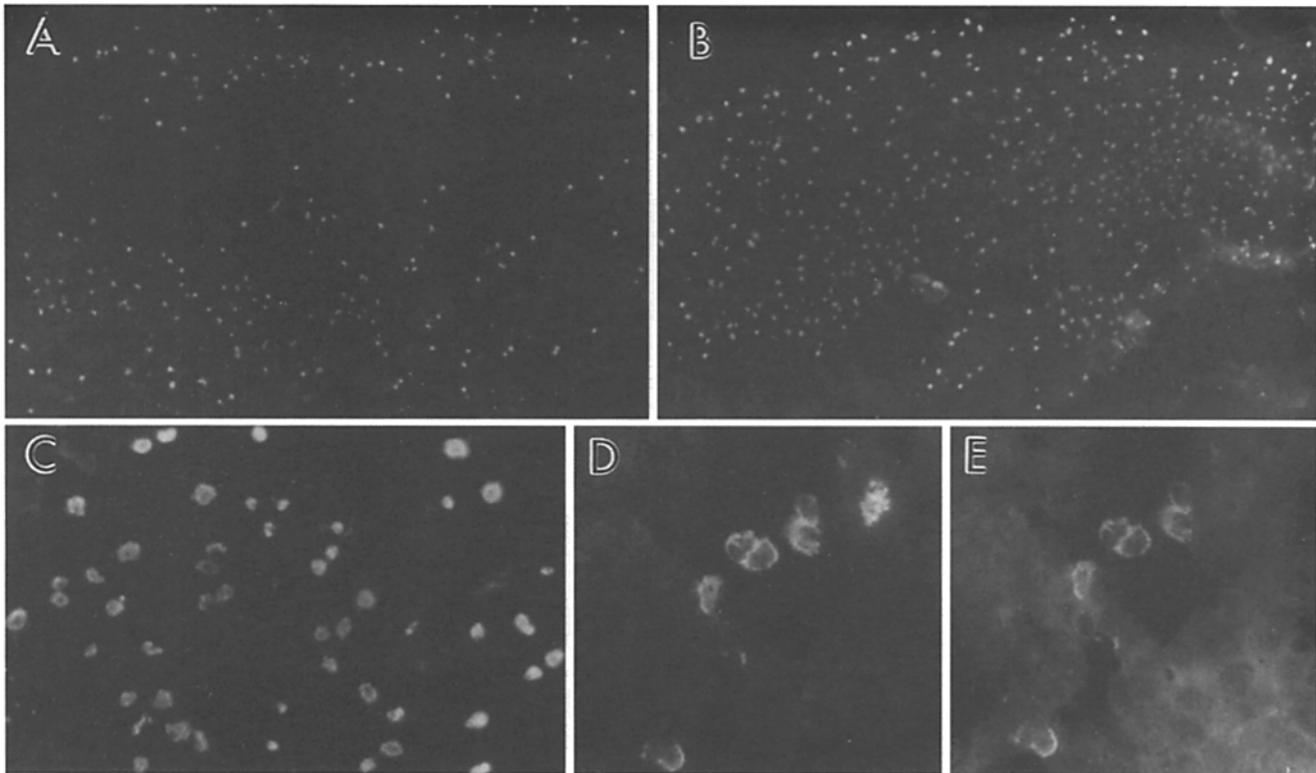
Figure 3. Immunostaining of tumors in C57/BL6 mice on day 18 after tumor cell injection. LGL-1-FITC labeling of subcutaneous tumors in (*A*) in untreated control mice and (*B*) mice treated with 100 μg/kg hIL-10 daily, starting on day 1. (*C*) Asialo-GM1-FITC labeling of subcutaneous tumors of IL-10 gene–transfected B16 cells. (*D*) Double immunostaining with asialo-GM₁-FITC and (*E*) LGL-1-Rhodamine of subcutaneous tumors in mice treated with 100 μg/kg hIL-10, on day 18.

Table 1. *Inhibition of Lung Metastases in Various Mouse Models*

Mouse model	Tumor	% Inhibition: 100 μg/kg hIL-10	% Inhibition hIL-10 gene transfection
nu/nu	B16-F10	65.6 %* ($P = 0.0019$)	100%
scid	LOX	80.5%‡ ($P = 0.002$)	ND
scid	B16-F10	64.3%* ($P = 0.004$)	100%
beige	B16-F10	14%*	33%
NK cell-depleted	B16-F10	19%§	ND

Tumor cells were transfected with the hIL-10 gene as described in the legend to Fig. 2, and B16-F10 clone #1A was used for the studies on inhibition with IL-10-gene transfected cells.
*Mice were inoculated with 5 × 10⁴ cells/animal. Treatment was begun on day 0. The mean number of lung metastases was counted on day 9.
‡Mice were inoculated subcutaneously with 5 × 10⁵ cells into the flank on day 0. Treatment was carried out on day 1 through day 22. The mean number of lung metastases was counted on day 22.
§Mice were depleted of NK cells with an anti-NK1.1 mAb, as described in Materials and Methods. In isotype control experiments with IgG2a antibodies, the same treatment with hIL-10 resulted in a 64% inhibition of tumor metastasis.

hibitory or stimulatory effects on the tumors in vitro or in vivo.

Immunohistological characterization led to the identification of mostly NK cells at the metastasis sites. These histological observations were confirmed by tumor metastasis measurements in different mouse models, which showed that there is good metastasis inhibition due to IL-10 in normal mice, athymic nu/nu mice (lacking T cells), and SCID mice (deficient in both B and T cells), but not in beige mice (displaying a functional deficiency of NK cells and macrophages) or mice specifically depleted of NK cells. Thus, while macrophages, CD4⁺ and CD8⁺ T cells, and NK cells have been observed in previous studies to have antitumoral effects in different situations, the preponderant role of NK cells in eliminating metastases in IL-10-treated mice is consistent with findings that IL-10 reduces CD4⁺ antigen-specific proliferation by downregulating MHC class II expression (10), and that it inhibits macrophage activation, cytokine and nitrogen oxide production, expression of B7, mycobacteriostatic activity, and tumor cytotoxicity (12–14, 25–28). Likewise, in primary mixed lymphocyte cultures, IL-10 inhibits allogeneic proliferative and cytotoxic responses of CD4⁺ and CD8⁺ T cells (29), and preincubation with IL-10 before exposure to tumor-associated antigens significantly inhibits delayed-type hypersensitivity responses (30). In contrast, IL-10 in combination with IL-2 increases IL-2-induced NK cell proliferation, and

IL-10 alone induces NK cytotoxicity against NK-resistant tumor cells in vitro (31). Furthermore, consistent with our results on the inhibition of tumor metastasis in vivo, IL-10 has been reported to render mouse lymphoma cells resistant to CTL and sensitive to NK-mediated lysis in vitro (32).

Nonetheless, the observation that both 100 µg/kg hIL-10 and cells transfected with the hIL-10 gene caused measurable inhibition in beige mice (14 and 33%, respectively) suggests that, although NK cells may be the predominant effector mechanism responsible for inhibition, IL-10 may also partially inhibit metastases through other means, such as through its ability to downregulate the production of pro-inflammatory cytokines and chemokines (12), which enhance the expression of tumor cell adhesion molecules and tumor cell migration (33, 34). In addition, it has recently been reported that transfected adenocarcinoma cells secreting high quantities of IL-10 (1,500–2,000 ng/million cells/48 h) are rejected by a combination of $CD8^+$ T lymphocytes, NK cells and neutrophils (20), which would also be consistent with our observation that our high IL-10 producing B16-F10 clone (secreting 400–500 ng/million cells/48 h) is rejected at the primary tumor stage in normal, nu/nu and SCID mice, and that there is still 33% inhibition of tumor metastasis in beige mice.

The IL-10-mediated activation/recruitment of NK cells observed in tumor metastases is in distinct contrast to the inhibition of NK cytokine production by monocytes/macrophages (15). While the mechanism used to activate and/ or recruit NK cells to sites of metastasis in vivo remains to be elucidated, it has recently been reported that IL-10 inhibits the synthesis of transforming growth factor (TGF)-β (35), which is known to suppress activation of NK cells (36, 37), and that NK cells express the IL-10 receptor (31). Given the many pleiotropic activities of IL-10, many of which may indirectly affect NK cell function, it will be interesting to identify the multiple pathways by which IL-10 may inhibit tumor metastasis.

The IL-10 protein appears to have its greatest effect on tumor metastasis, although we can not rule out at the moment the possibility that the marginal effects on primary tumor growth may be due to an inability of IL-10 injected intravenously to reach the tumor core, since one of our tumor cell clones transfected with the IL-10 gene (clone #1A) failed to establish primary tumors. Work in our laboratories is in progress to determine if a synergism, acting on both primary and metastasizing tumors, may exist between IL-10 and other cytokines, such as IL-2 and IL-4, which have previously been reported to inhibit primary tumor growth (1, 2, 7, 21). Cancer treatment with IL-10 to inhibit metastases, combined with other cytokines or surgical intervention to remove primary tumors, may thus provide an effective means of thwarting the spread of many tumors.

References

1. Fearon, E.R., D.M. Pardoll, T. Itaya, P. Golumbek, H.I. Levitsky, J.W. Simons, H. Karasuyama, B. Vogelstein, and P. Frost. 1990. Interleukin-2 production by tumor cells bypasses T helper function in the generation of an antitumor response. *Cell.* 60:397–403.

2. Tepper, R.I., P.K. Pattengale, and P. Leder. 1989. Murine interleukin-4 displays potent anti-tumor activity *in vivo. Cell.* 57:503–512.

3. Blankenstein, T., Z. Qin, K. Überla, W. Müller, H. Rosen, H.-D. Volk, and T. Diamanstein. 1991. Tumor suppression after tumor cell-targeted tumor necrosis factor α gene transfer. *J. Exp. Med.* 173:1047–1052.

4. Blankenstein, T., D.A. Rowley, and H. Schreiber. 1991. Cytokines and cancer: experimental systems. *Curr. Opin. Immunol.* 3:694–698.

5. Weber, J.S., G. Jay, K. Tanaka, and S.A. Rosenberg. 1987. Immunotherapy of a murine tumor with interleukin 2. *J. Exp. Med.* 166:1716–1733.

6. Silagi, S., R. Dutkowski, and A. Schaefer. 1988. Eradication of mouse melanoma by combined treatment with recombinant human interleukin 2 and recombinant murine interferon-gamma. *Int. J. Cancer* 41:315–322.

7. Roth, C., L.M. Mir, M. Cressent, F. Quintin-Colonna, V. Ley, D. Fradelizi, and P. Kourilsky. 1992. Inhibition of tumor growth by histoincompatible cells expressing interleukin-2. *Int. Immunol.* 4:1429–1436.

8. Dranoff, G., E. Jaffee, A. Lazenby, P. Golumbek, H. Levitsky, K. Brose, V. Jackson, H. Hamada, D. Pardoll, and R.C. Mulligan. 1993. Vaccination with irradiated tumor cells engineered to secrete murine GM-CSF stimulates potent, specific, and long-lasting anti-tumor immunity. *Proc. Natl. Acad. Sci. USA.* 90:3539–3543.

9. Yamada, G., Y. Kitamura, H. Sonoda, H. Harada, S. Taki, R.C. Mulligan, and H. Osawa. 1987. Retroviral expression of the human IL-2 gene in a murine T cell line results in cell growth autonomy and tumorigenicity. *EMBO (Eur. Mol.*

Biol. Organ.) J. 6:2705–2709.

10. de Waal Malefyt, R., J. Haanen, H. Spits, M.G. Roncarolo, A. te Velde, C. Figdor, K. Johnson, R. Kastelein, H. Yssel, and J.E. de Vries. 1991. Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression. *J. Exp. Med.* 174:915–924.

11. Go, N.F., B.E. Castle, R. Barret, R. Kastelein, W. Dang, T.R. Mosmann, K.W. Moore, and M. Howard. 1990. Interleukin 10 (IL-10), a novel B cell stimulatory factor: unresponsiveness of X chromosome-linked immunodeficiency B cells. *J. Exp. Med.* 172:1625–1631.

12. Moore, K.W., A. O'Garra, R. de Waal Malefyt, P. Vieira, and T.R. Mosmann. 1993. Interleukin-10. *Annu. Rev. Immunol.* 11:165–190.

13. de Waal Malefyt, R., J. Abrams, B. Bennett, C.G. Figdor, and J.E. de Vries. 1991. Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J. Exp. Med.* 174:1209–1220.

14. Bogdan, C., Y. Vodovotz, and C. Nathan. 1991. Macrophage deactivation by interleukin 10. *J. Exp. Med.* 174:1549–1555.

15. Tripp, C.S., S.F. Wolf, and E.R. Unanue. 1993. Interleukin 12 and tumor necrosis factor alpha are costimulators of interferon gamma production by natural killer cells in severe combined immunodeficiency mice with listeriosis, and interleukin 10 is a physiologic antagonist. *Proc. Natl. Acad. Sci. USA.* 90:3725–3729.

16. Silva, J.S., P.J. Morrissey, K.H. Grabstein, K.M. Mohler, D. Anderson, and S.G. Reed. 1992. Interleukin-10 and interferon-γ regulation of *Trypanosoma cruzi* infection. *J. Exp. Med.* 175:169–174.

17. Bermudez, L.F., and J. Champsi. 1993. Infection with *Mycobacterium avium* induces production of interleukin-10 (IL-10), and administration of anti-IL-10 antibody is associated with enhanced resistance to infection in mice. *Infect. Immun.* 61:3093–3097.

18. Zheng, L.M., D.M. Ojcius, and J.D.-E. Young. 1993. Distribution of perforin-containing cells in normal and pregnant mice. *Eur. J. Immunol.* 23:2085–2091.

19. Richter, G., S. Kruger Krasagakes, G. Hein, C. Huls, E. Schmitt, T. Diamantstein, and T. Blankenstein. 1993. Interleukin 10 transfected into Chinese hamster ovary cells prevents tumor growth and macrophage infiltration. *Cancer Res.* 53:4134–4137.

20. Giovarelli, M., P. Musiani, A. Modesti, P. Dellabona, G. Casorati, A. Allione, M. Consalvo, F. Cavallo, F. di Pierro, C. De Giovanni et al., 1995. Local release of IL-10 by transfected mouse mammary adenocarcinoma cells does not suppress but enhances antitumor reaction and elicits a strong cytotoxic lymphocyte and antibody-dependent immune memory. *J. Immunol.* 155:3112–3123.

21. Hock, H., M. Dorsch, U. Kunzendorf, Z. Qin, T. Diamantstein, and T. Blankenstein. 1993. Mechanisms of rejection induced by tumor cell-targeted gene transfer of interleukin 2, interleukin 4, interleukin 7, tumor necrosis factor, or interferon γ. *Proc. Natl. Acad. Sci. USA.* 90:2774–2778.

22. Hock, H., M. Dorsch, T. Diamantstein, and T. Blankenstein. 1991. Interleukin 7 induces CD4⁺ T cell-dependent tumor

rejection. *J. Exp. Med.* 174:1291–1298.

23. Kharkevitch, D.D., D. Seito, G.C. Balch, T. Maeda, C.M. Balch, and K. Itoh. 1994. Characterization of autologous tumor-specific T-helper 2 cells in tumor-infiltrating lymphocytes from a patient with metastatic melanoma. *Int. J. Cancer* 58:317–323.

24. Moormeier, J.A., and H.M. Golomb. 1991. Interferons: clinical applications. Hairy cell leukemia. *In* Biologic therapy of cancer. V. DeVita, S. Hellman and S. Rosenberg, editor. Lippincott, Philadelphia. 275–288.

25. Nabioullin, R., S. Sone, K. Mizuno, S. Yano, Y. Nishioka, T. Haku, and T. Ogura. 1994. Interleukin-10 is a potent inhibitor of tumor cytotoxicity by human monocytes and alveolar macrophages. *J. Leukoc. Biol.* 55:437–442.

26. Alleva, D.G., and K.D. Elgert. 1995. Promotion of macrophage-stimulated autoreactive T cell proliferation by interleukin-10: counteraction of macrophage suppressor activity during tumor growth. *Immunobiol.* 192:155–171.

27. Ding, L., P.S. Linsley, L.-Y. Huang, R.N. Germain, and E.M. Shevach. 1993. IL-10 inhibits macrophage costimulatory activity by selectively inhibiting the up-regulation of B7 expression. *J. Immunol.* 151:1224–1234.

28. Denis, M., and E. Ghadirian. 1993. IL-10 neutralization augments mouse resistance to systemic *Mycobacterium avium* infections. *J. Immunol.* 151:5425–5430.

29. Bejarano, M.T., R. de Waal Malefyt, J.S. Abrams, M. Bigler, R. Bacchetta, J.E. de Vries, and M.G. Roncarolo. 1992. Interleukin 10 inhibits allogeneic proliferative and cytotoxic T cell responses generated in primary mixed lymphocyte cultures. *Int. Immunol.* 4:1389–1397.

30. Beissert, S., J. Hosoi, S. Grabbe, A. Asahina, and R.D. Granstein. 1995. IL-10 inhibits tumor antigen presentation by epidermal antigen-presenting cells. *J. Immunol.* 154:1280–1286.

31. Carson, G.E., M.J. Lindemann, R. Baiocchi, M. Linett, J.C. Tan, C.-C. Chou, S. Narula, and M.A. Caligiuri. 1995. The functional characterization of interleukin-10 receptor expression on human natural killer cells. *Blood.* 85:3577–3585.

32. Salazar-Onfray, F., M. Petersson, L. Franksson, M. Matsuda, T. Blankenstein, K. Kärre, and R. Kiessling. 1995. IL-10 converts mouse lymphoma cells to a CTL-resistant, NK-sensitive phenotype with low but peptide-inducible MHC class I expression. *J. Immunol.* 154:6291–6298.

33. Rice, G.E., and M.P. Bevilacqua. 1989. An inducible endothelial cell surface glycoprotein mediates melanoma adhesion. *Science (Wash. DC).* 246:1303–1306.

34. Burrows, F.J., D.O. Haskard, I.R. Hart, J.F. Marshall, S. Selkirk, S. Poole, and P.E. Thorpe. 1991. Influence of tumor-derived interleukin 1 on melanoma-endothelial cell interactions *in vitro*. *Cancer Res.* 51:4768–4775.

35. Van Vlasselaer, P., B. Borremans, U. van Gorp, J.R. Dasch, and R. De Waal-Malefyt. 1994. Interleukin 10 inhibits transforming growth factor-β (TGF-β) synthesis required for osteogenic commitment of mouse bone marrow cells. *J. Cell Biol.* 124:569–577.

36. Moses, H.L., E.Y. Yang, and J.A. Pietenpol. 1990. TGF-β stimulation and inhibition of cell proliferation: new mechanistic insights. *Cell.* 63:245–247.

37. Sulitzeanu, D. 1993. Immunosuppressive factors in human cancer. *Adv. Cancer Res.* 60:247–267.