

Effect of Interleukin 1 Receptor Antagonist Gene Transduction on Human Melanoma Xenografts in Nude Mice

David M. Weinreich, Dina M. Elaraj, Markus Puhlmann, Stephen M. Hewitt, Nancy M. Carroll, Elizabeth D. Feldman, Ewa M. Turner, Paul J. Spiess, and H. Richard Alexander¹

The Surgical Metabolism Section [D. M. W., D. M. E., M. P., N. M. C., E. D. F., E. M. T., H. R. A.] and Immunology Section [P. J. S.], Surgery Branch and the Laboratory of Pathology [S. M. H.], Center for Cancer Research, National Cancer Institute, Bethesda, Maryland 20892

ABSTRACT

Interleukin (IL)-1 is a pleiotropic inflammatory cytokine that promotes angiogenesis and enhances tumor growth and metastases. We evaluated the effects of IL-1 receptor antagonist (IL-1ra) on tumor growth and metastases in human melanoma xenografts. We selected two human melanoma lines (SMEL and PMEL) with differential (high versus low, respectively) constitutive production of IL-1 by ELISA. The IL-1ra gene was isolated from monocyte RNA by PCR and retrovirally transduced into SMEL and PMEL. *In vitro* cell proliferation was evaluated using a WST-1 assay. Athymic nude mice received s.c. or i.v. injection with parental, vector-transduced, or IL-1ra-transduced melanoma cells, and tumor growth, lung metastases, and histology were characterized. IL-1 was produced by SMEL *in vitro* and *ex vivo* (117 and 67 pg/ml/10⁶ cells/24 h, respectively), but not by PMEL (15 and 0 pg/ml/10⁶ cells/24 h, respectively). Neither made IL-1ra natively. Gene-transduced cell lines secreted >1000 pg/ml/10⁶ cells/24 h of IL-1ra by ELISA. *In vitro* proliferation of each parental cell line was comparable to the proliferation rate of each transduced cell line. IL-1ra-transduced SMEL (SMEL/IL-1ra) showed significantly slower tumor growth compared with null-transduced and parental cell lines ($P < 0.001$, ANOVA-Bonferroni/Dunn). There was no difference in growth rates between PMEL and IL-1ra-transduced PMEL (PMEL/IL-1ra). A mixing study of SMEL and SMEL/IL-1ra showed significant inhibition of tumor growth at various ratios ($P < 0.001$, ANOVA-Bonferroni/Dunn). There were significantly fewer lung metastases with SMEL/IL-1ra versus SMEL ($P < 0.002$). IL-1ra decreases *in vivo* growth and metastatic potential of a human melanoma xenograft that constitutively secretes IL-1. This effect may be exploitable using clinically available IL-1ra for the treatment of human cancers.

INTRODUCTION

Tumors secrete multiple cytokines or factors that can alter the tumor microenvironment and promote tumor growth and metastases. Many of these factors promote an angiogenic phenotype in stromal or infiltrating host cells, resulting in the development of a neovasculature that provides nutrients for continued tumor growth and access to the vasculature for potential metastatic spread. Expression or overexpression of these tumor-derived angiogenic or inflammatory proteins *in vivo* has been shown to reflect an aggressive phenotype with respect to tumor growth, metastatic potential, and shortened survival (1–5). Antiangiogenic agents that inhibit proteins such as vascular endothelial growth factor or block matrix metalloproteinases are in clinical trials, alone or in combination with other antiangiogenic agents, chemotherapy, or radiation therapy (6–9).

IL-1² is a proinflammatory pleiotropic cytokine that has been shown to be an endogenous mediator of various acute and chronic inflammatory conditions such as endotoxic shock and RA (10, 11).

There is growing evidence that IL-1 promotes production of angiogenic proteins from host stromal or infiltrating cells in the tumor microenvironment that enhance tumor growth and metastases (12, 13). Investigators have shown an association between IL-1 production and altered tumor phenotype characterized by accelerated growth rates and increased metastases in experimental models (14). IL-1 production by tumors has been associated with a worse prognosis in patients with breast, lung, or head and neck cancers (15–17).

A naturally occurring antagonist to IL-1, the IL-1ra, has been extensively characterized and competitively blocks both isoforms of IL-1 at the receptor level (18–20). Previous work has shown that exogenously administered IL-1ra can block IL-1-induced experimental tumor metastases (14). Vidal-Vanaclocha *et al.* (21) have shown that development of hepatic metastases in a murine melanoma model could be inhibited by IL-1ra. Of note, others have shown that IL-1 may serve as an autocrine growth factor for some tumors (22), and La *et al.* (23) showed that IL-1ra directly inhibits proliferation of a murine skin carcinoma, suggesting that direct effects of IL-1ra on tumor cell proliferation may be responsible in part for effects on tumor growth or metastatic potential *in vivo*.

The current studies were performed to characterize the effects of IL-1ra gene transduction and protein overexpression on human melanoma cell lines and xenografts in nude mice. We selected two lines that differed in constitutive expression of IL-1 to demonstrate the specific effects of IL-1ra and show that IL-1ra inhibits tumor growth and metastases without direct effects on cellular proliferation *in vitro* or *in vivo*. The results suggest that clinically available recombinant IL-1ra may be useful in the treatment of human cancers that constitutively produce IL-1 and that further work with the recombinant protein is warranted.

MATERIALS AND METHODS

Isolation of the IL-1ra Gene and Cloning Strategy. Frozen peripheral blood mononuclear cells were obtained from a leukopheresis of a volunteer donor and frozen. The cells were subsequently thawed and plated in a culture flask containing Iscove's media supplemented with 10% AB serum and DNase. The cells were incubated for 3 h and washed twice with PBS. The RNA of the adherent monocytes was purified using Trizol (Invitrogen, Carlsbad, CA) following the manufacturer's protocol. cDNA was created from the purified RNA using SuperScript II (Invitrogen). The IL-1ra gene was amplified by PCR using gene-specific primers (5'-GATCAAGCTTATGGCTTTA-GAGAC-3' and 5'-GATCATCGATCTACTCGTCCTCT-3') and high-fidelity Pfx polymerase (Invitrogen). The amplified product was inserted into the blunt-4-TOPO cloning vector (Invitrogen) for sequencing using the manufacturer's protocol. The vector containing the IL-1ra gene was transduced into Max efficiency DH5 α cells (Invitrogen) and expanded overnight in LB broth with ampicillin (Stratagene, La Jolla, CA) to provide selection pressure. The plasmid was purified using a mini plasmid prep kit (Qiagen, Valencia, CA). The portion of the vector containing the IL-1ra gene was sequenced using an ABI Prism sequencer (Applied Biosystems, Foster City, CA). After the sequence was determined to be correct, the gene was excised from the TOPO vector using the appropriate restriction enzymes and inserted into pCLNCX, a custom retroviral expression plasmid using a cytomegalovirus promoter (24, 25). The null vector and the vector containing the gene for IL-1ra were expanded using DH5 α cells.

Received 4/17/03; revised 6/12/03; accepted 7/2/03.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ To whom requests for reprints should be addressed, at Head, Surgical Metabolism Section, Surgery Branch, Center for Cancer Research, Building 10, Room 2B07, National Cancer Institute, 10 Center Drive, Bethesda, MD 20892-1502. Phone: (301) 496-2195; Fax: (301) 402-1788; E-mail: Richard_Alexander@nih.gov.

² The abbreviations used are: IL, interleukin; IL-1ra, IL-1 receptor antagonist; RPMI-c, RPMI 1640 containing 10% FBS; RPMI-cg, RPMI 1640 supplemented with 10% FCS and 10 μ g/ml Geneticin; RA, rheumatoid arthritis.

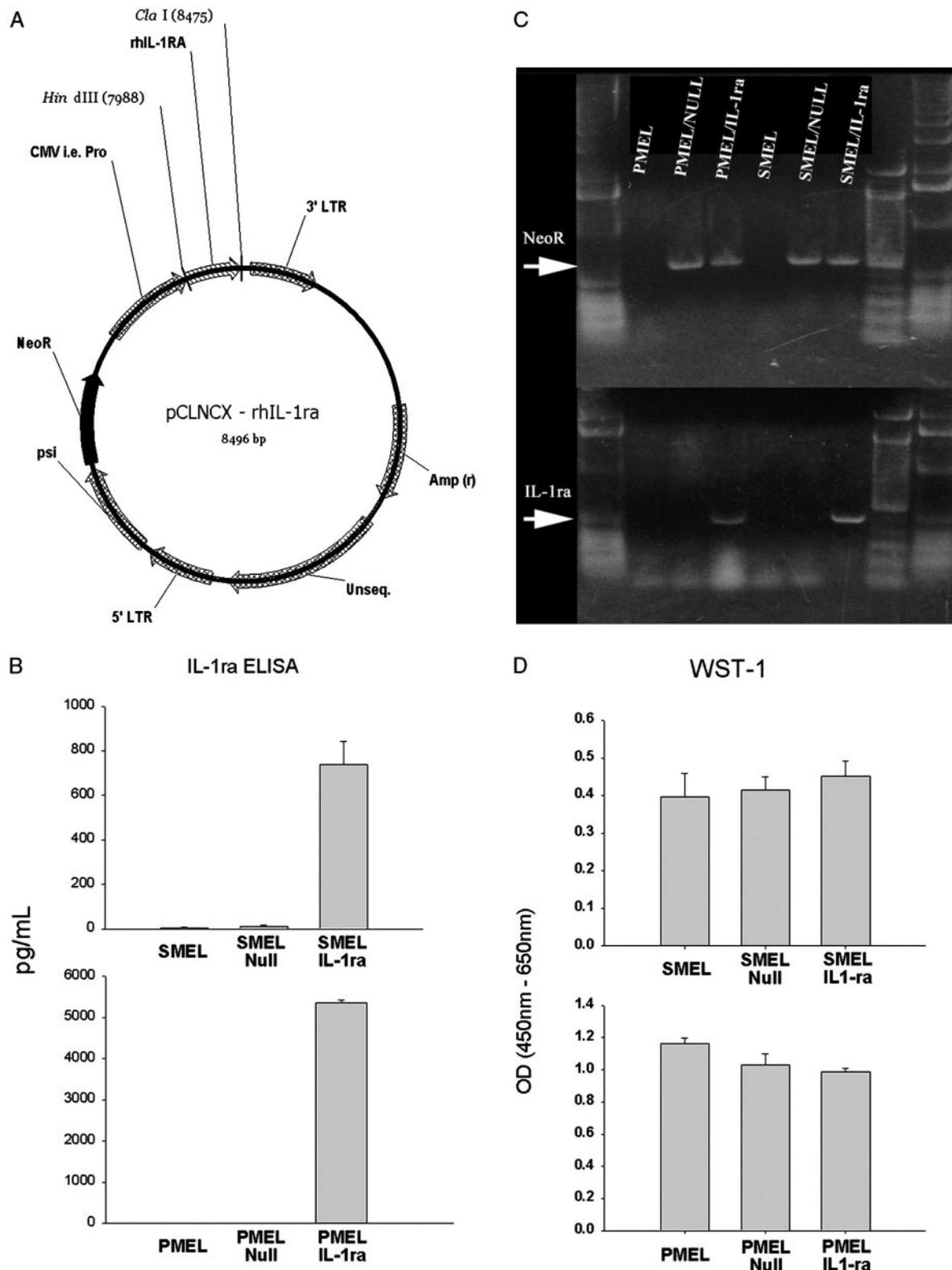


Fig. 1. A, vector map of the retroviral expression plasmid pCLNCX with the IL-1ra gene inserted 3' to the cytomegalovirus promoter. B, ELISA from the supernatant of each of the transduced and wild-type cell lines confirming production and secretion of IL-1ra by only the gene-transduced cell lines. C, PCR of the various clones demonstrating the presence of the neomycin resistance gene in all transduced cells and the presence of the gene encoding IL-1ra only in PMEL/IL-1ra and SMEL/IL-1ra (bottom). PMEL (wild type, Lane 1) and SMEL (wild type, Lane 4) do not contain either the IL-1ra or NeoR genes. PMEL/null (Lane 2) and SMEL/null (Lane 5) contain only the NeoR gene and lack the IL-1ra gene. PMEL/IL-1ra (Lane 3) and SMEL/IL-1ra (Lane 6) contain both the NeoR gene and the gene encoding IL-1ra. D, WST-1 reaction showing no difference in proliferation rates *in vitro*.

Selection of the Tumor Lines and Assay for IL-1 Expression. Two human melanoma tumor lines, SMEL and PMEL, which have been derived from patients and characterized previously (26), were grown in culture and plated at 1×10^5 cells/well in a 6-well plate with 2 ml of RPMI-c for 12 h, and

the supernatant was harvested. Simultaneously, 2×10^6 cells were injected s.c. into the flanks of athymic nude mice. The tumors were allowed to grow to 1 cm²; harvested; digested at 37°C with a triple digest mix of hyaluronidase, collagenase, and DNase for 3 h; filtered; and plated at 1×10^5 cells/well in a

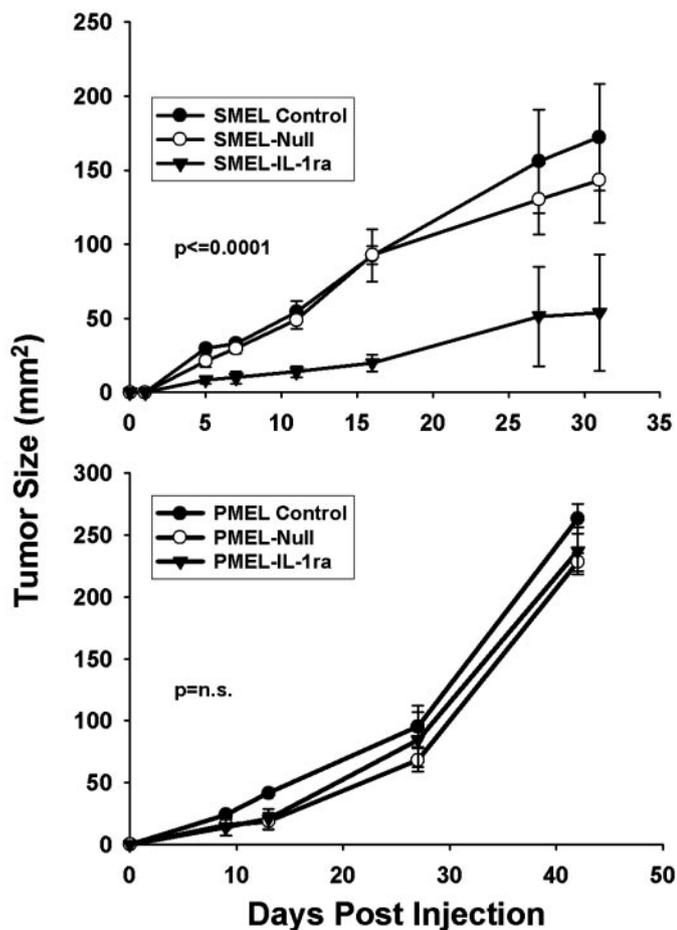


Fig. 2. Top panel shows marked growth inhibition of SMEL/IL-1ra xenografts compared with SMEL/null and parental SMEL controls. Bottom panel shows no effect on growth of PMEL/IL-1ra xenografts compared with null-transduced and wild-type controls.

6-well plate with 2 ml of RPMI-c media for 12 h; and the supernatant was harvested. An ELISA for IL-1 (Pierce, Rockford, IL) was performed.

Transformation of the Tumor Lines and Evaluation of *in Vitro* Effects of Transfection. GP293, a renal cell tumor line previously transformed with resistance to blasticidin (Calbiochem, San Diego, CA), was cotransfected with pCLNCX (null or encoding IL-1ra) and a packaging plasmid encoding the retroviral envelope protein using LipofectAMINE 2000 (Invitrogen) according to the manufacturer's protocol. Twenty-four h later, the PMEL or SMEL cells were exposed to the supernatant containing retrovirus. The IL-1ra-transduced cells were subcloned at 0.8 clone/well in a 96-well plate with 100 μ l of RPMI-cg. The supernatant was harvested, and an ELISA for IL-1ra was performed. The highest secreting clone was continually passaged in RPMI-cg. The parental cell line and the two transduced cell lines (null or gene transduced) were plated at 1×10^5 cells/well in a 6-well plate with 2 ml of RPMI-cg for 24 h. The supernatant was harvested, and an ELISA for IL-1ra was performed. The RNA from these cells was then purified using the Rneasy Mini Kit (Qiagen) for PCR analysis. To evaluate cell proliferation, cells were plated at 1×10^4 cells/well in 100 μ l of complete media in a 96-well plate, incubated for 24 h, and exposed to 10 μ l of WST-1 (Roche) per the manufacturer's protocol.

Animal Models for Evaluation of Tumor Xenografts. Tumor cells (2×10^6) were injected s.c. into the flanks of athymic nude mice, and the tumors were measured. Tumor areas were calculated by taking the product of the perpendicular diameters of each tumor over time. In a separate experiment, parental SMEL cells and SMEL/IL-1ra cells were injected s.c. into the flanks of athymic nude mice, and the mice were sacrificed, and tumors were harvested at approximately 16 mm² for quantitative vessel counts by a pathologist (S. M. H.) who was blinded to the nature of the experimental groups. In other experiments, parental SMEL cells were mixed *ex vivo* in different ratios with SMEL/IL-1ra cells to evaluate for a paracrine effect of IL-1ra production on

tumor growth. In other mice, 1×10^6 cells (SMEL, SMEL/null, or SMEL/IL-1ra) were injected via tail vein into athymic nude mice that were irradiated with 500 cGy in a Gammacell-40 irradiator (Nordian) just before tumor injection. Mice were euthanized on day 28, their trachea were cannulated with a 19-gauge needle, and the lungs were insufflated with India ink. The lungs were then harvested and washed in sterile PBS followed by Fecedes' solution. The lobes were then dissected, and the number of lung metastases was counted. For all animal experiments, the observer conducting the measurements of tumor size or counting of metastases was blinded to design of the experiment.

RESULTS

A vector map for the pCLNCX expression plasmid following insertion of the IL-1ra gene by restriction digest is shown in Fig. 1A. After transduction of SMEL and PMEL with either a null pCLNCX retroviral vector or pCLNCX-IL-1ra, only the cell lines transduced with pCLNCX-IL-1ra produced IL-1ra as detected by ELISA of the cell supernatant (Fig. 1B) or by PCR (Fig. 1C). All four transduced cell lines expressed the neomycin resistance gene, indicating a successful transduction (Fig. 1C). There were no differences in proliferation rates *in vitro* between the transduced and nontransduced cell lines, indicating that there were no autocrine proliferative effects of IL-1ra on these tumors (Fig. 1D).

To determine the amount of secretion of IL-1 α *in vitro* and in the tumor stroma *in vivo*, quantitative ELISA for IL-1 α was performed on supernatant from SMEL and PMEL melanoma cultured *in vitro* and *ex vivo* from cells harvested from tumor-bearing mice. The SMEL tumor line showed secretion of IL-1 α both *in vitro* and *ex vivo* (117 and 67 pg/ml/ 10^6 cells/24 h, respectively) whereas the PMEL tumor line produced very low or no detectable IL-1 α *in vitro* or *ex vivo* (15 and 0 pg/ml/ 10^6 cells/24 h, respectively). Transduction of the gene encoding for IL-1ra did not alter the amount of IL-1 α produced compared with the respective wild-type cell lines, and none of the cell lines produced IL-1 β by ELISA (data not shown).

Growth of s.c. SMEL/IL-1ra was statistically significantly decreased compared with the null-transduced or parental xenografts ($P < 0.0001$, ANOVA-Bonferroni/Dunn). However, PMEL/IL-1ra, PMEL/null, and parental PMEL xenografts, which do not produce IL-1, all grew at identical rates (Fig. 2). When SMEL was mixed at various ratios with SMEL/IL-1ra *ex vivo*, there was a significant inhibition of tumor growth *in vivo*, indicating a marked paracrine effect of locally produced IL-1ra (Fig. 3). Histological sections of tumor obtained from mice harboring SMEL or SMEL/IL-1ra tumors

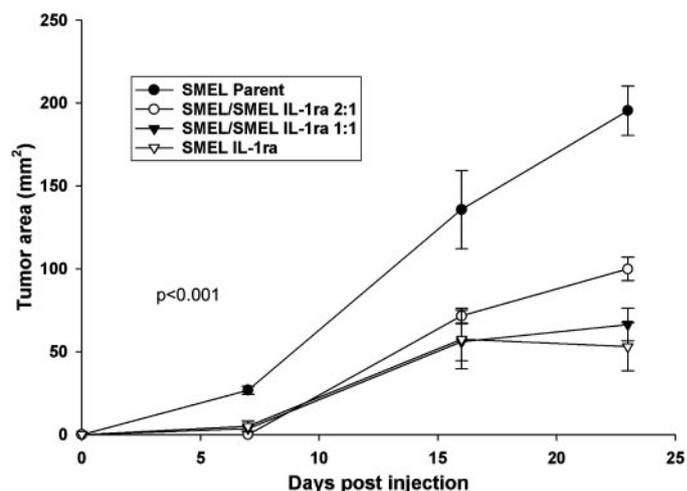


Fig. 3. SMEL was mixed at the indicated ratios with SMEL/IL-1ra *ex vivo* and injected s.c. into athymic nude mice. There was significant growth inhibition at all tested ratios, indicating a marked paracrine effect of IL-1ra on tumor growth.

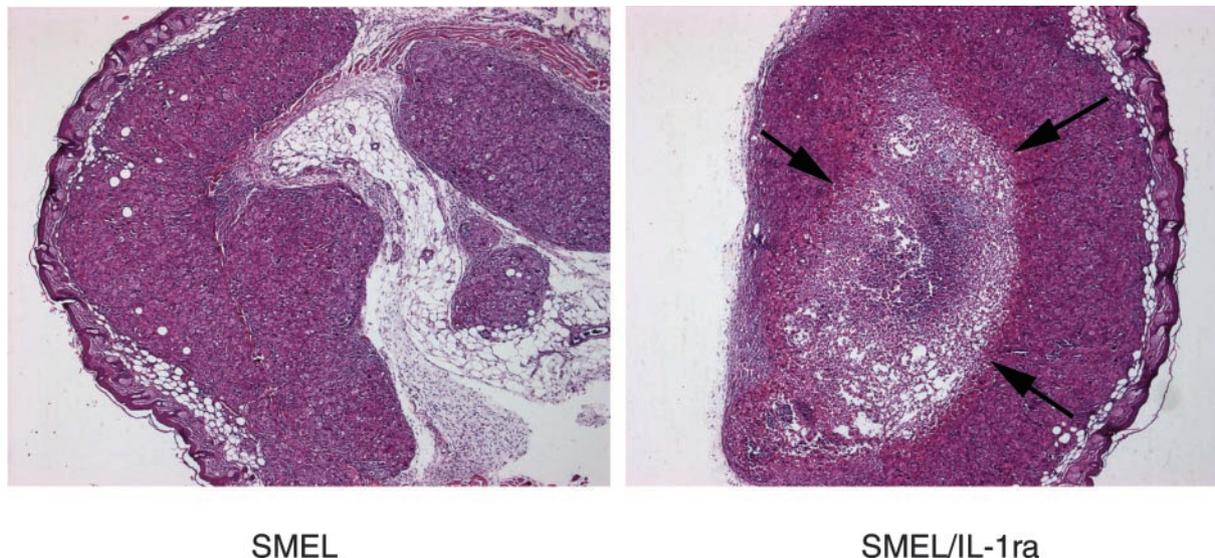


Fig. 4. Representative photomicrographs of H&E-stained tumors ($\times 25$ magnification) harvested from mice. Tumors were harvested at approximately 16 mm^2 . The SMEL parental tumor (left panel) shows a uniform pattern without any evidence of infiltrate or necrosis. The SMEL/IL-1ra tumor (right panel) shows marked central necrosis (denoted by arrows).

($n = 6$ mice/group) showed a large amount of central necrosis consistently present in SMEL/IL-1ra tumors that was not present in the parental SMEL tumors of equal size (Fig. 4). The mean number of mitotic tumor cells/high-powered field was 2.8 ± 0.37 in parental SMEL cells and 4.7 ± 0.53 in SMEL/IL-1ra cells ($P_2 = 0.054$), supporting the *in vitro* findings that IL-1ra did not alter tumor size by affecting cellular proliferation rates *in vivo*.

The SMEL/IL-1ra clones had decreased metastatic potential compared with SMEL cells as evaluated in a lung metastasis model. The numbers of lung metastases 28 days after i.v. tumor injection were significantly lower in SMEL/IL-1ra compared with both SMEL/null and parental SMEL (3.0 ± 0.4 versus 45.6 ± 4.7 and 35.7 ± 2.1 , respectively; $P < 0.002$; Fig. 5).

DISCUSSION

IL-1 is a pleiotropic angiogenic cytokine associated with an increase in the growth and metastatic potential of some human cancers such as squamous cell cancer of the head and neck or adenocarcinoma of the breast or lung (15, 16, 27). Previous work has shown that exogenously administered IL-1 can promote tumor growth and metastases in experimental tumor models (14, 17, 28), and IL-1ra administration has been shown to reduce the incidence of hepatic metastases in a murine melanoma model (21). The mechanisms for these observations are not known, but others have shown that IL-1 can cause autocrine proliferative effects on murine and human cancer cell lines, suggesting that IL-1ra may exert some antitumor activity *in vivo* by inhibiting IL-1-mediated tumor cell proliferation directly. The data from the current study support the prevailing theory that IL-1 produced by tumors *in vitro* and present in the tumor microenvironment *in vivo* may be important in tumor growth and metastatic potential. IL-1ra had no direct antiproliferative effects *in vitro* in two human melanoma lines that differed in their constitutive production of IL-1 and inhibited tumor growth *in vivo* selectively in the cell line that produced IL-1 natively.

It is also noteworthy that there was a significantly lower incidence of pulmonary metastases in SMEL/IL-1ra compared with wild-type or null-transduced SMEL; this supports a direct role for IL-1 in the metastatic phenotype of some human cancers. The mechanism of metastatic spread is a complex multistep process involving adhesion or trapping of tumor in the microcirculation, extravasation into the interstitium, and subse-

quent recruitment of host cells to establish a neovasculature (29–33). IL-1 has been shown to promote metastases in experimental tumors and may promote this process by up-regulation of endothelial cell surface expression of adhesion molecules or tissue factor, increased matrix metalloproteinase activity to promote tumor migration, and induction of localized vascular permeability with efflux of nutrient plasma and proteins into the interstitium (34–37).

The data reported in this study may have relevance to clinical cancer treatment. IL-1 is a known mediator of various acute and chronic inflammatory conditions, and a recombinant IL-1ra has been shown to have significant activity *in vivo* in abrogating the deleterious effects of endogenously produced IL-1 in experimental models of endotoxic shock and Gram-negative sepsis or models of chronic inflammation such as inflammatory bowel disease or arthritis (38, 39). The data suggest that IL-1 acts as an early mediator in the pathophysiology of these processes and promotes production of a cascade of downstream effector proteins from various cell types. It therefore represents an ideal target to abrogate or reverse these deleterious effects. In support of this concept, elevated levels of IL-1 in synovial

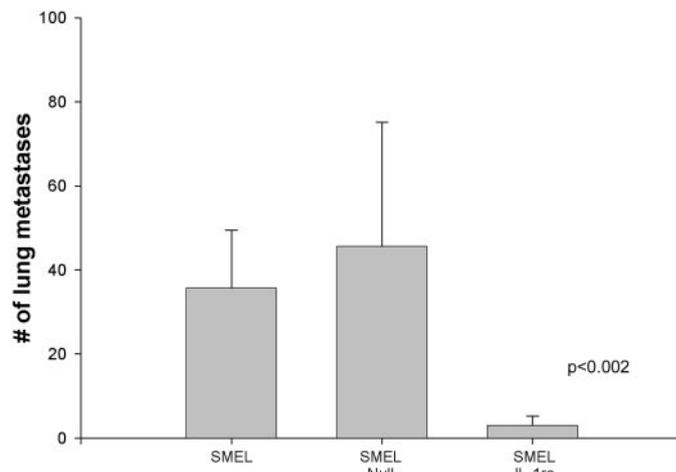


Fig. 5. There was marked inhibition of lung metastases from the SMEL/IL-1ra cells compared with the null-transduced and wild-type controls. Cells were injected via tail vein, and the lungs were harvested 28 days later as described in "Materials and Methods."

fluid of patients with RA are thought to mediate the characteristic articular joint destruction and neovessel formation in this disease (40, 41). Recombinant IL-1ra has been shown to ameliorate this condition and is now approved by the Food and Drug Administration for the treatment of patients with moderate to severe RA. The mechanisms through which IL-1ra may exert its therapeutic effects are not entirely known but most likely are related to inhibition of inflammation and neovessel formation in the articular space, a pathophysiology that shares some features with the neovascularization of growing tumors. Our data demonstrated a substantial bystander effect of IL-1ra *in vivo*, suggesting that local concentrations of the recombinant IL-1ra protein, when administered systemically, might also be sufficient to inhibit tumor growth or metastases. Further work characterizing the recombinant IL-1ra in experimental models of tumor growth and metastases is warranted, and we support further evaluation of the use of IL-1 blockade for the treatment of human cancers.

References

- Dias, S., Choy, M., Alitalo, K., and Rafii, S. Vascular endothelial growth factor (VEGF)-C signaling through FLT-4 (VEGFR-3) mediates leukemic cell proliferation, survival, and resistance to chemotherapy. *Blood*, 99: 2179–2184, 2002.
- Yuan, A., Yu, C. J., Chen, W. J., Lin, F. Y., Kuo, S. H., Luh, K. T., and Yang, P. C. Correlation of total VEGF mRNA and protein expression with histologic type, tumor angiogenesis, patient survival and timing of relapse in non-small-cell lung cancer. *Int. J. Cancer*, 89: 475–483, 2000.
- Grandis, J. R., Melhem, M. F., Gooding, W. E., Day, R., Holst, V. A., Wagener, M. M., Drenning, S. D., and Twardy, D. J. Levels of TGF- α and EGFR protein in head and neck squamous cell carcinoma and patient survival. *J. Natl. Cancer Inst.* (Bethesda), 90: 824–832, 1998.
- Salgado, R., Junius, S., Benoy, I., Van Dam, P., Vermeulen, P., Van Marck, E., Hugu, P., and Dirix, L. Y. Circulating interleukin-6 predicts survival in patients with metastatic breast cancer. *Int. J. Cancer*, 103: 642–646, 2003.
- Chen, J. J., Yao, P. L., Yuan, A., Hong, T. M., Shun, C. T., Kuo, M. L., Lee, Y. C., and Yang, P. C. Up-regulation of tumor interleukin-8 expression by infiltrating macrophages: its correlation with tumor angiogenesis and patient survival in non-small cell lung cancer. *Clin. Cancer Res.*, 9: 729–737, 2003.
- Eder, J. P., Jr., Supko, L. G., Clark, J. W., Puchalski, T. A., Garcia-Carbonero, R., Ryan, D. P., Shulman, L. N., Proper, J., Kirvan, M., Rattner, B., Connors, S., Keogan, M. T., Janicek, M. J., Fogler, W. E., Schnipper, L., Kinchla, N., Sidor, C., Phillips, E., Folkman, J., and Kufe, D. W. Phase I clinical trial of recombinant human endostatin administered as a short intravenous infusion repeated daily. *J. Clin. Oncol.*, 20: 3772–3784, 2002.
- Gingras, D., Boivin, D., Deckers, C., Gendron, S., Barthomeuf, C., and Beliveau, R. Neovastat: a novel antiangiogenic drug for cancer therapy. *Anticancer Drugs*, 14: 91–96, 2003.
- Marx, G. M., Pavlakis, N., McCowatt, S., Boyle, F. M., Levi, J. A., Bell, D. R., Cook, R., Biggs, M., Little, N., and Wheeler, H. R. Phase II study of thalidomide in the treatment of recurrent glioblastoma multiforme. *J. Neurooncol.*, 54: 31–38, 2001.
- Stopeck, A., Sheldon, M., Vahedian, M., Cropp, G., Gosalia, R., and Hannah, A. Results of a Phase I dose-escalating study of the antiangiogenic agent, SU5416, in patients with advanced malignancies. *Clin. Cancer Res.*, 8: 2798–2805, 2002.
- Dinarello, C. A. Biology of interleukin 1. *FASEB J.*, 2: 108–115, 1988.
- Dinarello, C. A., and Wolff, S. M. The role of interleukin-1 in disease. *N. Engl. J. Med.*, 328: 106–113, 1993.
- Salven, P., Hattori, K., Heissig, B., and Rafii, S. Interleukin-1 α promotes angiogenesis *in vivo* via VEGFR-2 pathway by inducing inflammatory cell VEGF synthesis and secretion. *FASEB J.*, 16: 1471–1473, 2002.
- Saijo, Y., Tanaka, M., Miki, M., Usui, K., Suzuki, T., Maemondo, M., Hong, X., Tazawa, R., Kikuchi, T., Matsushima, K., and Nukiwa, T. Proinflammatory cytokine IL-1 β promotes tumor growth of Lewis lung carcinoma by induction of angiogenic factors: *in vivo* analysis of tumor-stromal interaction. *J. Immunol.*, 169: 469–475, 2002.
- Chirivì, R. G., Garofalo, A., Padura, I. M., Mantovani, A., and Giavazzi, R. Interleukin 1 receptor antagonist inhibits the augmentation of metastasis induced by interleukin 1 or lipopolysaccharide in a human melanoma/nude mouse system. *Cancer Res.*, 53: 5051–5054, 1993.
- Chen, Z., Malhotra, P. S., Thomas, G. R., Ondrey, F. G., Duffey, D. C., Smith, C. W., Enamorado, I., Yeh, N. T., Kroog, G. S., Rudy, S., McCullagh, L., Mousa, S., Quezado, M., Herscher, L. L., and Van Waes, C. Expression of proinflammatory and proangiogenic cytokines in patients with head and neck cancer. *Clin. Cancer Res.*, 5: 1369–1379, 1999.
- Gemma, A., Takenaka, K., Hosoya, Y., Matuda, K., Seike, M., Kurimoto, F., Ono, Y., Uematsu, K., Takeda, Y., Hibino, S., Yoshimura, A., Shibuya, M., and Kudoh, S. Altered expression of several genes in highly metastatic subpopulations of a human pulmonary adenocarcinoma cell line. *Eur. J. Cancer*, 37: 1554–1561, 2001.
- Nozaki, S., Sledge, G. W., Jr., and Nakshatri, H. Cancer cell-derived interleukin 1 α contributes to autocrine and paracrine induction of pro-metastatic genes in breast cancer. *Biochem. Biophys. Res. Commun.*, 275: 60–62, 2000.
- Dinarello, C. A. Interleukin-1 and interleukin-1 antagonism. *Blood*, 77: 1627–1652, 1991.
- Dinarello, C. A. Interleukin-1, interleukin-1 receptors and interleukin-1 receptor antagonist. *Intern. Rev. Immunol.*, 16: 457–499, 1998.
- Dinarello, C. A. The role of the interleukin-1-receptor antagonist in blocking inflammation mediated by interleukin-1. *N. Engl. J. Med.*, 343: 732–734, 2000.
- Vidal-Vanaclocha, F., Amezcua, C., Asumendi, A., Kaplanski, G., and Dinarello, C. A. Interleukin-1 receptor blockade reduces the number and size of murine B16 melanoma hepatic metastases. *Cancer Res.*, 54: 2667–2672, 1994.
- Wolf, J. S., Chen, Z., Dong, G., Sunwoo, J. B., Bancroft, C. C., Capo, D. E., Yeh, N. T., Mukaida, N., and Van Waes, C. IL (interleukin)-1 α promotes nuclear factor- κ B and AP-1-induced IL-8 expression, cell survival, and proliferation in head and neck squamous cell carcinomas. *Clin. Cancer Res.*, 7: 1812–1820, 2001.
- La, E., Rundhaug, J. E., and Fischer, S. M. Role of intracellular interleukin-1 receptor antagonist in skin carcinogenesis. *Mol. Carcinog.*, 30: 218–223, 2001.
- Naviaux, R. K., Costanzi, E., Haas, M., and Verma, I. M. The pCL vector system: rapid production of helper-free, high-titer, recombinant retroviruses. *J. Virol.*, 70: 5701–5705, 1996.
- Feldman, A. L., Friedl, J., Lans, T. E., Libutti, S. K., Lorang, D., Miller, M. S., Turner, E. M., Hewitt, S. M., and Alexander, H. R. Retroviral gene transfer of interferon-inducible protein 10 inhibits growth of human melanoma xenografts. *Int. J. Cancer*, 99: 149–153, 2002.
- Jaskowiak, N., Yu, K. C., Alexander, H. R., Sarraf, P., and Fraker, D. L. Cancer cachexia in nude mice bearing human melanoma xenografts. Evaluation of model and cytokine profile. *Proc. Am. Assoc. Cancer Res.*, 36: 1995.
- Liss, C., Fekete, M. J., Hasina, R., Lam, C. D., and Lingem, M. W. Paracrine angiogenic loop between head-and-neck squamous-cell carcinomas and macrophages. *Int. J. Cancer*, 93: 781–785, 2001.
- Chen, Z., Colon, I., Ortiz, N., Callister, M., Dong, G., Pegram, M. Y., Arosarena, O., Strome, S., Nicholson, J. C., and Van Waes, C. Effects of interleukin-1 α , interleukin-1 receptor antagonist, and neutralizing antibody on proinflammatory cytokine expression by human squamous cell carcinoma lines. *Cancer Res.*, 58: 3668–3676, 1998.
- Bevilacqua, M. P., and Nelson, R. M. Endothelial-leukocyte adhesion molecules in inflammation and metastasis. *Thromb. Haemostasis*, 70: 152–154, 1993.
- Price, E. A., Coombe, D. R., and Murray, J. C. β_1 Integrins mediate tumour cell adhesion to quiescent endothelial cells *in vitro*. *Br. J. Cancer*, 74: 1762–1766, 1996.
- Morris, V. L., Schmidt, E. E., MacDonald, I. C., Groom, A. C., and Chambers, A. F. Sequential steps in hematogenous metastasis of cancer cells studied by *in vivo* videomicroscopy. *Invasion Metastasis*, 17: 281–296, 1997.
- Anastassiou, G., Schilling, H., Stang, A., Djakovic, S., Heiligenhaus, A., and Bornfeld, N. Expression of the cell adhesion molecules ICAM-1, VCAM-1 and NCAM in uveal melanoma: a clinicopathological study. *Oncology (Basel)*, 58: 83–88, 2000.
- Nam, J. S., Ino, Y., Sakamoto, M., and Hirohashi, S. Src family kinase inhibitor PP2 restores the E-cadherin/catenin cell adhesion system in human cancer cells and reduces cancer metastasis. *Clin. Cancer Res.*, 8: 2430–2436, 2002.
- Yoo, H. G., Shin, B. A., Park, J. S., Lee, K. H., Chay, K. O., Yang, S. Y., Ahn, B. W., and Jung, Y. D. IL-1 β induces MMP-9 via reactive oxygen species and NF- κ B in murine macrophage RAW 264.7 cells. *Biochem. Biophys. Res. Commun.*, 298: 251–256, 2002.
- Vecil, G. G., Larsen, P. H., Corley, S. M., Herx, L. M., Besson, A., Goodyer, C. G., and Yong, V. W. Interleukin-1 is a key regulator of matrix metalloproteinase-9 expression in human neurons in culture and following mouse brain trauma *in vivo*. *J. Neurosci. Res.*, 61: 212–224, 2000.
- Rao, V. H., Singh, R. K., Delimont, D. C., Schaefer, G. B., Bridge, J. A., Neff, J. R., Sanger, W. G., Sappenfield, J. W., Buehler, B. A., and Finnell, R. H. Interleukin-1 β upregulates MMP-9 expression in stromal cells of human giant cell tumor of bone. *J. Interferon Cytokine Res.*, 19: 1207–1217, 1999.
- Puhlmann, M., Turner, E., Weinreich, D. M., and Alexander, H. R. Permeability across functional endothelial cell (EC) monolayer by interleukin-1 β are dependent on induction of tissue factor (TF). *In: 88th Clinical Congress of the American College of Surgeons*. San Francisco: 2002.
- Alexander, H. R., Doherty, G. M., Venzon, D. J., Merino, M. J., Fraker, D. L., and Norton, J. A. Recombinant interleukin-1 receptor antagonist (IL-1ra): effective therapy against gram-negative sepsis in rats. *Surgery (St Louis)*, 112: 188–194, 1992.
- Dionne, S., Hiscott, J., D'Agata, I., Duhaime, A., and Seidman, E. G. Quantitative PCR analysis of TNF- α and IL-1 β mRNA levels in pediatric IBD mucosal biopsies. *Dig. Dis. Sci.*, 42: 1557–1566, 1997.
- Colville-Nash, P. R., and Scott, D. L. Angiogenesis and rheumatoid arthritis: pathogenic and therapeutic implications. *Ann. Rheum. Dis.*, 51: 919–925, 1992.
- Hirata, S., Matsubara, T., Saura, R., Tateishi, H., and Hirohata, K. Inhibition of *in vitro* vascular endothelial cell proliferation and *in vivo* neovascularization by low-dose methotrexate. *Arthritis Rheum.*, 32: 1065–1073, 1989.

Cancer Research

The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

Effect of Interleukin 1 Receptor Antagonist Gene Transduction on Human Melanoma Xenografts in Nude Mice

David M. Weinreich, Dina M. Elaraj, Markus Puhlmann, et al.

Cancer Res 2003;63:5957-5961.

Updated version Access the most recent version of this article at:
<http://cancerres.aacrjournals.org/content/63/18/5957>

Cited articles This article cites 37 articles, 14 of which you can access for free at:
<http://cancerres.aacrjournals.org/content/63/18/5957.full#ref-list-1>

Citing articles This article has been cited by 6 HighWire-hosted articles. Access the articles at:
<http://cancerres.aacrjournals.org/content/63/18/5957.full#related-urls>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
<http://cancerres.aacrjournals.org/content/63/18/5957>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.