Antimetastatic and Antitumor Activities of Interleukin 10 in Transfected Human Prostate PC-3 ML Clones: Orthotopic Growth in Severe Combined Immunodeficient Mice

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ABSTRACT
We have permanently transfected human prostate PC-3 ML tumor cells and examined the influence of interleukin 10 (IL-10) production on tumor growth and metastasis following orthotopic implantation in the prostate gland of severe combined immunodeficient mice. Measurements of tumor volume after 5, 8, and 12 weeks indicated that tumor volume was negatively correlated with the amount of IL-10 produced. Likewise, the extent of metastasis was inversely related to the amount of IL-10 produced. Following i.v. injection, the IL-10-expressing clones also failed to metastasize to the bone marrow. Controls showed that PC-3 ML and PC-3 ML mock clones grew rapidly and metastasized when implanted orthotopically or injected i.v. via the tail vein. Mouse survival curves showed that all of the mice injected orthotopically with the PC-3 ML clones died by about 14-16 weeks, whereas the PC-3 ML-IL10a or PC-3 ML-IL10b clones induced only 10-20% death after 23-24 weeks. Likewise, survival studies showed a high death rate by ~30 days with PC-3 ML mock cells but ~10% death by 12 weeks with the IL-10-transfected clones injected i.v. via the tail vein. The data strongly suggest that IL-10 production blocks tumor growth and metastasis in severe combined immunodeficient mice.

INTRODUCTION
IL-10² was identified as a cytokine synthesis inhibitory factor produced by murine type 2 helper T cells (1) and has since been found to be produced by both human type 1 and type 2 helper cells (2). The biological activities of IL-10 are only partially understood (3-4), but they include inhibitory effects on type 1 helper cells; blocking of monocyte and macrophage secretion of tumor necrosis factor α; and inhibition of monocyte secretion of IFN-γ, IL-1, IL-6, IL-8, granulocyte colony-stimulating factor, and reactive nitrogen or oxygen intermediates (5-9). IL-10 also stimulates monocyte expression of Fcγ receptors, which results in antibody-driven cytotoxicities (10).

In the past 5 years, the role of IL-10 in tumor growth and metastasis has been investigated. The biological effects of IL-10 on tumor growth have ranged from modulating tumor growth (via indirect effects on the immune system) to inhibiting tumor angiogenesis and metastasis. Huang et al. (11) found that human melanoma A375P cells transfected with a murine IL-10 cDNA exhibited reduced growth and metastatic abilities, which correlated with a significant decrease in neovascularity of the tumors. IL-10 produced by the A375P-IL-10 cells was found to downregulate expression of vascular endothelial growth factor, IL-1β, tumor necrosis factor α, IL-6, and MMP-9 in activated macrophages that normally infiltrated the tumor tissues. The authors suggested that the production of IL-10 by tumor cells might inhibit macrophage-derived angiogenic factors to block tumor growth and metastasis indirectly. Alternatively, IL-10 might sensitize tumor cells to NK cells, which block metastasis, as shown in a murine model of breast cancer (12). In similar studies, Richter et al. (13) reported that IL-10 blocked tumor growth, apparently by blocking angiogenesis and macrophage penetration of the tumor tissue. Kundu et al. (12) also found, with studies of IL-10-transfected murine mammary tumor cell lines, that tumor growth was completely inhibited and metastasis was reduced by ~90% in syngeneic BALB/cByJ mice. The effect appeared to be independent of T-cell activity but was dependent on NK cell function. These observations suggest that the production of IL-10 by tumor cells might inhibit macrophage-derived angiogenic factors to block tumor growth, angiogenesis, and metastasis indirectly (12). In apparent contrast to these findings, Sato et al. (14) found that IL-10 expression was characteristic of human metastatic melanomas, implying that IL-10 might down-regulate the antitumor activities of monocytes and macrophages, blocking production of antitumor effector molecules (15), thereby enabling metastasis (14). Further studies are required to resolve the role of IL-10 in tumor growth and metastasis as a function of the tissue, origin, or the type of cancer and whether differences exist between tumors in rodent and human.

In this study, we permanently transfected human prostate PC-3 ML tumor cells (a bone-metastasizing subclone of PC-3 cells) and examined the influence of IL-10 production on tumor growth and metastasis following orthotopic implantation in the prostate gland and i.v. injection via the tail vein of SCID mice. The results showed that IL-10 inhibited tumor growth and metastasis to the liver, lung, peritoneum, and bone marrow. Mouse survival curves showed that IL-10 expression was directly correlated with a significant increase in mouse survival (>90%). We suggest that IL-10 might be an important adjuvant.
therapy for treatment of primary cancer and the prevention of tumor invasion and metastasis.

MATERIALS AND METHODS

Tumor Cell Lines. The metastatic PC-3 ML subclones were derived in our laboratory from human prostatic PC-3 parent cells (16). PC-3 ML clones and IL-10-transfected clones were maintained at low passage (< 10 passages) for all of the experiments and grown in the presence of 10% fetal bovine serum according to published methods (16). Conditioned medium was prepared from PC-3 ML cells or IL-10-transfected clones incubated in serum-free medium for 48 h according to published methods (16). Protein levels were measured according to the method of Bradford (17).

IL-10 Transfection. Total cytoplasmic RNA was isolated from 10^7 human peripheral blood lymphocytes treated with 10 μg/ml Con-A. RNA was reverse transcribed into cDNA using oligo(dT)20 primer as described by others (18) and then amplified 40 cycles using two oligonucleotide primers derived from a published human IL-10 sequence (19), including 5'-AATGGATATCGCGAATTC(TTCAAGCTG-3' and 5'-AATGGATATCGCGAATTC(TTCAAGCTG-3'. Incorporating an SdrI and an EcoRV site, respectively (underlined). The PCR was carried out for 20 s at 94°C, 20 s at 60°C, and 20 s at 72°C for 40 cycles followed by a 10-min extension at 72°C. The PCR product was then cloned into a Bluescript vector (Stratagene, La Jolla, CA), and several independent clones were sequenced to confirm that the IL-10 gene had been cloned. One of the cDNAs was subcloned into a expression vector pCPE4 (Invitrogen, San Diego, CA). PC-3 ML cells were transfected with the pCPE4 vector alone (PC-3 ML mock) or pCPE4-human IL-10 vector using the LipofectAMINE method (Oncogene Science). Following transfection, hygromycin (400 μg/ml)-resistant cells were selected over a 2-month period, the cells were pooled, and single cell clones were generated by limited dilution in 96-well dishes. Four different clones were generated and characterized (PC-3 ML-IL10a, b, c, and d). Preliminary ELISAs showed that each of the clones produced IL-10 (>10 pg/ml) when seeded at 10^5 cells/ml in 1 ml of serum-free medium for 48 h. The clones were amplified in five passages, and cells were frozen in liquid nitrogen for future studies. Cells were revived and used at a final passage <10 in all experiments. Reverse transcription-PCR analysis (see methods above) of the mRNA isolated from each of the clones (passage 10) confirmed that they each expressed the IL-10 gene. ELISAs (see methods below) with IL-10 antibodies (courtesy of Dr. S. Narula, Schering-Plow, Kenilworth, NJ) confirmed that each of the clones still produced IL-10 (>10 pg/ml).

Cellular Cytotoxicity Assays. It was important to assess whether the IL-10 gene-transfected PC-3 ML clones produced IL-10. Because MC/9 cells proliferate in response to picogram levels of IL-10 (20), this assay was used to assess production by the different clones. Methods described previously by Giovarelli et al. (20) were followed. In brief, the MC/9 cells were seeded at 10^5 cells/well in 96-well plates for 72 h in the presence of 200 μl of conditioned medium from the different PC-3 ML clones. Cell number was then measured by the MTT assay at an absorbance of A 570 nm. Cell viability in the experiments was estimated by trypan blue exclusion assays as >95%.

PC-3 ML Cell Proliferation Assay. The biological activity of IL-10 was determined using the colorimetric MTT (Sigma Chemical Co., St. Louis, MO) dye-reduction assay (21–22). In brief, 1 × 10^4 PC-3 ML cells/well in 200 μl of medium containing 5–30 pg/ml IL-10/ml in a 96-well microtiter plate were treated for 72 h. Ten μl of 5 mg/ml MTT were added and incubated for 4 h; the cells were then detergent-lysed in absolute ethanol, and the plates were read for absorbance at 570 nm.

ELISAs. ELISAs were carried out as described previously using well-characterized IL-10 monoclonal antibodies (Ref. 23; courtesy of Dr. N. Sawant, Schering-Plow). ELISAs (A400) were performed using aliquots of conditioned medium protein to coat the enzyme immunoabsorbent plates (Dynatech, Chantilly, VA). After blocking with 5% BSA, excess primary antihuman IL-10 antibody (100 μg/ml) was added and incubated at 37°C for 2 h. A Vector Elite kit (Vector Laboratories, Inc., Burlingame, CA) was used to detect the bound antibody with goat antimouse 2nd antibody and o-phenylenediamine as peroxidase substrate.

The absorbance readings (A400) were obtained for three protein concentrations of the conditioned medium (0.25, 0.50, and 1.0 mg of protein), and the actual amounts of IL-10 produced by each cell line were determined from a standard curve. The data were then normalized for 1.0 mg of conditioned medium. IL-10 receptor antibodies were provided courtesy of Kevin Moore (DNAX Corp., San Diego, CA; Ref. 23).

Mice. Pathogen-free male mice SCID/SCID mice were purchased from Taconic Laboratories (Albany, NY) and housed in barrier cages in a barrier facility. Mice were fed a standard laboratory chow ad libitum and were used at 8–10 weeks of age. Orthotopic injection was carried out according to methods described by Fidler and colleagues (25). Following experimental treatment, the mice were sutured with staples and watched for several days to ensure recovery.

Bone Metastasis Studies. PC-3 ML cells and IL-10-transfected PC-3 clones were injected i.v. via the tail vein (~2 × 10^5 cells in 0.2 ml; Ref 16) of SCID mice. The presence of tumors was determined by gross dissection of the mice after 30–40 days (or up to 100 days in mouse survival studies) and by histological examination of serial sections of the lumbar vertebrae according to published methods (16).

Immunohistochemistry. Tissues were processed for antibody labeling according to previously described methods (26) using primary IL-10 antibodies (Schering-Plow) at a dilution of 1:500 and secondary peroxidase-antiperoxidase antibodies (Sigma) at a dilution of 1:400. At least three sections were labeled from each tissue sample, and one section was labeled with 2μ antibody only as a control. One additional section was stained with H&E for identification of the cells.

Statistical Analysis. Differences in the number of colonies of the lung, liver, and peritoneum were analyzed using the Mann-Whitney U test. Statistical significance was determined by a two-way ANOVA or by the two-tailed Student’s t test (Minitab, Statistical Software, version 8.2), and results are ex-
RESULTS

IL-10 Influence on PC-3 ML Cell Proliferation in Vitro.

Preliminary MTT assays (22–23) revealed that the proliferation rate of the PC-3 ML cells was not significantly different in the absence or presence of IL-10 (P > 0.001). That is, cells plated at 1 × 10⁴/well in the presence of IL-10 (5, 10, and 30 pg/ml for 72 h) proliferated to the same extent as untreated cells (data not shown). Also, trypan blue exclusion assays indicated >95% cell viability in the presence of 5–30 pg/ml for 72 h.

Transfection of PC-3 ML Cells. PC-3 ML tumor cells were transfected with the IL-10 gene. Four different clones that Northern blots indicated expressed IL-10 mRNA were grown for further analysis. Relative IL-10 activity in the conditioned medium of the clones (i.e., medium from clones plated at 10⁴ cells/ml for 72 h in 35-mm dishes) was assayed based on the influence of the conditioned medium on MC/9 cell proliferation (Table 1). The data indicated that PC-3 ML cells and PC-3 ML mock-transfected cells failed to stimulate MC/9 cell proliferation (a cell line known to proliferate in response to IL-10, 20), indicating that they probably express very little or no IL-10. In contrast, all four IL-10-transfected clones induced MC/9 cell proliferation, indicating that they expressed IL-10. Note that the relative IL-10 activity differed among the clones (i.e., PC-3 ML-IL10a > PC-3 ML-IL10b > PC-3 ML-IL10c > PC-3 ML-IL10d clones). In the presence of IL-10 receptor antibodies (1:100 dilution), the conditioned medium from the PC-3 ML-IL10c clones failed to stimulate a significant increase in MC/9 cell proliferation compared to untreated controls (P < 0.005), confirming that active IL-10 was in fact produced by the cells.

ELISAs. ELISAs with IL-10 antibodies confirmed that PC-3 ML and PC-3 ML mock-transfected cells failed to secrete IL-10, whereas the IL-10 levels produced by the IL-10-transfected cells was statistically significant compared to the PC-3 ML and PC-3 mock clones (P < 0.001).

Table 1  IL-10 expression by transfected PC-3-ML clones

<table>
<thead>
<tr>
<th>Cell line</th>
<th>IL-10 activity (A570)</th>
<th>IL-10 levels (pg/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC-3 ML</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>PC-3 ML mock</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>PC-3 ML-IL10a</td>
<td>0.10 ± 0.01</td>
<td>12 ± 0.5</td>
</tr>
<tr>
<td>PC-3 ML-IL10b</td>
<td>0.45 ± 0.05</td>
<td>10 ± 1.0</td>
</tr>
<tr>
<td>PC-3 ML-IL10c</td>
<td>0.97 ± 0.08</td>
<td>33 ± 6.5</td>
</tr>
<tr>
<td>PC-3 ML-IL10d</td>
<td>0.65 ± 0.06</td>
<td>22 ± 4.4</td>
</tr>
</tbody>
</table>

* IL-10 activity was determined by in vitro MC/9 cell proliferation assays (see “Materials and Methods”). MC/9 cells were seeded at 10⁴ cells/well in 96-well plates for 72 h in the presence of 200 µl of conditioned medium from the different PC-3 ML clones. Total numbers of MC/9 cells were measured by the MTT assay, and the plates were read at an absorbance of A570 after 72 h. Statistical comparison indicated that the values from the IL-10 transfected clones were significant compared to the PC-3 ML and PC-3 mock clones (P < 0.001).

RESULTS

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Preliminary MTT assays (22–23) revealed that the proliferation rate of the PC-3 ML cells was not significantly different in the absence or presence of IL-10 (P > 0.001). That is, cells plated at 1 × 10⁴/well in the presence of IL-10 (5, 10, and 30 pg/ml for 72 h) proliferated to the same extent as untreated cells (data not shown). Also, trypan blue exclusion assays indicated >95% cell viability in the presence of 5–30 pg/ml for 72 h.

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ELISAs. ELISAs with IL-10 antibodies confirmed that PC-3 ML and PC-3 ML mock-transfected cells failed to secrete IL-10 in the medium after 48 and 96 h. In comparison, IL-10 was secreted by IL-10-transfected PC-3 ML clones at levels of 55, 41, 30, and 55 pg/mg total protein, after a 48-h period (P < 0.001 compared to PC-3 ML mock clones). ELISA measurements of the IL-10 levels in the plasma of SCID mice 12 weeks after orthotopic inoculation of the tumor cell line (1 × 10⁴ cells), Values were averaged from triplicate measurements in three experiments for the activity measurements and from five mice for protein levels. Values represent the mean ± SD. Statistical evaluation indicated that the IL-10 levels produced by the IL-10-transfected cells was statistically significant compared to PC-3 ML cells or PC-3 ML mock clones (P < 0.001).

Values averaged from n = 5 mice/data point.
Antimetastatic and Antitumoral Activities of IL-10

**Fig. 2** Number of SCID mice with lung (A), liver (B), and bone (C) metastasis following i.v. injection with histogram PC-3 ML cells (column 1); PC-3 ML mock cells (column 2); PC-3 ML + PC-3 ML-IL10b cells (50:50 mixture; column 3); three IL-10-transfected clones: PC-3 ML-IL10a (column 4), PC-3 ML-IL10b (column 5), and PC-3 ML-IL10c (column 6); and IL-10-pretreated PC-3 ML cells (column 7).

IL-10d, respectively (Table 1), indicating plasma levels of IL-10 correlated inversely with observed tumor size ($P < 0.001$). Finally, pretreatment of the PC-3 ML cells with IL-10 (30 ng/ml for 4 h) prior to injection reduced the size of the tumors formed by about 30% after 12 weeks (data not shown).

**Metastasis of Orthotopic Implants.** Metastasis of the different clones was assessed based on the numbers of lung nodules, liver nodules, and bone metastases formed following orthotopic injection of the cells at $1 \times 10^5$ cells/site for 12 weeks (Fig. 2). For comparison, mice ($n = 10$ per experiment) were injected with PC-3 ML, PC-3 ML mock, and PC-3 ML cells pretreated with IL-10 (30 ng/ml for 4 h); PC-3 ML-IL10b, c, and d clones; and PC-3 ML cells mixed with PC-3 ML-IL10b cells (1:1; see Fig. 2, A and B, columns 1–7, respectively). The data showed that PC-3 ML and PC-3 ML mock clones formed numerous nodules (>150/lung; >60/liver), whereas the IL-10-expressing clones formed few nodules (<5/lung or liver; Fig. 2, A and B, columns 4–6). Statistical comparisons confirmed that the IL-10-expressing clones produced significantly fewer tumor nodules than the PC-3 ML mock or PC-3 ML clones ($P < 0.001$).

Immunohistochemistry of the nodules from the IL-10-transfected cells indicated that the nodules formed did not express IL-10 ($n = 20$ nodules/organ site), indicating that a loss of IL-10 expression was responsible for the behavior of the cells. Interestingly, the PC-3 ML cells pretreated with IL-10 showed a significant reduction in metastasis (<70/lung; <50/liver) compared to untreated cells ($P < 0.05$). Likewise, the mixture of PC-3 ML cells with PC-3 ML-IL10b cells showed a significant reduction in lung (<80) and liver (<40) metastases compared to controls (Fig. 2, A and B; $P < 0.05$).

It was difficult to track bone metastases by gross dissection, but histological sections of the common sites of metastasis (lumbar vertebrae, iliac crest, ribs, femur, and fibia) indicated that mice exhibited only one metastasis on average (usually to the lumbar vertebrae). Of ten mice examined per cell line, PC-3 ML and PC-3 ML mock cells produced at least one bone metastasis in 7 of 10 and 8 of 10 mice, respectively (Fig. 2C, columns 1 and 2), whereas only the PC-3 ML-IL10c clones...
exhibited a single bone metastases in the 10 mice examined (Fig. 2C, see columns 4–6; \( P < 0.001 \) compared to PC-3 ML mock cells). The IL-10 pretreated PC-3 ML cells showed only 5 of 10 mice with bone metastasis (Fig. 2C, column 7), and the mixture of PC-3 ML cells with PC-3 ML-IL-10b cells showed 2 of 10 mice with bone metastasis (Fig. 2C, column 3; \( P < 0.005 \)).

**Survival Curves.** Mouse survival curves (Fig. 3) showed that the PC-3 ML and PC-3 ML mock clones implanted orthotopically produced 80% death by about 15–16 weeks (with no increased death over 26 weeks), whereas the PC-3 ML-IL10a, PC-3 ML-IL10b, and PC-3 ML-IL10c clones induced only 10–20% death (\( n = 10 \) mice) by up to 24 weeks (\( P < 0.001 \) compared to controls). In mice injected with a mixture of PC-3 ML-IL10a plus PC-3 ML cells (50:50 mixture), about 40% of the mice died by about 16 weeks (with no increased death by 24 weeks), indicating that IL-10 expression reduced the rate of tumor growth (Fig. 3; \( P < 0.05 \) compared to controls). Finally, the PC-3 ML cells pretreated with IL-10 showed a slight reduction in mouse mortality to 70% by \( \sim 16–23 \) weeks (Fig. 3).

**Tail Vein Injection Experiments.** We originally characterized the PC-3 ML cells based on their ability to metastasize to the bone (>70% efficiency) following i.v. injection via the tail vein (16). Identical studies here confirmed that the PC-3 ML and PC-3 ML mock cells formed lumbar vertebrae bone marrow metastasis in at least 70% of the mice following i.v. injection via the tail vein with no incidence of metastasis to other tissues, including the liver and lung (Table 2). Identical studies showed that the PC-3 ML-IL10a, b, and c clones failed to metastasize to the bone marrow or to the liver and lung tissues. However, the PC-3 ML-IL-10d clones exhibited limited metastasis to the bone (\( n = 2 \) of 10), liver (\( n = 3 \) of 10), and lungs (\( n = 4 \) of 10), but the number of nodules detected in the latter cases were \(<3\) nodules/organ (Table 2). Finally, in mice injected with a mixture of PC-3 ML-IL10a plus PC-3 ML cells (50:50 mixture), most of the mice exhibited lumbar bone metastasis (\( n = 7 \) of 10), but no metastasis to other organ sites was detected. Comparisons of the IL-10-transfected cells with the mock-transfected cells indicated that the data were significant (\( P < 0.001 \)).

Fig. 4 illustrates by immunolabeling with IL-10 antibodies that the majority of the tumor cells from PC-3 ML-IL-10a cells were positively labeled in a tumor from a mouse sacrificed at 12 weeks (Fig. 4b). In contrast, none of the cells were labeled in liver nodules resulting from PC-3 ML-IL-10a cell metastasis (Fig. 4c). Surveys of two different lungs (20 nodules total), two livers (20 nodules total), and two bone metastases (2 nodules total) indicated that these tumors failed to express detectable IL-10, indicating that a loss of IL-10 expression might be associated with the development of the metastatic phenotype.

**DISCUSSION**

In this paper, we examined the malignant behavior of PC-3 ML human prostate cancer clones permanently transfected with the IL-10 gene. The data showed that IL-10 expression resulted in inhibition of tumor growth and metastasis following orthotopic implantation in the prostate of SCID mice. In the few cases in which metastasis occurred, immunolabeling indicated that the metastatic lesions \( (n = 22 \) total) failed to express IL-10. Thus, in contrast to Sato et al. (Ref. 14; see "Introduction"), these data suggest that a loss of IL-10 expression enables tumor escape and metastasis. However, the mechanism of IL-10 action is not understood and could arise from direct effects on either gene expression by the tumor cells, tumor angiogenesis, or indirect effects on macrophage infiltration or immune responses by NK cells. Recent studies have indicated that IL-10 is a potent inhibitor of angiogenesis in IL-10-transfected M-MSV-BALB/3T3 tumor cells grown in BALB/c or nu/nu mice (27). In addition IL-10 can block monocyte and macrophage secretion of IL-1 and tumor necrosis factor \( \alpha \) (28–30), presumably to inhibit macrophage infiltration and destruction of the tumor cells. In agreement, Richter et al. (13) further found that IL-10 gene transfection of tumor cells resulted in a direct inhibition of macrophage infiltration. The implication is that infiltrating macrophages are necessary for the growth of the tumor. Im et al. (27) also found that IL-10-transfected melanoma cell tumors...
failed to promote macrophage infiltration or angiogenesis of the tumor, indicating that cytokine effects on neovascularization might be critical. Alternatively, CD8+ lymphocytes, neutrophils, and NK cells may be equally important antitumor activities (20). It is also possible that the influence of IL-10 on angiogenesis might block WBC penetration of the tumor tissue (i.e., by inhibiting MMP-2/9 secretion; Ref. 31) and thereby limit tumor growth and metastasis from the prostate gland (32-35).

In this study, we demonstrated that IL-10 gene-transfected PC-3 ML cells failed to metastasize to the liver, lung, peritoneum, or bone tissues. On the basis of previous studies (and data presented here), which showed that IL-10 pretreatment of the PC-3 ML cells blocked tumor seeding in the bone marrow (and metastasis) following i.v. injection via the tail vein (31), we postulate that direct effects of IL-10 on tumor cell behavior (i.e., invasive properties and metalloproteinase production) might account for the antitumor activity of IL-10 in the experiments reported here. In this respect, we have also shown that the antitumor activity of IL-10 on PC-3 ML cells correlated with IL-10 induction of TIMP-1 expression and the down-regulation of MMP-2 and MMP-9 levels (31). Likewise, evidence that IL-10 up-regulated TIMP-1 mRNA in primary epithelial cultures derived from human prostate cancer (24) suggested that IL-10 might normally activate TIMP-1 to block metastasis in vivo. Recently, we have demonstrated that IL-10 ligand receptor activation of a novel signal pathway activated transcription factor binding to a novel enhancer element located in the 5’ promoter region of the TIMP-1 gene (36). These data suggest that specific signal transduction pathways might control TIMP-1 expression to modulate tumor growth and metastasis in vivo (36). However, IL-10 has been found to modulate the expression of several other genes, including elastin (37) and follistatin (38), and expression of these genes (or unknown candidate genes) might also influence the growth and related metastatic behavior of the cells.

The natural regulation of gene expression and metastasis is no doubt strongly influenced by the host tissue stroma and microenvironment (25), and these are important considerations for interpretation of the gene-specific activity of IL-10. In an elegant study, Fidler and colleagues (25) have shown that several genes expressed by highly metastatic PC-3M cells were up-regulated by implantation in the prostate gland and down-regulated by implantation s.c. in nude mice. Highly metastatic PC-3M cells expressed high levels of epidermal growth factor receptor, basic fibroblast growth factor, MMP-2, MMP-9, and multidrug resistance-1 mRNA transcripts following orthotopic implantation in the prostate gland compared to the low levels of these genes expressed by PC-3M cells injected ectopically (25). More importantly, they reported a direct correlation between the orthotopic implantation, an elevated expression of these genes, and an increased frequency of metastasis (25), indicating that the regulation of gene expression by the host tissue plays a major role in modulating metastasis. In agreement, the PC-3 ML cells, which normally produce high levels of MMP-2 and MMP-9 and low amounts of TIMP-1 (24), exhibited a high degree of metastasis to different sites (i.e., bone marrow, lung, liver, and peritoneum) following orthotopic injection.

The ability of IL-10 to down-regulate MMP-2 and MMP-9, while simultaneously up-regulating TIMP-1 in IL-10-transfected PC-3 ML cells (35), might therefore account for the significant reduction of tumor growth and elimination of metastasis by orthotopic implants in SCID mice. That is, TIMP-1 over-expression could block growth and metastasis from the prostate gland to distant sites. This possibility is further supported by the fact that IL-10-transfected PC-3 ML clones failed to metastasize following i.v. injection via the tail vein in SCID mice.

In summary, because IL-10 appears to have little toxicity when administered systemically to mice or humans (39), we suggest that IL-10 might be an important adjuvant for treatment of primary cancer where localized spreading has occurred.

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