Rat Prostate Adenocarcinoma Cells Disseminate to Bone and Adhere Preferentially to Bone Marrow-derived Endothelial Cells

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ABSTRACT

Approximately 70% of patients with prostatic cancer develop bone metastases. Metastatic prostate adenocarcinomas are associated with high mortality rates and represent a leading cause of cancer-related deaths among males. To study the host-tumor interactions underlying the predilection of prostate cancer cells for skeletal bone, an experimental model was developed using rat Dunning carcinoma Mat-LyLu cells. Inoculations of these cells into the left ventricle of the heart led to the development of spinal metastases in 100% of inoculated animals. A subline of Mat-LyLu (Mat-LyLu-B5) was subsequently selected through the sequential inoculation of bone marrow-derived carcinoma cells into the left ventricle and was found to have an increased metastatic potential compared to the parental line. The possible role of tumor cell adhesion to host cells in the process of bone marrow colonization was then investigated in vitro using the metastatic line and primary cultures of rat bone marrow-derived stromal cells. It was found that the adhesion of the metastatic Mat-LyLu cells to a bone marrow stromal cell culture highly enriched for endothelial cells was significantly higher than the adhesion to other bone-derived cells, including nonendothelial bone marrow stromal cells (3.5×) and osteoblasts (1.7×). This result suggests that the adhesion of prostate carcinoma cells to the bone marrow endothelium may play a role in their metastasis to bone.

INTRODUCTION

The incidence of and mortality from prostate cancer are increasing yearly, and carcinoma of the prostate is now the second major cause of cancer deaths in males in the United States. One out of 11 men in the United States is expected to be diagnosed with carcinoma of the prostate, and of these a third are expected to die from their cancer (1). With an increasingly aging population, the mortality rate could increase again by 50% in the next 15 years (1).

The skeleton is the major site of metastasis for prostate carcinoma. Approximately 70% of patients with prostate cancer will develop bone metastases (1). These metastases, which are situated predominantly in red bone marrow (most commonly in the spine), are generally associated with a poor prognosis. The development of new and effective therapeutic regimens for the management of prostate carcinoma depends therefore on a better understanding of the mechanisms which underlie the predilection of this malignancy to bone.

In recent years, it has become clear that the selective patterns of secondary organ colonization, characteristic of many types of cancers, cannot be entirely explained by anatomical and mechanical trapping theories of tumor cell dissemination (2, 3). Several specific factors have been shown to regulate this process, known as site-specific metastasis (4). Among them the preferential adhesion of cancer cells to the organ extracellular matrix proteins, parenchymal cells, and in particular organ-specific receptors expressed on the luminal side of the vascular endothelium has been shown to play an important role (5–7). This adhesive interaction is thought to trigger tumor cell invasion and promote cellular proliferation (4).

To understand the mechanisms underlying the predilection of prostate cancer to bone, we developed an animal model using Dunning prostate carcinoma cell line R3327-Mat-LyLu. The intracardiac inoculation of these cells into the left ventricle of syngeneic rats resulted in the development of bone marrow metastases. Bone marrow-derived carcinoma cells as well as the parental line were then used to analyze the role of cell-cell adhesion in this metastatic process.

MATERIALS AND METHODS

Animals

Indbred male Copenhagen rats weighing 150–200 g were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis, IN). Female Wistar rats weighing 100–150 g were obtained from Charles River (Montréal, Québec, Canada).

Tumor Cells

The Dunning R3327-Mat-LyLu cell line (8) was obtained courtesy of Dr. J. T. Isaacs (John Hopkins School of Medicine). It was maintained in vivo in Copenhagen rats by s.c. inoculation of 10⁶ cells. The animals usually developed lymph node and lung metastases within 3 weeks, but bone metastases were not observed (9). The tumors were resected when their diameter reached approximately 3 cm, and the tumor cells were enzymatically dispersed in a solution of 0.05% trypsin (Gibco, Burlington, Canada). In vitro the cells were maintained in RPMI 1640 supplemented with 2 mM L-glutamine (MA Bioproducts), 100 units/ml of penicillin-streptomycin sulfate (Gibco), 250 mM dexamethasone, and 0.2% gentamycin (Sigma).

TMT-081, a metastatic rat mammary carcinoma line, was obtained from Dr. U. Kim (Roswell Park Memorial Institute, Buffalo, NY). The maintenance and the tumorigenic and metastatic properties of these tumor cells were described in detail elsewhere (10).

Bone Metastasis Assay

Lumbar bone metastases of the Dunning tumor were obtained following the injection of Copenhagen male rats with 5 × 10³ or more Mat-LyLu cells into the left ventricle (i.e. injection) as described elsewhere (11). The animals developed hindleg paralysis within 2–3 weeks (depending on inoculum size) due to spinal cord compression by tumor cells extending from the vertebral body, as confirmed by histopathology (see below in "Results"). Tumor cells were harvested from the lumbar region and maintained in culture for 2–3 weeks, after which some of the cells were used in the adhesion assays. The remaining cells were reinjected i.e. into new rats to obtain the next generation of bone-metastasizing cells. This procedure was repeated six times.

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3 The abbreviations used are: FBS, fetal bovine serum; DiI-Ac-LDL, acetylated low-density lipoprotein labeled with 1,1'-dioctadecyl 1-1-3,3',3'-tetramethyl-indo-carbocyanine perchlorate; BM, bone marrow; BMEC, BM cultures enriched for endothelial cells; BMSC, BM stromal cells; vWF, von Willebrand factor; PBS, phosphate-buffered saline; FACS, fluorescence-activated cell sorter; ECGF, endothelial cell growth factor.
Osteoblasts

The isolation of primary osteoblasts from rat fetal calvariae was carried out as previously described by Bernier et al. (12). Cell viability was assessed with trypan blue exclusion dye, and the cells were plated in 24-well plates at a density of $1.5 \times 10^5$ cells/well in RPMI containing 10% FBS and cultured for 72 h prior to use in the adhesion assays.

Fibroblasts

Primary cultures of rat fibroblasts were prepared from third trimester fetuses using established procedures (13). The cells were cultured in RPMI-FBS at a density of $1.5 \times 10^5$ cells/well in 24-well plates.

Bone Marrow Stromal Cells

Bone marrow cells were obtained from the femoral bone of Wistar rats by flushing the marrow cavity with 10 ml RPMI-FBS through a 23-gauge needle. The marrow suspension was filtered through a 100-µm mesh sieve, centrifuged at 1200 rpm for 10 min, resuspended, and plated at a density of $10^6$ cells/well in 24-well plates (Nunc) or $4 \times 10^6$ cells/well in 6-well plates (Nunc). The culture medium was Dulbecco's modified Eagle's medium (Gibco) supplemented with 20% FBS, antibiotics (as described for RPMI), and 0.1 mg/ml heparin (Organon, Toronto, Ontario, Canada). To enrich BM endothelial cells, some of the cultures were supplemented with 200 µg/ml ECGF (Vec Tec, Schenectady, NY) on the day of plating and thereafter on alternate days for a total period of 3 weeks.

Endothelial Cells

 Cultures of bovine aortic endothelial cell were prepared using the procedure described by Gospodarowicz et al. (14). The cells were maintained as described for BM stromal cells in medium supplemented with ECGF.

Human umbilical vein-derived endothelial cells, obtained as described elsewhere (15), were kindly provided by Dr. J. Gordon (Depart-ment of Surgery, McGill University). The cells were cultured in McCoy's 5A medium (Flow Laboratories) containing 20% FBS.

Rat hepatic endothelial cells were isolated and cultured as described in detail elsewhere (16).

FACS Analysis

Uptake of Ac-LDL. BMSC cultured for 2–4 weeks with or without ECGF were labeled by incubation with 10 µg/ml Dil-Ac-LDL (Biomedical Technologies, Inc., Stoughton, MA) for 4 h at 37°C. The labeled cells were dispersed with PBS/EDTA, centrifuged, and resuspended in RPMI-FBS containing 10 mM Ar-2-hydroxyethylpiperazine-Ar'-2-ethane sulfonic acid. Fluorescent cells were analyzed by FACS (Becton-Dickinson FACSTAR) at an excitation wavelength of 514 nm. A total of $10^4$ cells from each sample were analyzed. Fibroblasts and bovine aortic endothelial cells, the latter previously shown to express receptors for Ac-LDL (17), were used as negative and positive controls, respectively.

von Willebrand Factor. BMSC were also analyzed for expression of von Willebrand factor. Three-week-old cultures were dispensed with PBS/EDTA, centrifuged, and resuspended in culture medium containing rabbit antiserum to vWF (Dakopatts, Glostrup, Denmark) at a dilution of 1:100 (normal rabbit serum was used as the control). Incubation was at room temperature for 90 min. The cells were washed three times and incubated with 10% normal swine serum in PBS for 30 min to block nonspecific binding. The second antibody, i.e., trime-thyl-rodamine-isothiocyanate-conjugated swine-anti-rabbit antiserum (Nordic, Capistrano Beach, CA), was added at a dilution of 1:20 (both primary and secondary antibodies were diluted in PBS containing 3% normal swine serum) for a 30-min incubation at room temperature. The unbound antibody was washed with PBS, and cells were resuspended in Dulbecco's modified Eagle's medium. Fluorescent cells were analyzed by FACS. Fibroblasts and human umbilical vein-derived endothelial cells, the latter previously reported to express vWF (18), were used as negative and positive controls, respectively.

Adhesion Assays. Mat-LyLu cells in log phase were labeled with Na$^+$Cr. To monolayers of primary rat cell cultures in 24-well plates, $10^3$ labeled tumor cells were added for a 60-min incubation at 37°C. Nonadherent cells were removed by repeated washing with PBS, and adherent cells were lysed with 1 M NaOH. Radioactivity associated with the lysate was monitored in a gamma counter.

The number of stromal cells/well at the time of the assay was determined by dispersing and counting the cells from three wells. The average number of cells/well was calculated and used to standardize the results, which are expressed as the proportion of prostate carcinoma cells which adhered per 150,000 stromal cells.

Statistics

Student's $t$ test and the Mann-Whitney $U$ test were used for the statistical analyses.

RESULTS

During the course of this study Copenhagen rats were inoculated with Mat-LyLu cells by the i.c., i.v., or s.c. routes. The inocula ranged from $5 \times 10^3$ to $2 \times 10^3$ tumor cells. The results obtained following these injections are shown in Table 1. All

<table>
<thead>
<tr>
<th>Type of cells</th>
<th>No. of cells inoculated</th>
<th>Route of inoculation</th>
<th>No. of days prior to hindleg paralysis</th>
<th>Incidence of Metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mat-LyLu-Pr</td>
<td>$5 \times 10^4$</td>
<td>i.c.</td>
<td>14 (13–14)</td>
<td>5/5</td>
</tr>
<tr>
<td>Mat-LyLu-B5</td>
<td>$2 \times 10^4$</td>
<td>i.c.</td>
<td>12 (11–12)</td>
<td>5/5</td>
</tr>
<tr>
<td>Mat-LyLu-B6</td>
<td>$5 \times 10^3$</td>
<td>i.c.</td>
<td>13 (12–14)</td>
<td>0/5</td>
</tr>
<tr>
<td>Mat-LyLu-B4</td>
<td>$5 \times 10^3$</td>
<td>i.c.</td>
<td>12 (11–13)</td>
<td>0/5</td>
</tr>
<tr>
<td>Mat-LyLu-B5</td>
<td>$5 \times 10^3$</td>
<td>i.v.</td>
<td>19 (18–21)</td>
<td>5/5</td>
</tr>
<tr>
<td>Mat-LyLu-B5</td>
<td>$5 \times 10^3$</td>
<td>s.c.</td>
<td>12 (11–14)</td>
<td>0/5</td>
</tr>
<tr>
<td>Mat-LyLu-B5</td>
<td>$5 \times 10^3$</td>
<td>i.c.</td>
<td>12 (12–14)</td>
<td>0/5</td>
</tr>
<tr>
<td>Mat-LyLu-B5</td>
<td>$5 \times 10^3$</td>
<td>i.v.</td>
<td>NS</td>
<td>0/5</td>
</tr>
<tr>
<td>Mat-LyLu-B5</td>
<td>$5 \times 10^3$</td>
<td>s.c.</td>
<td>NS</td>
<td>0/5</td>
</tr>
<tr>
<td>TMT-011</td>
<td>$5 \times 10^4$</td>
<td>i.c.</td>
<td>NS</td>
<td>0/5</td>
</tr>
</tbody>
</table>

* Median and range.

Literature cited.

1. BM, bone marrow; LN, lymph node; NS, not seen.

2. Parental Mat-LyLu line.

3. Bone metastasis-derived Mat-LyLu line.

4. Statistically significant delay ($P < 0.008$) in onset of hindleg paralysis as compared to Mat-LyLu-B5 or Mat-LyLu-B6 as determined by the Mann-Whitney test.
animals which received i.c. inoculations developed hindleg paralysis followed by death 4–5 days later. No animals inoculated i.v. or s.c. with the tumor cells developed hindleg paralysis, and metastases were observed in the lungs and lymph nodes only (Table 1). In animals inoculated i.c. with $2 \times 10^4$ to $5 \times 10^5$ cells, paralysis was apparent by days 12–14. In autopsies, a distended bladder due to failure of evacuation was usually found. No macroscopic metastases could be detected in any of the major organs except occasionally in the adrenal glands and kidneys. Histological examination confirmed that there were metastatic lesions in the bone marrow of the lumbar and lower thoracic vertebrae of these animals. A tumor mass was also found in the spinal canal extending from the bone marrow and compressing the spinal cord. Typical histological findings are shown Fig. 1. As is frequently the case for BM metastases of human prostatic carcinoma both osteoblastic and osteolytic lesions of the tumor were observed (Fig. 2). Micrometastases were also observed in the adrenal glands and kidneys but were not detected in the prostate gland, seminal vesicles, sternum, or femurs or in the lungs, lymph nodes, liver, or spleen.

To test whether tumor cells derived through repeated sequential isolation and reinjection of BM metastases had an increased bone-metastasizing potential, the ability of such cells to form BM metastases following i.c. inoculation was compared to that of the parental line. As shown in Table 1, no significant difference in incidence or in the time interval preceding onset of the paralysis was observed when the animals were inoculated i.c. with $2 \times 10^4$ to $5 \times 10^5$ parental (Mat-LyLu-P) or metastatic (Mat-LyLu-B) cells. However, when the tumor cell inoculum was reduced to $5 \times 10^3$ cells/animal, a significant acceleration in the onset of paralysis was noted in animals inoculated with the metastases-derived tumor cells. While rats inoculated with Mat-LyLu-P cells developed paralysis by day 19, rats inoculated with Mat-LyLu-B5 or Mat-LyLu-B6 cells developed paralysis by days 12–14, approximately 1 week earlier (Table 1). As a control the metastatic rat mammary carcinoma line TMT-081 was used. When $5 \times 10^4$ cells of this tumor were injected i.c. into six syngeneic rats they failed to give rise to bone metastases for up to 8 weeks following the inoculation (Table 1).

To study the role of cell-cell adhesion in BM colonization by Mat-LyLu cells, cultures of BMSC were prepared and characterized by cell surface marker analysis. Uptake of Ac-LDL has been widely used to distinguish vascular endothelial cells from fibroblasts and mesenchymal cells (19). The proportion of Ac-LDL(+) cells in the BM cultures was therefore determined. The
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Fig. 3. FACS analysis of rat bone marrow stromal cells labeled with Dil-Ac-LDL. Cells were labeled with rhodamine-conjugated Ac-LDL. The cells to the right of the vertical line were considered positive. Shown are results of one FACS analysis which were typical of six performed. a, fibroblasts; b, bovine aortic endothelial cells; c, unlabeled BMSC; d, unlabeled BMEC; e, BMSC labeled with Dil-Ac-LDL; f, BMEC labeled with Dil-Ac-LDL.

results shown in Fig. 3 and summarized in Table 2 revealed that there was a significant enrichment of Ac-LDL(+) cells in cultures of BMSC grown in the presence of ECGF.

Similarly, when the cells were analyzed for expression of vWF, a significant increase in the proportion of vWF+ cells was noted, as shown in Fig. 4 and Table 2. Under phase contrast microscopy these cells exhibited a cobblestone morphology typical of endothelial cells, while BM stromal cells grown in the absence of ECGF were mainly fibroblast-like in appearance (Fig. 5).

Adhesion of Mat-LyLu cells to primary cultures of BM stromal cells and to osteoblasts, fibroblasts, and liver-derived endothelial cells was subsequently measured. The results shown in Fig. 6 demonstrate that the adhesion to BMEC was significantly higher than the adhesion to primary cultures of fibroblasts (5.5x), nonenriched BM-derived stromal cells (3.0x), and, to a lesser extent, osteoblasts (1.40x). It was also significantly higher than the adhesion to rat liver sinusoidal endothelial cells (7.0x).

To determine whether adhesion to BMEC correlated with metastatic potential in the Mat-LyLu model, the adhesion of Mat-LyLu-B5 cells was measured and compared to the adhesion of the parental line. We found that the adhesion of Mat-LyLu-B5 cells to BMEC but not to hepatic endothelial cells, fibroblasts, or osteoblasts was significantly increased compared to the parent line (Fig. 6).

DISCUSSION

Despite the prevalence of BM metastases in the pathology of human prostate carcinoma, little progress has been made in understanding the host-tumor interactions underlying this metastatic process. This is due in large measure to the lack of an animal model in which the patterns of dissemination of human prostatic carcinoma can be reproduced.

Prostate carcinoma metastasis to bone has recently been described in two animal models where the i.v. route of inoculation was used in combination with ligation of the inferior vena cava in order to divert the tumor cells to the vertebral venous plexus and away from the lung capillary bed (20, 21). Although these models helped demonstrate that animal prostate carcinoma cells, in a manner similar to that of the human carcinoma, can produce osseous metastases when access to the microcirculation of the bone marrow is provided, they are limited by two drawbacks: (a) the method of inoculation is cumbersome and requires an invasive procedure; and (b) the lumbar vertebrae metastases which are produced do not mimic the patterns of bone metastasis seen in human cancer (22).

In the present study metastases were found almost exclusively in the BM following the inoculation of the tumor cells into the left ventricle. This occurred despite the fact that the total skeletal blood flow represents less than 10% of the cardiac output (23). The patterns of bone colonization, i.e., the initial
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Fig. 4. Immunofluorescence analysis of rat bone marrow stromal cells with antibodies to vWF. The first antibody was rabbit antibody to vWF and the second trimethyl-rhodamine-isothiocyanate-conjugated swine-anti rabbit antiserum. The profile shown is representative of four profiles obtained from FACS analyses. a, fibroblasts; b, human umbilical vein-derived endothelial cells; c, BMSC incubated with the second antibody only; d, BMEC incubated with the second antibody only; e, BMSC labeled with antibodies to vWF; f, BMEC labeled with antibodies to vWF.

Table 2 Results of FACS analysis using two different markers for endothelial cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>Dil-Ac-LDL added</th>
<th>MIF&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% of +ve cells&lt;sup&gt;b&lt;/sup&gt;</th>
<th>TRITC-anti&lt;sup&gt;c&lt;/sup&gt;</th>
<th>MIF&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% of +ve cells&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIBRO</td>
<td>+</td>
<td>167</td>
<td>0.1 ± 0.5</td>
<td>+</td>
<td>130</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>BAE/HUVE</td>
<td>+</td>
<td>335</td>
<td>78 ± 4</td>
<td>+</td>
<td>1509</td>
<td>80 ± 3</td>
</tr>
<tr>
<td>BMSC</td>
<td>-</td>
<td>74</td>
<td>0.3 ± 0.2</td>
<td>-</td>
<td>117</td>
<td>1.0 ± 0.4</td>
</tr>
<tr>
<td>BMEC</td>
<td>-</td>
<td>137</td>
<td>0.5 ± 0.3</td>
<td>-</td>
<td>119</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>BMSC</td>
<td>+</td>
<td>215</td>
<td>12 ± 3</td>
<td>+</td>
<td>580</td>
<td>30 ± 6</td>
</tr>
<tr>
<td>BMEC</td>
<td>+</td>
<td>1200</td>
<td>70 ± 5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>+</td>
<td>1209</td>
<td>65 ± 4.5&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> MIF, mean intensity of fluorescence. Results of experiments described in Fig. 3 and 4 are shown.

<sup>b</sup> Results are expressed as mean and SD of 6 experiments with Dil-Ac-LDL and 4 experiments with antibody to vWF.

<sup>c</sup> TRITC, trimethyl-rhodamine-isothiocyanate; FIBRO, primary culture of fibroblasts; BAE, bovine aortic endothelial cells used as a positive control for Ac-LDL expression; HUVE, human umbilical vein endothelial cells used as a positive control for vWF expression; BMSC, bone marrow stromal cells cultured without ECGF; BMEC, bone marrow stromal cells cultured in presence of ECGF to enrich the endothelial cell subpopulation.

<sup>d</sup> Statistically significant difference (P < 0.005) compared to BMSC.

growth of the tumor cells in the bone marrow of the vertebral bodies with subsequent invasion into the spinal canal, closely resembled the pathological picture normally associated with the metastasis of human prostate carcinoma to vertebral bone. It appears therefore that the intracardiac route of inoculation provides a means for reproducing more closely the pattern of organ site-specific metastasis characteristic of metastatic prostate carcinoma.

The reasons for the failure of Mat-LyLu cells to metastasize to bone following s.c. or i.v. inoculation are unclear. It is possible that in the rat, tumor cell arrest in the lung capillaries may bring about the death of the majority of cancer cells (24) and/or blockage of their recirculation into the arterial blood flow. Alternatively, it may lead to alterations in the surface molecules required for adhesion to osseous tissues and BM metastasis formation. Since i.c. inoculation allows the tumor cells to bypass the lung capillary bed, it may result in the release of sufficient numbers of metastatic cancer cells into the circulation and subsequently into the BM vasculature. Therefore, cell access to the target organ vascular bed as well as specific interactions with the organ microenvironment appear to play a role in the patterns of dissemination in the present model. When a similar experiment was carried out with the highly metastatic rat breast carcinoma line TMT-081 (10), no bone metastases were detected 8 weeks after the inoculation of 5 × 10<sup>4</sup> cells. These findings as well as studies reported by others (25) confirm that vascular access, although required, is not sufficient for the establishment of BM metastases.
The selective adhesion of tumor cells to microvessel endothelial cells derived from the preferred target organ of metastasis has been demonstrated in various tumor systems. Using the B16 melanoma model, Nicolson et al. (26) have shown, that brain-colonizing tumor cells are significantly more adherent to brain-derived endothelial cells than are lung-colonizing B16 cells. Auerbach et al. (6) demonstrated selective attachment between hepatoma cells and liver-derived vascular endothelium, between glioma cells and brain-derived endothelial cells, and between teratoma cells and ovary-derived endothelium. Our findings suggest that in a manner similar to that of vascular endothelium, BM endothelial cells may serve as a site of specific adhesion for bone-homing prostate carcinoma cells.

Positive characterization of BM endothelial cells in the present study was made on the basis of the two markers Ac-LDL and vWF. Although Dil-Ac-LDL uptake is also characteristic of macrophages (27), results of latex particle ingestion studies (not shown) indicated that only approximately 3% of the cells in the 3-week-old BMEC cultures were phagocytic, suggesting that the great majority of the Ac-LDL(+) cells were endothelial.

Our results suggest that BM-derived endothelial cells express adhesion ligands for prostatic cancer cells which are not expressed on hepatic endothelial cells or on nonendothelial cells of the bone marrow. This is in accordance with other studies in which the expression of organ-specific ligands on the vascular endothelium has been documented (6). Extracellular matrix proteins underlying the endothelium have been implicated in the regulation of expression of such ligands (7), but the mechanisms involved are not yet clear.

To test whether attachment to the subendothelial matrix proteins is involved in the adhesion to BMEC we recently compared the adhesion of Mat-LyLu-P and Mat-LyLu-B cells to various isolated extracellular matrix proteins. Results (not shown) of these adhesion assays revealed that Mat-LyLu-B cells were significantly more adherent than Mat-LyLu-P cells to fibronectin and laminin but not to vitronectin, osteopontin, and types I and IV collagen. However, the proportions of cells which adhered to fibronectin and laminin during a 60-min incubation were 15% and 10%, respectively. As adhesion to BMEC reached 43% during the same time interval, it appears that cell-cell rather than cell-matrix interactions mediated the adhesion to BMEC. It should be noted in this context that a relatively high level of adhesion of Mat-LyLu-B5 cells was also seen on cultured osteoblasts (26% adhesion as compared to 44% on BMEC). This suggests that the adhesion ligands recognized by the carcinoma cells on BM endothelium may represent organ-specific determinants also expressed on other bone cells. The osteoblastic response seen in BM colonized by the Mat-LyLu cells and similar osteoblastic metastases associated with human prostate carcinoma metastases also imply a close interaction between these tumor cells and osteoblasts (28).

The adhesion of hematopoietic stem cells to BM stromal cells, including stromal cells expressing endothelial cell markers, has been shown to play a central role in hemopoiesis, leading to proliferation and differentiation of the stem cells (29). The lymphoid cell surface receptor CD44 which appears to mediate adhesion to the proteoglycan hyaluronate, as well as epidermal growth factor receptors associated with the stromal cells, have been implicated in this adhesive interaction (30). Studies with other tumor models have already shown that adhesion receptors of blood cells may be expressed on tumor cells.
of diverse origin and mediate tumor cell adhesion and metastasis (31, 32). Recent reports have in fact implicated a variant of the CD44 receptor in tumor metastasis (33). Consequently, the possibility exists that adhesion of prostatic cancer cells to bone marrow stromal cells may involve adhesion ligands for hematopoietic cells. We are currently exploring this possibility.

While prostate carcinoma cells are generally slow growing in the primary site, their growth rate is often enhanced in the bone marrow lesions (34, 35). This suggests that the microenvironment of the bone marrow may be a rich source of growth-promoting factors for the prostatic cells. In a recent study, Zetter et al. (36) have shown that the growth of human prostate carcinoma PC-3 cells can be stimulated by conditioned medium derived from cultured stromal cells of bovine, rat, or human BM. The source of the growth-stimulating factor(s) is not as yet known. In light of our present results and in view of the studies which link adhesion and proliferation during hemopoiesis in the bone marrow, it is conceivable that prostate carcinoma cell adhesion to the BM endothelium may serve to augment the release of, and response to, growth-promoting cytokines secreted by endothelial and/or other stromal cells of the bone marrow (29). This hypothesis is the subject of our current investigations.

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