Disseminated Growth of Murine Plasmacytoma: Similarities to Multiple Myeloma

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ABSTRACT

Murine plasma cell tumors share a number of common features with human multiple myeloma, suggesting their possible use as a model for this disease. However, one major difference between the two is the peritoneal localization of murine tumors as opposed to bone marrow residence of malignant plasma cells in early stages of multiple myeloma. We have thus examined the ability of murine plasmacytoma to produce disseminated growth similar to that seen in myeloma or other lymphoid neoplasias. Of four murine cell lines evaluated, all were demonstrated to effect highly metastatic disease involving multiple organs, although variation was observed between lines. A temporal analysis was accordingly performed with the S107 line to assess the pattern of cellular localization. Both light microscopy and PCR analysis revealed that engraftment of plasma cells occurs first in the bone marrow, followed by dissemination to other sites including the spleen, lung, and liver. Cells passed in vivo through the bone marrow display an entirely different metastatic pattern with no homing preference to bone marrow or any other organ, suggesting the occurrence of a phenotypic change. Microscopic osteolytic lesions were observed adjacent to plasma cell tumor masses in the bone marrow, indicating early stages of bone disease. These findings demonstrate previously unrecognized similarities between the murine and human diseases and suggest the use of this in vivo model for experimental approaches to the treatment of human disease.

INTRODUCTION

Multiple myeloma is an invariably fatal form of human cancer caused by an accumulation of neoplastic plasma cells. Initial stages of the disease are represented by a long indolent phase in which malignant plasma cells are largely restricted to the bone marrow. After treatment, most patients enter a plateau, from which they eventually progress to a blast phase, leading to widely disseminated metastatic disease. As with most human diseases, the development of appropriate animal models would facilitate both an understanding of the biology of the disease as well as provide a system for testing novel therapeutic approaches. One candidate for such use is the murine plasmacytoma system. Plasma cell tumors can be induced in the peritoneum of the BALB/c mouse by a variety of agents including plastics (1), certain solvents, such as pristane (2, 3), and silicone (4). These agents induce the formation of complex granulomatous tissue in the peritoneal cavity within which plasma cell tumors arise. Tumors arising from these induction schemes requiring granuloma formation by agents such as pristane can only be successfully transplanted into recipients that have previously received pristane i.p. (priming) and are, therefore, dependent on the granuloma environment. After several transplant generations, the priming dependence is lost, and tumors can be transplanted into untreated animals. Thus, murine plasmacytoma is generally regarded as a neoplasm that arises in, and is restricted to, the peritoneal cavity. To assess possible similarities between the murine disease and human myeloma, we have performed experiments to determine whether murine plasma cell tumors are capable of disseminated extraperitoneal growth with particular attention to the question of bone marrow involvement.

MATERIALS AND METHODS

Cell Lines and Inoculations. Four plasmacytoma cell lines were used in the present studies. Two of these, S107 and X24, are long-term culture lines derived from parental tumors after sufficient in vivo passages to attain priming independence. The 5-25 cell line was derived from a primary plasmacytoma adapted to culture by growth on stromal cell feeder layers (5). Two stromal cell independent sublines were established, one of which is IL-6 independent (5-25 IL-6 Ind) and the second, IL-6 and priming dependent (5-25 IL-6 Dep). The S107 subline containing the neomycin resistance gene (S107 PASS3) was constructed by transfection of a neo cassette (6). Cells were maintained in RPMI 1640 (Life Technologies, Inc., Gaithersburg, MD) supplemented with 10% of heat-inactivated fetal bovine serum (Biofluids, Inc., Rockville, MD), 50 μg/ml streptomycin, 50 IU/ml penicillin, 2 mmol/L-l-glutamine, and 50 μM β-mercaptoethanol. Prior to injection, cells were washed twice in PBS and resuspended at 1–5 × 10^7/ml. One hundred μl of cell suspension was inoculated either into the lateral tail vein or i.p. All recipient and control BALB/c mice were irradiated at 300 R 24 h before injection.

Histotechnology. Tissue samples from lung, liver, spleen, and ovary were fixed in 10% neutral formalin for 24 h. Spine fragments, ribs, and femurs were dissected free of adhering tissue, the ends were cut with a scalpel blade, and the opened bone was immersed in 10% neutral formalin for 24 h. Decalcification was performed in EDTA for 2–3 weeks (American Histolabs, Gaithersburg, MD). Tissue samples including bones were dehydrated and embedded in paraffin blocks by standard procedure (American Histolabs). Four-μm sections on silanated slides were used for all histochemical staining, H&E, Giemsa, and TRAP staining were performed according to standard protocols (American Histolabs).

In Situ Hybridization. Riboprobes specific for the heavy chain variable regions expressed by the S107 and X24 lines were prepared from cloned DNA segments. A 5-25-specific probe was generated by reverse transcription-PCR using a universal 5’ primer ACAAGCTTAGGC(GA)CA(GA)CTGCAGC-GA/GTCAT(AG)G (7) and 3’ primers corresponding to the four heavy chain JH segments. The first, IL-6 dependent (5-25 IL-6 Ind) and the second, IL-6 and priming dependent (5-25 IL-6 Dep). The S107 subline containing the neomycin resistance gene (S107 PASS3) was constructed by transfection of a neo cassette (6). Cells were maintained in RPMI 1640 (Life Technologies, Inc., Gaithersburg, MD) supplemented with 10% of heat-inactivated fetal bovine serum (Biofluids, Inc., Rockville, MD), 50 μg/ml streptomycin, 50 IU/ml penicillin, 2 mmol/L-l-glutamine, and 50 μM β-mercaptoethanol. Prior to injection, cells were washed twice in PBS and resuspended at 1–5 × 10^7/ml. One hundred μl of cell suspension was inoculated either into the lateral tail vein or i.p. All recipient and control BALB/c mice were irradiated at 300 R 24 h before injection.

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Immunohistochemistry. Four-μm tissue sections were cut from formalin-fixed, paraffin-embedded blocks and placed on silanated slides. Before immunohistochemical staining, slides were deparaffinized in xylene and rehydrated using serial treatments in 100% ethanol, 70% ethanol, and PBS. All procedures were carried out at room temperature. Slides were blocked for 20 min in PBS containing 2% of normal rabbit serum and incubated at room temperature with 1 ml of rabbit anti-mouse IgA antibody (Southern Biotechnology, Birmingham, AL) diluted in the same buffer to a concentration of 2 μg/ml. Slides were washed twice for 5 min in PBS and developed using a Vectastain ABC kit (Vector, Burlingame, CA). To quench the endogenous peroxidase activity, slides were incubated in 3% H$_2$O$_2$ in methanol. After staining, slides were counterstained with hematoxylin, dehydrated, and mounted in Permount (Fisher, Fair Lawn, NJ).

DNA Isolation. Portions of different organs (50–300 mg by weight) were placed in 2 ml of lysis buffer containing 0.1 M Tris-HCl (pH 8.5), 5 mM

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2 The abbreviations used are: IL, interleukin; TRAP, tartrate-resistant acid phosphatase.
Table 1: Appearance of tumor cells in BALB/c mice

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<th>Tumor</th>
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* Animals with no indication of disease were sacrificed between 69 and 95 days after receiving tumor cells and tissues taken for *in situ* analysis.

EDTA, 0.2 M NaCl, 0.2% SDS, and 100 μg/ml proteinase K (ICN Biomedicals, Inc., Aurora, OH) and incubated overnight at 55°C with gentle shaking. The resulting lysate was passed through a QIAshredder (Qiagen, Inc., Chatsworth, CA) to reduce the viscosity of the solution. DNA was further purified with a GeneClean kit (Biolol, Vista, CA) using limiting amounts of Glass Milk to ensure an equal concentration of DNA in different samples. Briefly, 700 μl of NaI solution were added to 200 μl of the lysate, followed by incubation with 5 μl of Glass Milk. After being washed, the DNA was recovered in 50 μl of water.

**PCR Analysis.** A 4-μl aliquot of DNA sample was subjected to 35 cycles of PCR in a 50-μl reaction mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.1% gelatin, 200 μM deoxynucleotide triphosphates, and 1 μM each primer. PCR was carried out in a PTS-100 programmable thermal controller (MJ Research, Inc., Watertown, MA) under the following conditions: initial denaturation at 95°C for 15 s, followed by 35 cycles of denaturation at 95°C for 15 s; annealing at 60°C for 15 s; and elongation at 72°C for 40 s. neo-specific primers were used to detect metastatic plasma cells: Neo-1, 5'-CTCCTGGCGAGAAGATCTCCATC-3'; and Neo-2, CCA-CAGTCGATGAATCCAGAG-3'. A control reaction was carried out using primers specific for the β chain of the IL-2 receptor: IL-2R/1, 5'-CAAGGCGGTCAGCAGTCCGTC-3'; and IL-2R/2 5'-CCATAGGCAGT-GTTGAGGTCT-3'. PCR products were visualized on ethidium bromide-stained, 1% agarose gels.

**RESULTS**

**Assessment of Metastatic Potential.** In initial studies, the ability of four cell lines, S107, X24, 5-25 IL-6 independent, and 5-25 IL-6 dependent, to evidence metastatic spread was analyzed in BALB/c recipient mice. Following i.p. injection, large peritoneal tumors were observed in 9 of 17 animals within 35 days (Table 1). Of the remaining eight, six demonstrated metastatic plasma cell disease upon *in situ* analysis. All animals with no obvious disease symptoms were sacrificed between 69 and 95 days after inoculation. Paraffin sections were obtained from tissues of each animal and hybridized with a heavy chain variable region probe corresponding to the expressed gene in each cell line.

As might be expected, variation was observed between animals in all groups. However, the metastatic pattern exemplified by the S107 and X-24 cell lines in a 3-4-week interval after injection was quite similar. Plasmacytoma cells could be typically detected in nearly all tissues (Figs. 1 and 2), including the red pulp of the spleen, endometrium of the uterus, stroma of the ovaries, periportal spaces of the liver, interalveolar septa of the lungs, bone marrow, brain, and meninges. Control tissues hybridized with the same probe (data not shown) were negative because only plasma cells are detected under...
the conditions used. It can be noted that in some organs, nearly all normal tissue is replaced by tumor cells. Metastatic spread was also significantly more pronounced in i.v.-injected animals. An example of the variation exhibited between animals is presented for the S107 and X-24 cell lines in Table 2.

i.p. injection of 5-25 IL-6 dependent cell line resulted in a minimal disease pattern in all animals with scattered tumor cells found only in the lungs in three of four animals. However, when mice received pristane prior to tumor cells, metastatic disease comparable to that observed with S107 and X-24 was observed in three of three recipients (Table 1). i.v. injection resulted in a minimal disease pattern with scattered tumor cells localized to the lungs as seen in the i.p., non-pristane-treated group. The enhanced dissemination in pristane-treated animals suggests a requirement for growth factors induced by this agent. The 5-25 IL-6 independent cell line produced minimal metastatic disease except in animals pretreated with pristane. This was the only line in which metastatic disease did not develop after i.v. inoculation.

Irrespective of route of administration, the organ most frequently (~75% of animals) involved in metastatic spread was found to be the lung with a predominant presentation of numerous single cells in interalveolar spaces, suggestive of hematogenous spread. Liver involvement was noted in approximately 50% of animals, with tumor cells detected primarily in the sinusoids forming large masses in late

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Table 2 Metastatic spread of S107 and X-24 in normal mice

* Tissue abbreviations are: Spl, spleen; Thy, thymus; Ut, uterus; Ov, ovary; Liv, liver; Lag, lung; Adr, adrenal gland; Kid, kidney; BM, bone marrow; Brn, brain; Men, brain meninges; Pan, pancreas; PM, peritoneal masses; PB, peripheral blood; SC, subcutaneous mass; Sum, sum of metastatic scores in all tissues examined. The presence of tumor cells was scored numerically with the corresponding assignment: 0, absence of tumor cells; 1, single, isolated tumor cells; 2, tumor cells in small clusters; 3, large accumulations of tumor cells replacing host tissue.
DISSEMINATED GROWTH OF MURINE PLASMACYTOMA

Fig. 3. Representative organ metastases of plasma cell tumor lines. a. Liver metastases 81 days after i.v. injection of X-24 cells. H&E counterstained autoradiogram of tissue hybridized with a 35S-labeled X-24 heavy chain variable region probe (×40). b. Tumor infiltration of the spinal column 31 days after i.v. administration of S107 cells. A darkfield view of the autoradiogram in which tumor cells are identified by silver grains is shown. Tumor cells originating in the bone marrow (upper right) penetrate and extensively fill the spinal column surrounding the spinal cord (center; ×40).

Stage animals (Fig. 3a). Among i.v. recipients, approximately one-half became emaciated, demonstrated ruffled fur, and frequently displayed hind leg paralysis, suggestive of spinal compression. Paraplegia probably results from bone marrow-localized tumor cells breaking through into the spinal canal and subsequently causing cord compression as seen in Fig. 3b.

Kinetics of Engraftment of Different Organs of BALB/c Mice by Plasmactoma Cell Line S107. The detection of tumor cells within the bone marrow suggested a previously unobserved similarity to human myeloma. Based upon the above described results, a temporal study of engraftment following i.v. inoculation was, therefore, undertaken using the S107 cell line. To unambiguously monitor small numbers of tumor cells and avoid detection of normal plasma cells expressing the same heavy chain variable region, the S107 line was “marked” by transfection of the neomycin resistance gene for detection by PCR using neo-specific primers. A G418-selected subclone, S107 PAS/3, which in preliminary experiments uniformly produced hind leg paralysis and 100% mortality, was used for subsequent studies. Upon autopsy at the third week following injection of this subclone, curving of the spine was observed in all animals with no visual changes in other organs (except ovaries in ~20% of mice), indicating significant bone marrow involvement. Mice were subsequently injected with 10^6 or 10^8 cells and sacrificed at various time intervals, and organs were taken for DNA and histological analysis.

In preliminary experiments in which S107 PAS/3 DNA was serially diluted in normal liver DNA, it was found that the detection limit of the method was at least 1% of genomic equivalents of S107 PAS/3 DNA in a total DNA sample. As a control for DNA quantity and integrity, samples from the same organs were coanalyzed with IL-2 receptor β chain-specific primers. Results presented in Fig. 4 demonstrate that the invasion of neo-positive tumor cells into bone marrow occurs as early as 1 week after injection, whereas all other tested organs remained negative at this time interval. During the second week, the proportion of neo-positive cells increased significantly in the bone marrow, whereas other organs still remained negative. At the third week after injection, all tested organs, except the liver, were positive.

The neo-positive cells evidenced significant IgA synthesis as demonstrated by immunoperoxidase staining (data not shown) of paraffin sections taken from the same animals subjected to PCR analysis. By 2 weeks after injection, massive infiltration of the bone marrow by IgA-positive plasma cells was observed, whereas only single IgA-positive cells were occasionally found in the spleen, confirming the PCR analysis.

Changes in Metastatic Behavior of Plasma Cells during Bone Marrow-involved Growth. The observation that S107 PAS/3 cells engrafted in the bone marrow prior to dissemination suggested that the bone marrow environment might induce a phenotypic change facilitating metastatic spread. To test this possibility, a series of experiments were performed in which bone marrow from S107 PAS/3-inoculated mice was removed at the appearance of disease symptoms, and the tumor cells expanded for 2-3 days in culture. These cells were then re-introduced i.v. into new recipients, and the process was repeated for a total of four in vivo passages. Following the fourth passage, dissemination patterns were assessed by PCR analysis of DNA samples isolated from different organs at the second week after...
injection. In control mice injected with parental S107 PAS/3 cells (Fig. 5; G0), bone marrow was still the only organ demonstrating engraftment by neo-positive cells at the 2-week interval, whereas mice injected with the fourth passage (G4) of the same cell line showed no tissue preference in dissemination pattern. Microscopic analysis of cytospin preparations stained with H&E and Giemsa stain did not reveal any significant differences either in nuclear/cytoplasmic morphology or mitotic rate between the two generations of S107 PAS/3 cells (data not shown). Tumor cells obtained from these mice were subsequently grown in culture for 4 weeks and then reinjected into recipients. The dissemination pattern observed after 2 weeks was identical to that of the in vivo passaged G4 and did not revert to that of the parental line, indicating that the change in metastatic pattern likely reflected a genotypic event.

Changes in Bone Marrow Architecture after Plasma Cell Injection. The neoplastic cells infiltrating the bone marrow were detected at the histological level by the second week after injection (Fig. 6). Normal hemopoietic tissue was focally replaced by tumor cells containing prominent nucleoli and exhibiting a high mitotic rate. Four weeks after injection, most of the neoplastic tissue appeared as a highly malignant and necrotic tumor that occupied large areas of the bone marrow cavity. As demonstrated by TRAP staining of paraffin sections, increased numbers of osteoclasts associated with cortical and trabecular bone were observed in all samples (Fig. 7a). Resorption pits were mainly located in areas adjacent to neoplastic cells, in contrast with the smooth contour of the bone adjacent to uninvaded marrow (Fig. 7b). Four weeks after injection, in regions of heavy tumor infiltration, bone surfaces appeared extensively scalloped, sometimes without associated osteoclasts (Fig. 7c). In some cases, cortical destruction and extensive infiltration of tumor tissue into the surrounding soft tissues were observed. This was particularly frequent in the rib area (Fig. 7d).

DISCUSSION

The development of appropriate animal models is of obvious importance to both an understanding of disease processes and a development of new therapeutic approaches. In this regard, we have undertaken an assessment of murine plasma cell tumors in terms of similarities to corresponding human plasma cell diseases, with particular attention to multiple myeloma. Multiple myeloma accounts for approximately 1% of human cancers and is invariably fatal, with patient life expectancies largely unchanged in the last 20 years (9).

We have noted previously that the murine tumors share a number of parallels with human myeloma: (a) T cells have been shown to play an important role in the progression of "transformed" B cells to terminally differentiated plasma cell tumors (10). This situation appears reflected in human myeloma in that, in a number of instances, "abnormal" B cells have been described in the periphery of patients which, in some instances, have been shown to be clonally related to the neoplastic plasma cells localized in the bone marrow (11–13). Thus, normal T cells may similarly drive these abnormal B cells to terminally differentiated, neoplastic plasma cells; and (b) IL-6 has been suggested to be the major growth factor involved in multiple myeloma (14–17). Recent studies have demonstrated that IL-6 knockout mice are completely resistant to the development of plasma cell tumors, except by agents that constitutively activate the IL-6 signal transduction pathway (18, 19), establishing the obligatory role of this cytokine in BALB/c plasma cell disease.

Although these similarities suggest a commonality to the murine and human diseases, one obvious difference is that murine tumors are induced in the peritoneal cavity to which they are thought to be restricted. In contrast, early-stage human myeloma is primarily a bone...
marrow disease which in late stages may metastasize to a variety of organs. The present studies provide important new data revealing previously unrecognized parallels between the two. The studies described herein demonstrate that murine tumors are not restricted to growth in the peritoneal cavity but are capable of metastatic spread (Figs. 1 and 2). Most intriguingly, experiments with the S107 line revealed that after i.v. injection, tumor cells preferentially colonize the bone marrow (Fig. 4), mimicking early-stage human disease. At later time points, dissemination occurs to other organs. Dissemination to a variety of organs, including spleen, lymph nodes, liver, and kidney, although not usually recognized as a clinical feature of human disease, has similarly been described as a common occurrence in terminal-stage myeloma (20, 21). It is noteworthy that cells passaged through the bone marrow display an entirely different metastatic pattern than the initial S107/PAS3 cells (Fig. 5), suggesting that they have undergone a phenotypic change. Furthermore, it would seem likely that this phenotypic change reflects a corresponding genotypic alteration because the new phenotype is maintained in culture and does not revert to that of the parental cells. Such a putative change might be equivalent to that occurring in progressing myeloma patients, which allows bone marrow-resident tumor cells to migrate to peripheral tissues. Thus, the two cell lines described provide a valuable model for future studies on molecular changes leading to the disseminated phase of this disease.

Dissemination of S107 cells is accompanied by an increase in osteoclasts and microscopic lesions in areas of bone adjacent to tumor cells (Fig. 7), indicative of initial stages of bone disease, a common clinical manifestation in multiple myeloma patients (22). The overall disease pattern thus appears, in many respects, to represent a time compression of that seen in myeloma, with all mice becoming moribund within 28–31 days. Animals uniformly exhibit loss of weight and hind leg paralysis caused by penetration of plasma cells from the vertebral bone marrow into the epidural space, with subsequent compression of the spinal cord (Fig. 3).

A number of attempts have been made to develop models for plasma cell disease and multiple myeloma both in terms of understanding the disease process and in developing therapeutic approaches. It has been demonstrated that fresh human myeloma cells will survive in the SCID mouse, but expansion is extremely limited (23). Other studies have shown that certain human myeloma cell lines produce a disease in SCID mice resembling multiple myeloma and may thus prove useful in drug testing (24–28) but are clearly limited by the xenogeneic nature of the system. Radl and coworkers (29, 30) have described a murine plasma cell disease occurring spontaneously in a
small percentage of old C57BL mice that appears to originate in the bone marrow and may be useful as a disease model. The present data demonstrate that BALB/c plasma cell tumors share a number of previously unrecognized features with human myeloma and suggest the use of this system for evaluating therapeutic approaches to plasma cell disease. Furthermore, the ability to identify small numbers of "marked" tumor cells by PCR provides an opportunity to carefully monitor disease progression as well as residual disease during therapeutic trials.

REFERENCES

Disseminated Growth of Murine Plasmacytoma: Similarities to Multiple Myeloma

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