Metastatic Patterns in the Ta4 Transplantable Rat Myeloma

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Introduction

Multiple myeloma is a human neoplasm of relatively high incidence. Current therapy consists of cytotoxic agents, which normally reduce the tumor burden but do not result in cures (6). More studies are needed in myeloma therapy.

The IR162 rat myeloma is a potential therapeutic model for human multiple myeloma. The tumor was derived from a spontaneous ileocecal lymph node tumor in a LOU/C rat, and it was readily transplantable by subcutaneous or intraperitoneal routes into LOU/C and LOU/M rats, which are histocompatible (2, 3). The tumor cells have been reported free of oncogenic viruses (4), and this appears to reflect the human situation (12). IR162 cells secrete a monoclonal IgE. The tumor has been established in cell culture as the Ta4 line, which has remained tumorigenic and continued to secrete IgE *in vitro* (7, 9). In this report the metastatic properties of tumors initiated from the Ta4 line (Ta4 tumors) were studied.

Materials and Methods

The IR162 tumor was obtained from Dr. H. Bazin in Louvain, Belgium. The Ta4 line was developed at the University of Notre Dame Lobund Laboratory (7, 9).

Louvain/Wsl/M (LOU/M) rats were given Teklad L485 diet and tap water *ad libitum* (10). Subcutaneous tumors, initiated by the injection of cultured Ta4 cells, were minced, diluted in balanced salt solution, and one to three million viable cells were inoculated subcutaneously into young adult male rats between the ages of three and four months. Tumor growth was evaluated two to three times per week, and tumor size was calculated as the product of two perpendicular diameters.

Peripheral blood and bone marrow smears were stained with the Wright-Giemsa procedure (15).

Linear regression analysis and T test were performed using the Minitab program (14).

Results

Subcutaneous tumors initiated by the injection of first or second animal passage Ta4 cells (Ta4 tumors) were well vascularized. Serum from these rats contained large amounts of monoclonal IgE as detected by double gel diffusion or single radial immunodiffusion. In Table 1 data from 23 tumor bearers in four separate experiments are presented. Metastasis occurred primarily to ipsilateral axillary, inguinal, and retroperitoneal lymph nodes, as well as to parathymic lymph nodes. Splenomegaly was a consistent characteristic and was directly related to the weight of the primary tumor ($r = .9$). Tumor bearing rats were anemic, and the hematocrit was inversely related to the tumor burden ($r = .9$).

Following a lag period after inoculation, tumors became palpable, and tumor size increased linearly with time ($r = .9$). At autopsy tumor weight was highly related to calculated tumor size ($r = .7$).

Differential counts on femoral bone marrow smears were made on groups of rats at weekly intervals before tumors became palpable, and on rats with large

TABLE 1. *Pathology of Rats Bearing Transplanted TA4 Subcutaneous Tumors*

	Tumor-Free Controls[a]	Subcutaneous Tumor
Number of Observations	8	23
Tumor Weight (gm.)	0	21.7 ± 14.6
Spleen Weight (%)		
Body Weight	.17 ± .02	.23 ± .12
Hematocrit (%)	ND	37 ± 8
Peripheral White Cell Count (/m^3)	7147 ± 3962	8419 ± 2624
Incidence of Lymph Node Tumors		
Axillary	– – –	7/18
Inguinal	– – –	5/18
Lumbar or Renal	– – –	8/18
Mesneteric	– – –	0/18
Omental	– – –	0/18
Parathymic	– – –	11/18

a. Data are presented as the mean ± standard deviation.

tumors. Marrow metastasis was not detected in rats sacrificed before tumors became palpable. Injection of peripheral blood, spleen cells, and bone marrow cells from these experimental rats intraperitoneally into normal rats failed to produce tumors. Rats bearing large tumors had metastases in bone marrow, accompanied by a reduction in the erythrocytic, neutrophilic, and eosinophilic series (Table 2). Differential counts on peripheral blood revealed significant increases in lymphocytes (56% to 65%) although immature forms were not seen. Polymorphonuclear leucocytes were decreased from 40% to 30%. Red cell morphology was consistent with the presence of anemia.

TABLE 2. *Differential Results of Bone Marrow Smears*

Maturation Series (Percent)	Controls c	Tumor Bearing
Neutrophilic	[a]43.8 ± 3.6	34.5 ± 5.6[b]
Eosinophilic	5.1 ± 3.1	1.5 ± 2.1[b]
Basophilic	1.1 ± 1.2	1.4 ± 1.8
Erythrocytic	33.4 ± 2.9	25.5 ± 4.7[b]
Lymphocytic	12.8 ± 2.9	13.0 ± 1.9
Mature Plasma Cells	0.7 ± 0.5	1.0 ± 0.9
Megakaryocytes	1.8 ± 1.0	2.0 ± 1.2
Imature Plasma Cells or or Tumor Cells	0	20.3 ± 2.7
Tumor Weight (gm.)	0	32.4 ± 10.8

a. Data are presented as the mean ± standard deviation
b. Significant difference at the 0.5 level by the two sample T test
c. There were 10 rats in each group

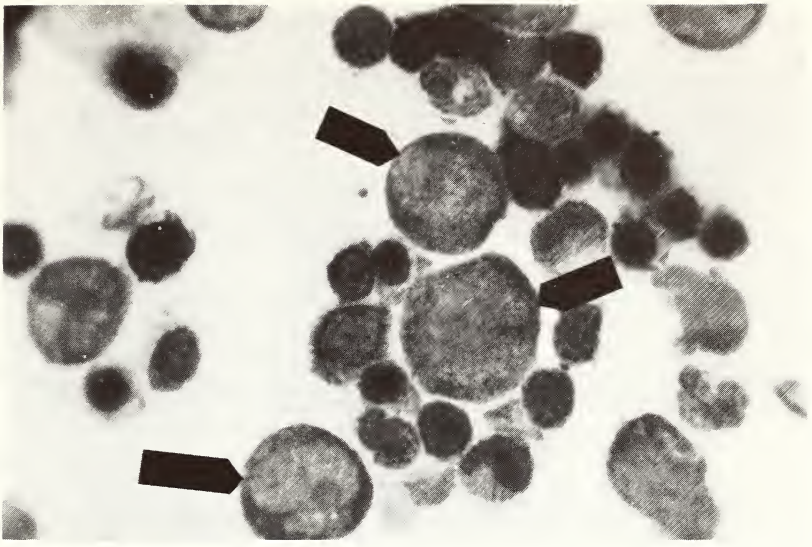

FIGURE 1. *Ta4 Tumor Metastasis in Bone Marrow*

Tumor cells had large nuclei, prominent golgi apparatus, and would be classified as immature plasma cells, plasmacytoid cells, or plasmablasts (Figure 1).

Discussion

The Ta4 cells in culture and Ta4 tumor present a useful model for the therapy of human multiple myeloma. The Ta4 cell line has been useful in screening putative cytoreductive and immunomodulatory agents (8). We wished to characterize the *in vivo* properties of the Ta4 tumor and evaluate it as a model for multiple myeloma.

Both the rat Ta4 and human myeloma are plasma cell tumors, and both secrete immunoglobulins. Because of the very low levels of IgE in normal rats (5), it is easy to detect the monoclonal IgE in serum of tumor bearing rats.

A multiple myeloma model should be highly metastatic, and the subcutaneous Ta4 tumor metastasizes readily to draining lymph nodes. However, the probable site of origin and predominant site of growth in multiple myeloma is the bone marrow (12). The transplanted Ta4 cells did infiltrate the bone marrow, but this was a late event in the course of the tumor. Anemia is a distinguishing characteristic in both the rat and human diseases (6, 12), as is the related reduction in cells of the erythrocytic series in the bone marrow. The splenomeagaly also appeared to reflect metastasis. The enlarged spleens in tumor bearing rats were previously reported to have abnormal cellular architecture and to contain large numbers of tumor cells (7).

A subcutaneous inoculation route was chosen for two reasons. 1) Since chemotherapeutic agents are often administered intraperitoneally to rodents, we wished to separate the sites of primary tumor growth and drug presentation. 2) Subcutaneous inoculation allowed the evaluation of tumor development by measurement of external size. When therapy is administered and then discontinued, the length of the lag before tumor appearance may reflect the extent to

which the tumor cells were killed. This is a reasonable assumption since tumor growth was linear with time after the tumor became palpable and therapy trials with methotrexate indicated that it was probably the case (Manuscript in preparation). Serum IgE levels may also be used to periodically monitor tumor burden although this would not be the method of choice because bleeding causes additional stress to the rats.

Current therapy of multiple myeloma generally reduces the tumor burden, but it does not result in cures (6). The disease is itself immunosuppressive, as are the chemotherapeutic agents (6, 11, 12, 13). We were therefore particularly interested in a model which could be used to evaluate putative immunotherapies, which would be useful after the tumor burden was reduced. Therefore, we chose an inoculum which resulted in a low tumor burden and a relatively long lag before tumors became palpable. During that period of low tumor burden, potential immunotherapies could be tested. The Ta4 cells are susceptible to antibody-complement mediated cell lysis *in vitro* (unpublished), and they appear to be sensitive to immunotherapy *in vivo* (1). In conclusion the Ta4 cell line and Ta4 tumor appear to provide a usable model for studying both cytotoxic and immunomodulatory therapies for human myeloma.

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