Factors Controlling Metastasis of Experimental Breast Cancer\footnote{Supported in part by a grant from the Hartford Foundation and Contract SA-43-ph-2398 with the Cancer Chemotherapy National Service Center, National Cancer Institute, NIH.}

UNTAE KIM

Roswell Park Memorial Institute, New York State Department of Health, Buffalo, New York

Summary

Rats bearing a large mammary adenocarcinoma, induced by methylcholanthrene (MCA), whether it is an autochthonous or a transplanted isologous tumor, often had an enlarged spleen without metastatic involvement of any organs. Such spleens contained many tumor cells in the venous sinuses. When blood and spleen-mince obtained from the tumor host were inoculated separately into the peritoneal cavity of young adult isologous rats, they produced numerous tumor nodules in the abdomen in all rats. It was concluded, therefore, that the rationale for not finding metastasis in the rat bearing an MCA-induced mammary tumor is in the host immune state evoked by the strong specific antigenicity of the tumor. The possible mechanism controlling metastasis of breast cancer is also discussed.

Introduction

A carcinoma of the breast in women usually kills the host by invading vital organs or by massive dissemination resulting in cachexia. In laboratory animals, however, breast cancer seldom metastasizes spontaneously, whether the tumors are primary or transplanted. When an animal dies with a tumor, it is usually not from the metastasis but from cachexia caused by a localized but overgrown tumor mass. Either blood-borne or lymphatic metastasis is a customary event in advanced human breast cancer, whereas it is an extreme rarity in animals.

The author observed that a rat bearing an outsized MCA-induced mammary adenocarcinoma, whether primary or transplanted, often had a large spleen (Fig. 1) without any significant lymphadenopathy or hepatomegaly and without any visible metastatic foci. Such spleens were usually dark red, unlike splenomegaly in leukemic rats. The present communication is a report of findings in such rats that may be related to host factors controlling the dissemination of breast cancer.

Materials and Methods

BLOOD AND SPLEEN DONORS. The donors were a 9-month-old inbred F/Fu female rat bearing a large MCA-induced mammary adenocarcinoma (11) in the right thoracic region and a 6-month-old inbred W/Fu rat bearing a large transplanted, autonomous MCA-induced mammary adenocarcinoma in the right inguinal region (12).

EXPERIMENT 1. From the primary tumor-bearing F/Fu rat whole blood was obtained by tapping the abdominal aorta, and 0.2 ml was injected i.p. into each of six 50-day-old inbred F/Fu rats. The enlarged spleen was minced in tissue culture medium 199 (1:1 ratio) and injected similarly into another group of 6 rats.

EXPERIMENT 2. From the transplanted autonomous tumor-bearing W/Fu rat, blood and spleen were obtained, and 0.5 ml was injected similarly into each rat in 2 groups of six 50-day-old inbred W/Fu female rats.

A complete autopsy was performed on the donor and recipient rats, and histologic sections were taken from lungs, liver, spleen, regional lymph nodes, and kidneys. Blood smears and spleen imprints were stained with Wright-Giemsa stain. The blood- or spleen-injected rats were kept until death or killed in extremis.

Results

Microscopic examination of the primary and transplanted tumor-bearing rats revealed no metastatic tumor foci in any of the organs studied. However, in the spleen imprints there were many small clusters of recognizable carcinoma cells. Even in the histologic sections there were clusters of large, distinctly recognizable carcinoma cells in sinuses. The blood smears contained occasional tumor cells.

In the 1st experiment, most rats from either group died in 3-5.5 months, and the last rat died 13 months after the inoculation. Autopsy disclosed that the abdominal cavity of all rats was filled with numerous tumor nodules measuring from 1 to 15 mm in average diameter, and the tumor nodules were found even in the liver (Fig. 2). The histologic pattern of the nodules was that of adenocarcinoma similar to that in the donor rat (Fig. 3).

In the 2nd experiment, in which young adult W/Fu female rats received whole blood and the spleen-mince i.p., all rats died within 6 months, with the massive tumor dissemination in the peritoneal cavity. The nodules were adenocarcinoma similar to the transplanted autonomous tumor in the donor rat.

Discussion

Metastases of neoplastic cells to a distant site may be controlled by the following conditions: (a) the nature of the growth or invasiveness of the tumor cell (this may not necessarily coincide with the growth rate, since as in certain occult thyroid and gastric carcinomas, the metastatic tumor is often found long before the primary lesion is discovered); (b) the size of the vein and its proximity to the tumor (vascular lumen large enough to permit free flow of the liberated tumor cells); (c) the receptiveness of the secondary host organs or tissues (26); and (d) the antigenicity of neoplastic cells or the immunologic sensitivity of the host.
Since the arterial blood taken from the tumor host produced a massive miliary dissemination of the host tumor, there seems to be no question about the ability of rat MCA-induced mammary adenocarcinoma cells to penetrate into the systemic circulation. Although Willis (26) doubted that tumor cells could traverse the pulmonary circulation to enter the greater circulation without establishing metastatic foci in the lung, the evidence noted in this experimental tumor system indicates that large numbers of tumor cells do escape entrapment in the pulmonary vasculature. The failure to find either a macroscopic or a microscopic secondary tumor in all the organs examined does not rule out the possibility of hidden tumor foci. However, in view of the innumerable tumor nodules found in the peritoneal space following inoculation of a small amount of blood, it is unlikely that these circulating tumor cells originated in the undetectable secondary lesion. Circulating tumor cells in blood have been found in cancer patients (20, 22) and in sarcoma-bearing laboratory animals (5, 6, 9, 25), and inoculation of the latter produced sarcomas (3, 6, 8, 9, 25). Therefore, it seems quite clear that malignant solid tumors are being steadily liberated into the general circulation.

The spleen was the only organ that was enlarged in the MCA-induced mammary tumor host, and the tumor cells were found only in the venous sinuses of the spleen. Flaks (6) successfully produced sarcoma with lungs and lymph nodes draining a transplanted Jensen-sarcoma in mice, but failed to do so with liver, heart, testis, brain, bone marrow, thymus, or lymph nodes other than those draining the tumor. Although the tumor producibility of other organs from the mammary tumor host was not tested, the cells appeared to be concentrated mostly in the spleen. It is well known that certain tumors have “preferential sites” of metastasis or certain organs and tissues have receptiveness to particular tumors. However, our present knowledge cannot account for this phenomenon by any explanation beyond the so-called “soil” hypothesis (cf. Ref. 26).

The tumor cells were in the greater circulation, and many of them were trapped in the spleen; yet their ability to settle and establish a proliferating metastatic nodule seemed to have been contained or neutralized. It is also interesting to note that many of the tumor cells were found in the spleen, pointing to the possible role of spleen rendering the tumor cell innocuous. Since Foley (7) first described the existence of specific antigenicity in MCA-induced mouse sarcoma, many tumor-specific antigens have been discovered in various experimental solid tumors and leukemias (21), including a specific isoantigenicity of MCA-induced mouse mammary adenocarcinoma (23). Such specific immune state was found even in the autochthonous tumor host (15, 24). Administration of cortisone (2, 18), X-ray (4, 10, 18), or potent antitumor chemotherapeutic agents (16–18) that also have immunosuppressive activity caused metastasis of transplanted tumors in laboratory animals. Thus, the evidence gathered here seems to indicate that the immune state of the host plays a major role in controlling dissemination of malignant solid tumors and that the absence of metastasis in the MCA-induced mammary tumor host is caused by a specific auto- and iso-antigenicity of the tumor. Specific antigenicity in spontaneous mammary tumors has not been identified, and it may be weakly antigenic, as in the cellophane-induced sarcoma (14). Therefore, processes that might evoke host immune response, as described by Baldwin (1) and others (13, 19), may be effective methods of controlling tumor metastasis.

References


Fig. 1. A 6-month-old W/Fu rat bearing a large transplanted MCA-induced mammary adenocarcinoma in the right inguinal region. Note the enlarged spleen.

Fig. 2. Two F/Fu rats that received an i.p. injection of 0.2 ml of blood and spleen-mince 5.5 months prior to death from an MCA induced autochthonous mammary-adenocarcinoma-bearing F/Fu rat.

Fig. 3. A photomicrograph of the mammary adenocarcinoma nodules shown in Fig. 2. H & E, × 400.

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