The Kinetics of the Organ-specific Metastasis of a Transplantable Reticuloendothelial Tumor

H. Ira Pilgrim
The Germfree Laboratory, Department of Surgery, University of Utah College of Medicine, Salt Lake City, Utah 84112

SUMMARY

Reticuloendothelial tumors tend to metastasize to specific organs and are, therefore, ideal tools for testing the “seed-soil hypothesis” of metastasis.

A transplantable reticulum-cell sarcoma which selectively metastasizes to the spleen was used. Equal numbers of cells were injected into both kidney and spleen of six C3H mice. At the time of sacrifice, growth in the spleen was always considerably greater than in the kidney. In no case was the mitotic index greater in the spleen than in the kidney. Since the growth rate equals the mitotic rate minus the cell loss rate, one may conclude that the reason for the relative lack of growth in the kidney is that the rate of tumor cell loss is greater there than in the spleen. Additional studies demonstrate that these tumor cells will migrate to the spleen if implanted in other locations, including the kidney. This tendency to migrate out of organs other than the spleen may account for the apparent selective metastatic behavior of this tumor.

INTRODUCTION

Most of the work on the metastasis of carcinoma indicates that its spread and growth is determined largely by the anatomic distribution of tumor cells and emboli (15, 24, 27, 28). Kinsey (12) showed that the Cloudman melanoma had a clear-cut predilection for lung tissue, regardless of whether the mouse’s lung was located in the thoracic cage or was transplanted to one of the limbs of the host animal. Green and Harvey (9) tested for the presence of a hamster melanoma in various organs, following intravenous injection of the tumor, by transplanting the organs of the injected animals into a new host to see if they contained viable tumor cells. They found that the melanoma cells were present in the lung at all times, while the presence of tumor in organs such as the spleen, kidney, brain, and liver was quite variable. A similar pattern was found with a Schwann-cell tumor. A lymphoma, on the other hand, showed that cells from an injected lymphoma could be demonstrated in all organs tested at all times following intravenous injection. They concluded that the behavior of lymphocytic tumors was quite different from that observed in melanomas or Schwann-cell tumors.

The tendency of reticuloendothelial tumors to metastasize selectively is well established. Lymphocytic tumors in the mouse generally involve the lymph nodes, spleen, and liver (6); histiocytomas involve the lymph nodes and liver (3, 6); Hodgkin-like tumors involve the liver, spleen, and lymph nodes (6). Histiocytomas tend to metastasize to the liver when transplanted subcutaneously or injected intravenously (3). Transplatable plasmacytomas have a predilection to metastasize to the bone marrow and produce “punched-out” lesions in the bone (13, 17). Murine lymphocytic neoplasms, which usually originate in the thymus (18), tend to metastasize to all of the lymphoid organs, as do Hodgkin-like tumors (19). This tendency of specific lines of reticuloendothelial tumors to metastasize selectively to specific organs following transplantation has been confirmed in our laboratory. The metastatic pattern is so well fixed that it is possible to identify the particular tumor line by its metastatic pattern.

The tendency of certain tumors to metastasize selectively to specific organs led to the development of Paget’s “Congenital Soil” theory, which states that certain tumors will grow only in organs which provide favorable conditions for their growth. The theory has been well reviewed by Leighton (15), Willis (27), and Cameron (2). Despite a good deal of speculation, there is no direct evidence demonstrating that tumor cells will multiply better in one site than another. The purpose of the experiments being reported was to see if cells from a tumor with clear-cut metastatic preferences would multiply better in their preferred site than in a nonpreferred site. Equal numbers of tumor cells were injected into the preferred metastatic site and also to a nonpreferred site. If the soil-seed hypothesis is correct, the rate of growth in the preferred site should be greater than that in the nonpreferred site. This should be reflected in the mitotic rate in the preferred site being greater than the mitotic rate in the nonpreferred site.²

MATERIALS AND METHODS

The spleen and left kidney of six mice were injected with 121,000 tumor cells in two microliters of Hanks’ balanced salt solution. The animals were sacrificed seven days after injection. Photographs were taken of the tumors, and the spleen and kidney were fixed. After fixation with Fekete’s formol-

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²The author is indebted to Dr. Leo Samuels for pointing out that the rate of tumor cell division in an organ should be related to the metastatic preference of the tumor for that organ.
acetic alcohol, the kidneys and spleens were sliced with a razor blade. The free-hand sections which showed the largest amount of metastasis were embedded in paraffin, sectioned at five microns, and stained with hematoxylin and eosin. All transplants were made in syngeneic C3H/Pi or C3Ha/Pi mice.

Tumor. The tumor is a Hodgkin's-like tumor (reticulum cell sarcoma, Type B of Dunn). It originated at the ileocecal junction of a C3Ha/Pi mouse and was transplanted for 20 generations in this laboratory. It had been stored in a frozen state prior to its use in these experiments. In its early stages of transplantation, it consisted of a mixture of reticulum cells and lymphocytes (Fig. 1). After four transplant generations, the lymphocytes disappeared, leaving only the reticulum cells (Fig. 2). The tumor invariably metastasizes to the spleen (Fig. 3). If transplanted subcutaneously, it will also metastasize to the regional nodes. There are occasional metastases to the liver. Metastasis to the kidney is extremely rare.

Counting Methods. To eliminate variations in tissue thickness, the tissues from each animal were fixed and embedded in the same block. Tissues were fixed in Fekete's formol-acetic alcohol (8) and stained with hematoxylin and eosin. Cells were counted at a magnification of X 900, using an eyepiece reticle. All tissues were scanned to ascertain that there was a uniform distribution of cells. All of the cells in 10 adjacent fields were counted and the mitotic figures recorded and classified as to phase. Ten additional fields were scanned only for mitotic figures, and the mitotic figures were classified. It was assumed that the same number of cells were present in the second ten fields. Between 1,200 and 1,600 cells were counted per specimen. An "undetermined" category was included, which consisted of mitotic figures in which the phase could not be determined. This was usually due to the fact that only a portion of the chromatin was present in the section.

Statistical Methods. Before proceeding with the experiment, it was decided that if the mean counts in the kidney were greater than the mean counts in the spleen, it would constitute statistical evidence in favor of the hypothesis of no difference in mitotic rate, and we would accept the hypothesis that the mitotic rate in the kidney and in the spleen were the same. If, on the other hand, the mitotic rate in the kidney was less, a one-tailed t test would be performed, using a confidence limit of P = 0.01. The results were such that a t test was unnecessary.

RESULTS

The results of the experiment, in which equal numbers of cells were injected into the kidney and spleen of individual animals, are presented in Chart 1. The camera lucida drawings illustrate the large discrepancy between the size of the tumor mass in the spleen and kidney. The amount of tumor in the spleen is always considerably larger than that in the kidney. The tumor cell mitotic index in every case proved to be somewhat greater in the kidney than in the spleen. This may be a spurious correlation, or may represent a real increase in the mitotic index of the tumor cells in the kidney. Preliminary studies using labeling with tritiated thymidine indicate that there is probably little or no difference between the mitotic rate in the tumor in kidney and in the spleen. There is, however, no question that the tumor mitotic index is not greater in the spleen.

Table 1 shows the distribution of mitoses by phase. There is little or no difference between the phase distribution of mitotic figures in tumor in the kidney and in tumor in the spleen. Since the duration of each phase of the mitotic cycle is directly proportional to the frequency with which mitotic figures are seen in that stage of the cycle (Wright's Law), we may conclude that there is no difference in the mitotic time of those phases which are visible, relative to one another. We cannot, of course, draw any conclusions about the synthetic phases which cannot be seen with the technic used, nor exclude the possibility that the total cycle time is uniformly altered in one organ.

A second experiment was designed to ascertain whether cells injected into the kidney would metastasize to the spleen. Two microliters of a tumor cell suspension were injected directly into the kidney of 21 C3H mice. Animals were sacrificed at 10 and 14 days after transplantation. In every case, the tumor was present in the spleen. In the animals sacrificed 10 days after transplantation, the tumor in the spleen had a pattern of individual nodules, while at 14 days after injection the spleen consisted of a confluent tumor mass.

To test for the presence of an oncogenic virus, 3.3 grams of tissue were homogenized in 25 ml of phosphate-buffered tissue culture saline, using an Elvejhem-Potter homogenizer. The cell suspension was centrifuged twice for 15 minutes at 6500 rpm in an angle centrifuge to remove the cells. The supernatant was checked microscopically for the presence of cells and none were found. One-half to 1.0 ml was injected subcutaneously into 12 young C3H mice. Eleven months after injection there was no sign of tumor either at the injection site or in the spleen.

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Table 1

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<th>Animal number</th>
<th>Organ</th>
<th>Prophase</th>
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<th>Telophase</th>
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Count of mitotic figures in the tumor, by phase, after injection of equal numbers of tumor cells into the kidney and spleen. The mitotic index is the percentage of tumor cells in mitosis. The number in the columns under “Phase of mitosis” is the percentage of all mitotic figures in the phase indicated. Note that there is little difference between kidney and spleen with regard to the percentages in different phases of mitosis.

DISCUSSION

Before we can analyze the experiments reported here, it is first necessary to understand the kinetics of expanding cell populations. Logic dictates that the mass of a given system is regulated by what goes into the system and what leaves it. If one deals with rates, we can say that the growth rate equals the rate at which substance is added to the system, minus the rate at which substance leaves the system (11, 16). In the case of tumors, most of the addition to the system is a function of cell division, while the loss from the system consists of cells which either migrate, or die and are lysed. The concept of a dividing or nondividing fraction is important, is taken into account when the mitotic rate (M) is calculated on the basis of the total cell population, rather than simply the proliferating fraction. Chart 2 presents a simple diagram illustrating the basic equation. “G” represents the rate at which the cell population is expanding; “M” represents the rate at which the cells are being added, i.e., the mitotic rate; and “L” represents the rate of cell loss.

Most of the work done on cell kinetics has been done under situations where the growth rate was zero and the rate of cell division could be directly equated with the rate of cell loss (23). Baserga (1) discussed the mitotic cycle in tumors. Hoffman et al. (10), Collins et al. (4), Laird (14), and Durbin et al. (7) studied the growth rates of tumors. Collins et al. (4) and Hoffman et al. (10) discuss the logarithmic growth characteristics of tumors, and Laird (14) and Durbin et al. (7) consider the deviations from a logarithmic growth pattern and point out that tumors appear to follow a Gompertz distribution rather than a logarithmic one. Steel (20, 21), Steel et al. (22), and Iversen (11) analyzed the growth of tumors and presented detailed discussions of the parameter of cell loss. Dethlefsen et al. (5) used a computer in an attempt to collate the large amount of data on the growth of tumors.

Chart 2. A simplified diagram illustrating the principle of growth kinetics. The rate of growth in any system is equal to the rate at which material is going into the system, minus the rate at which material is leaving the system. In the case of most tumors, the growth rate may be expressed as the increase in cell numbers, or a value that can be equated with cell number. The input is largely the mitotic rate; and the rate of cell loss expresses cells which are lysed within the tumor mass or cells which leave the tumor mass. G, the rate at which the cell population is expanding; M, the mitotic rate, or the rate at which the cells are being added; L, the rate of cell loss.
number of variables in tumor growth and attempted to estimate cell loss. Weiss and Kavenau (26) discussed the theoretical considerations involved in growth and growth control. They pointed out that "growth, as the measured gain of organic mass of a circumscribed living system is the net balance of mass produced and retained (incremental component) over mass destroyed and otherwise lost (decremental component) within the given boundaries." In other words, the mass of a living system is equal to what goes in, minus what comes out.

If we consider the experiment performed in light of the growth equation which we have just discussed, we find that, if the rate of growth of the tumor in the spleen is greater than the rate of growth of tumor in the kidney, and the mitotic rate in the kidney is equal to the mitotic rate in the spleen, then it follows that the rate of cell loss in the kidney must be greater than the rate of cell loss in the spleen. The reason for the obvious difference in metastatic preference is not due to a differential rate of cell division, but to a differential rate of cell loss in which the preferred organ appears to retain cells more readily than the other organs, which appear to lose cells more readily.

If the rate of cell loss is greater in the kidney, by what mechanism does this cell loss take place? Are the cells lysed more rapidly, or do they migrate away from the kidney mass? The answer to this question may be found in part in the results of the experiment in which the kidney alone is injected. Ten days after injection of tumor into the kidney alone, tumors were evident in the spleen, indicating that the cells had migrated out of the kidney and lodged in the spleen. We cannot say whether cell destruction is an important parameter in the system; we can only conclude that migration from a nonpreferred organ is one important parameter in determining selective metastatic behavior.

We conclude that, within the system tested, the differential rate of cell division is not the controlling factor in producing selective metastasis. We also know that cells appear to emigrate more readily from nonpreferred metastatic sites than they do from preferred sites. Weiss and Andres (25) used dissociated embryonic skin cells and were able to show that these cells will more readily from nonpreferred metastatic sites than they do from preferred sites. Weiss and Andres (25) used dissociated embryonic skin cells and were able to show that these cells will

REFERENCES

Fig. 1. Section of the Hodgkin’s-like tumor in its first transplant generation. Note the mixture of reticulum cells and lymphocytes. H & E, × 1000.

Fig. 2. The same tumor in its 22nd transplant generation. Notice the loss of lymphocytes. H & E, × 1000.

Fig. 3. Mouse injected in the right axilla, with Hodgkin’s-like tumor. Note the metastasis to the right inguinal node and the massive metastasis to the spleen.

Fig. 4. Animal injected with Hodgkin’s-like tumor. Equal numbers of cells were injected into the kidney and spleen. This photograph was taken 7 days after tumor injection.
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