The Metastatic Behavior of a Spleen-tropic Reticulum Cell Sarcoma in Splenectomized Mice (35856)

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A transplantable line of reticulum cell sarcoma type B of Dunn (Hodgkin's-like tumor) metastasizes selectively to the spleen. Regardless of where the tumor is injected, the syngeneic host animal invariably develops massive metastasis to the spleen. With the exception of the regional lymph nodes, the metastasis of the tumor to other sites is variable, and usually does not occur following subcutaneous implantation. When equal numbers of tumor cells are transplanted into the spleen and kidney, the tumor mass in the spleen is always considerably greater than that in the kidney (1). One would expect that this could be accounted for by a more rapid rate of cell division. The mitotic index of the tumor implanted in the spleen is not greater than the mitotic index of the tumor implanted in the kidney. This can only mean the rate of cell loss in the kidney is greater than the rate of cell loss in the spleen. If the tumor is implanted in the kidney alone, the tumor is found to metastasize rapidly to the spleen. The implication of this study is that the cells of this tumor tend to remain in the spleen and migrate out of organs other than the spleen.

Since the spleen sequesters these tumor cells, and they migrate out of other organs, I wanted to determine what the behavior of this tumor would be in the absence of the spleen. Would the animals live longer, since the spleen is not essential to life? Where would it metastasize as its second choice? This communication reports an experiment designed to answer these questions.

Materials and Methods. The tumor used is a Hodgkin's-like tumor (reticulum cell sarcoma, type B of Dunn). It originated at the ileocecal junction of a C3Hf/Pi mouse, and had been stored in a frozen state prior to its use in these experiments. It was in the 24th transplant generation at the time of its use. This tumor consisted of a mixture of reticulum cells and lymphocytes. After four transplant generations, the lymphocytes disappeared, leaving only the reticulum cells. The metastatic pattern has not changed in the course of transplantation, and the tumor invariably metastasizes to the spleen and the regional lymph nodes. Metastasis to other organs is rare following subcutaneous transplantation.

The mice used were of the C3Hf/Pi strain. They were maintained 8/cage, and were fed autoclaved Wayne Autoclavable Lab-blox.

At the age of 2 months, the mice were ear-tagged and randomized by number to two groups, which remained assorted in their respective original cages. Each animal was designated as to whether it would be splenectomized, or subject to a mock operation. The mock operation was identical to that performed for splenectomy except that the spleen was merely examined and not removed. The animals were kept in their original cages following treatment, and each cage contained mice that were intact and splenectomized. Twenty-five days after the operation, each animal received an inoculation) into the subcutaneous tissue of the flank of 0.05 ml of a tumor cell suspension containing approximately 37,000 cells/mm^3. Each cage was checked daily in the morning and late afternoon to ascertain if any animals had died. Dead animals were autopsied.

There were 25 splenectomized mice and 26 mock-operated controls.

It should be noted that when this tumor is inoculated into the flank by trocar, that local growth occurs as well as growth in the

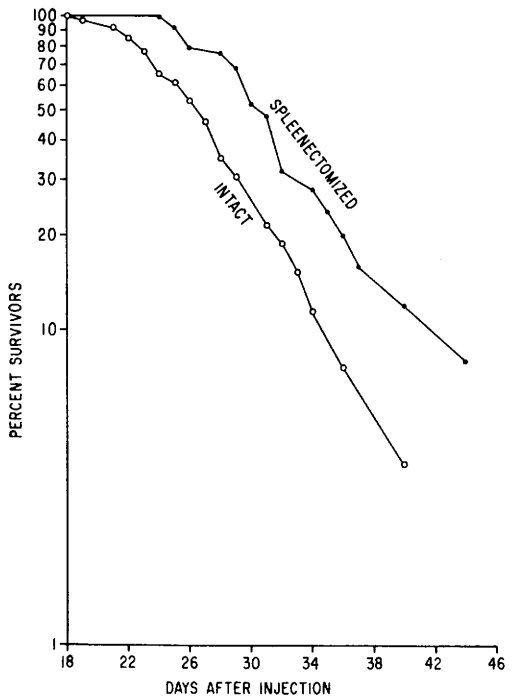

FIG. 1. A survivorship plot of mice injected with a splenotropic reticulum cell sarcoma: There were 25 splenectomized mice and 26 intact mock-operated controls.

inguinal and axillary lymph nodes on the same side. When injected as a cell suspension, local growth rarely occurs, but the regional node still shows tumor metastasis.

Results. Contrary to our expectations, the splenectomized animals started dying later, and survived longer than the intact (Fig. 1). The first death in the intact group occurred at 19 days following tumor injection, while the first death in the splenectomized group occurred 6 days later at 25 days. The median age at death was 27.5 days for those animals with intact spleens and 30.5 days for those with their spleen removed. Figure 1 represents a plot of the logarithm of the percentage survivors against time. When plotted in this manner, the slope of the line represents the death rate. It can be seen that there is a delay in the onset of death, but that once it starts both the splenectomized and intact animals died at approximately the same rate.

At the time of autopsy, all of the control animals had massive spleens and there was obviously an immense amount of tumor present. The spenectomized animals, in contrast, had what subjectively appeared to be a small volume of tumor when compared to the animals with their spleens. Those that had been splenectomized showed tumor metastasis to the liver, kidneys, and lymph nodes. In no case, however, was there the massive amount of tumor that was found in the animals with intact spleens.

Discussion. Our expectation was that, since the spleen is not an organ essential to life, the sequestration of cells in the spleen would enhance the survival of animals inoculated with the tumor. This did not prove to be the case. On the contrary, animals with their spleens succumbed sooner than did the splenectomized ones. The subjective observation that at the time of their death that there was more tumor present in animals possessing their spleens than in those without would appear to indicate that it does indeed take a smaller amount of tumor to kill an animal without its spleen. How, then, can we account for the fact that animals that had been splenectomized survived for a longer period of time. Again, we have the paradox of having greater growth of tumor cells in animals with their spleens intact than in animals with their spleens removed. If we extrapolate from earlier studies (1), it is necessary to conclude that the rate of cell loss (or cell death) is greater in splenectomized mice. We can postulate that cells sequestered in the spleen are not destroyed, while a certain rather high percentage of circulating cells are destroyed. That circulating cells are subject to mass destruction appears to be confirmed by the work of Parks (2) who showed that tumor cell death occurs rapidly following intravenous injection, with 80% of the injected cells being destroyed within 24 hr (he used the same tumor that has been used in this study).

The second question was what would the alternative site of metastasis be in the absence of the spleen. The most consistent finding in splenectomized animals was metastasis to the surface of the liver and the kidney. This metastatic pattern is similar to

that found in a line of transplantable reticulum cell sarcoma which is being transplanted in this laboratory; this tumor is morphologically different, being the type of tumor that tends to form multinucleate giant cells.

Summary. The effect on survival of mice innoculated with a splenotropic reticulum cell sarcoma was tested in splenectomized and mock-operated syngeneic mice. The splenectomized animals survived longer. Evidence was presented that this is probably due to rapid destruction of circulating cells, and that the tumor cells are protected by being sequestered in the spleen.

1. Pilgrim, H. I., Cancer Res. **29**, 1200 (1969).
2. Parks, R. C., Anat. Rec. **169**, 396 (1971).

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