Organ Localization and the Effect of Trauma on the Fate of Circulating Cancer Cells

DOMENICO AGOSTINO AND EUGENE E. CLIFTON

Division of Experimental Surgery and Physiology, Sloan-Kettering Institute for Cancer Research, and Department of Surgery, Cornell University Medical College, New York, New York

SUMMARY

The systemic inoculation of 100,000 Walker 256 cancer cells into rats under control conditions produced metastases in the lungs of over 90% of the animals. No metastases were seen in the other organs. If the animals were fed CCL4, hepatic metastases were also produced in 73% of the animals. Implantation of glass fragments into organs before inoculation of the tumor cells resulted in tumor growth in the liver, kidney, and mesentery.

Viable tumor cells were shown to be present in the liver, kidney, heart, testicle, and brain by s.c. inoculation of organ homogenates, obtained 4 hr after the i.v. injection of tumor cells. There was no evidence that tumor cells lodged or grew in the spleen (Tables 1, 4, 5).

From these results it is evident that tumor cells lodge not only in the lungs but also in other organs throughout the body. Local trauma apparently is of major importance in the localization and growth of circulating tumor cells. The importance of the clotting mechanism and fibrin formation in the lodging of tumor cells and their growth has been discussed.

Cancer spreads by direct extension, by lymphatic permeation, and by the blood stream. In many locally curable lesions the final outcome is dependent upon distant metastases, presumably by cancer cells that enter the blood and are carried to distant sites, where they may lodge and grow (15, 25). Tumor cells have been demonstrated in the blood of patients with cancer (17, 27, 28, 30). There is question concerning the true nature of these cells, and many cells reported to be cancer cells are thought to be bone marrow cells such as megakaryocytes (24).

The fate of the cancer cells that enter the circulating blood cannot be predicted. The majority apparently perish, others may lodge and remain dormant, and some may produce early metastases. The reason for specific organ localization of different tumors has not been well established even with animal experiments. Tissue specificity (23) and vascular peculiarities (9, 16) have been suggested.

In our investigations of the factors affecting the dissemination of cancer cells and development of metastases in experimental animals, particular attention has been given to the effect of the coagulation mechanism. With the administration of heparin or fibrinolysin, metastases were significantly reduced with the V2 and Brown-Pearce carcinomas of the rabbit (14) and with the Walker 256 carcinomas of the rat (1, 21). When these agents were combined with nitrogen mustard (3, 4) or irradiation (2, 11), metastases were further reduced. In contrast, when hypercoagulability was induced by a high fat diet (13) or by the administration of fibrinolysin inhibitors (12) the incidence of metastases was increased significantly. Wood (33) recently has demonstrated the effect of fibrinolysin on tumor clumping by cinematography.

With the systemic inoculation of a large number of cancer cells (100,000–300,000), pulmonary metastases occurred in the majority of animals (1, 4, 21). Other organs remained free of obvious malignant tumor growth. The reason for this has not been established. It might be considered that all tumor cells were filtered out in the lungs, but this seems unlikely since they were found in the aortic blood. The viability of the cancer cells was also shown by s.c. inoculation of the blood into new recipient animals (7, 22). Tumor cells therefore must reach other organs. Why did they not produce metastases? Were the tissues of these organs not susceptible to the tumor? Did the tumor cells pass through the organ without lodging, or did they lodge and remain dormant?

The present studies were undertaken in an attempt to find some answer to these questions.

MATERIALS

Wistar female rats (about 200–250 gm) and weanlings (about 30–40 gm) were used. Glass fragments were pre-
pared by smashing with a hammer; the largest fragments were about 0.5 mm in diameter. Carbon tetrachloride, 320 mg in 0.2 ml, was given by stomach tube each day for 3 consecutive days. The following high fat diet was used: butter, 40%; casein, 20%; cholesterol, 5%; Celluflor (a cellulose flour), 5%; Wesson's salt mixture (32), 4.0%; choline chloride, 0.2%; and vitamin mixture (General Biochemicals, Inc., Laboratory Park, Chagrin Falls, Ohio), 2.0%.

Walker 256 carcinosarcoma has been carried in the ascitic form for several years in our laboratory (22). The fluid containing tumor cells was aspirated from the peritoneal cavity of donor animals and diluted with saline to a concentration of 100,000 cells/ml.

METHODS

Glass fragment implant.—Glass (2–3 pieces) was implanted surgically into various organs; 3 days later 100,000 cancer cells were inoculated i.v. (femoral vein). No glass was seeded in 10 control rats.

Carbon tetrachloride.—Carbon tetrachloride (320 mg, in 0.2 ml) was administered by stomach tube daily for 3 consecutive days. On the 4th day the animals were anesthetized with ether, and 100,000 cancer cells (1 ml) were injected i.v. (femoral vein).

High fat diet.—These animals were fed the high fat diet for 3 months. They were then inoculated with 100,000 Walker 256 cancer cells and continued on the high fat diet for another month. Following this they were returned to normal diet (Purina chow).

Secondary transplant of organ homogenates.—Cancer cells (100,000) were inoculated i.v. into untreated animals. Four hr after the i.v. inoculation, the animals were sacrificed and the organs were removed, minced, and homogenized with saline. (a) In the 1st group (Table 4), each organ or pair of organs from 2 animals was homogenized with 10 ml of saline. One ml of this homogenate was then inoculated s.c. into each of 10 young rats (30–40 gm). (b) In a 2nd series the organs were homogenized in different amounts of saline (see Table 5) to give a relatively greater mass of heart, brain, testicle, and spleen per 1 ml. This was done to have an organ mass similar to that of the liver. One ml of homogenate was inoculated s.c. into each of 10 weanling animals.

RESULTS

Glass Fragment Implant (Table 1)

Control (no glass, 10 rats).—Of these animals, 4 died with lung metastases within 2 weeks. Six died in 3 weeks and all had pulmonary metastases. No metastases were seen in the liver, spleen, kidney, or any other organ studied.

Liver (10 rats).—Two of the 10 rats died within 2 weeks; both had pulmonary metastases, and 1 also had a tumor mass in the liver at the site of the glass implant. The 2nd rat had a large liver abscess. The remaining 8 rats died 3 weeks after inoculation; 8 had lung metastases, and 6 had tumors at the site of the glass implant. All 10 animals had pulmonary metastases, and 7 had hepatic metastases.

Kidney (10 rats).—Two of these rats died within 2 weeks with pulmonary metastases; neither had kidney metastases. Eight died at 3 weeks and all had pulmonary metastases; 1 also had a large tumor in the kidney at the site of the glass implant. Pulmonary metastases were present in all 10 rats and kidney tumor in 1.

Mesentery (10 rats).—Of these rats, 1 died within 2 weeks and had a very large tumor at the site of the glass implant, as well as pulmonary metastases. The remaining 9 animals died within 3 weeks, and all had pulmonary metastases only. The incidence of pulmonary metastases in this group was 100% and of mesenteric tumor, 10%.

Spleen (10 rats).—Six of these rats died within 2 weeks after the tumor cell inoculation, and 4 died in 3 weeks. All animals had pulmonary metastases. No tumor was seen in the spleen in any of the animals.

Carbon Tetrachloride (Table 2, Chart 1)

Controls (10 rats) (no CCl₄).—Of these animals 2 died within 2 weeks, 3 at 3 weeks, 2 at 4 weeks, and 3 at 5 weeks. At autopsy all had pulmonary metastases. None had apparent metastases in the liver.

Carbon tetrachloride (11 rats).—CCl₄ was fed for 3 days and the cancer cells were infused i.v. Three rats died in 3 weeks, and all had metastases in the lungs and liver. Six died within 4 weeks; 4 had both lung and liver metastases, and 2 had only pulmonary metastases. Two died at 5 weeks; 1 had lung and liver metastases, and the other had only pulmonary metastases. Lung tumors were present in 11 rats (100%); and liver in 8 (73%).

| TABLE 1 | Metastatic Tumor Reproduced in 4 Organs of Rats by Implantation of Glass Fragments* |
|---------------------|---------------------|---------------------|---------------------|---------------------|
|                     | Control (no glass) | Glass fragments implanted |                         |                         |
|                     |                    | Liver                | Kidney               | Mesentery             | Spleen               |
| Total No. of animals| 10                 | 10                   | 10                   | 10                    | 10                    |
| Pulmonary metastases| 10 (100%)          | 10 (100%)            | 10 (100%)            | 10 (100%)            | 10 (100%)            |
| Liver metastases    | 0                  | 7 (70%)              | 0                    | 0                     | 0                     |
| Kidney metastases   | 0                  | 0                    | 1 (10%)              | 0                     | 0                     |
| Mesentery           | 0                  | 0                    | 0                    | 1 (10%)              | 0                     |
| Spleen              | 0                  | 0                    | 0                    | 0                     | 0                     |

* In each animal, 100,000 Walker 256 carcinosarcoma cells were inoculated via the femoral vein.

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TABLE 2
INCIDENCE OF METASTASES IN LUNG AND LIVER IN CONTROLS AND IN RATS FED CARBON TETRACHLORIDE

<table>
<thead>
<tr>
<th></th>
<th>Controls (no CCl₄)</th>
<th>Fed CCl₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total No. of animals</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Pulmonary metastases</td>
<td>10 (100%)</td>
<td>11 (100%)</td>
</tr>
<tr>
<td>Liver metastases</td>
<td>0</td>
<td>8 (73%)</td>
</tr>
</tbody>
</table>

Three survived 9 weeks and at this time were sacrificed and autopsied. No tumor was found in either the lungs or the liver. Lung metastases were present in 93%, and liver metastases in none.

Test animals.—Twenty-five rats were fed the high fat diet for 3 months and then were inoculated with cancer cells. Five rats died in less than 2 weeks with only pulmonary metastases. Six died at 2 weeks. All had pulmonary metastases, and 1 had liver metastases. Five died at 4 weeks; all had lung metastases, and 1 had liver metastases. Six died at 5 weeks with only pulmonary metastases.

Controls (15 rats fed a normal diet).—Five animals died at 2 weeks, 6 at 3 weeks, and 3 at 4 weeks. All animals had pulmonary metastases. No tumor was seen in the liver of any of these animals. One animal survived 9 weeks. At autopsy no metastases were seen in either the lungs or the liver. Lung metastases were present in 93%, and liver metastases in none.

SECONDARY TRANSPLANT OF ORGAN HOMOGENATES (TABLE 4)

1. Table 4.—Lungs (10 rats): All 10 developed s.c. tumors at the site of inoculation within 10 days.

Liver (10 rats): Eight of these animals developed s.c. tumors at the site of implant, within 10 days. The remaining 2 were observed for 9 weeks and then sacrificed. At autopsy no tumor was found.

Heart (10 rats): The tumor grew s.c. in 3 animals (30%). The remaining 7 were sacrificed at 9 weeks and no tumor was found.

Kidney (10 rats): A solid tumor became apparent in about 2 weeks in 2 rats. The remaining 8 were kept for 9 weeks and then were sacrificed and autopsied. None had a tumor.

Blood (10 rats): The blood was withdrawn from the inferior vena cava 4 hr after inoculation of 100,000 cancer cells. One ml of this blood was inoculated s.c. into 10 rats, 1 of which developed a s.c. tumor at the site of injection after 2 weeks. The remaining 9 were kept for 9 weeks and then were sacrificed and autopsied. No tumor was found.

Spleen (10 rats): None of these animals developed a tumor by 9 weeks. At that time they were sacrificed; at autopsy no tumor was found.

Testicle (10 rats): None of these animals developed a s.c. tumor. All were sacrificed at 9 weeks. An autopsy showed no tumor in any animal.

HIGH FAT DIET (TABLE 3)

Controls (15 rats fed a normal diet).—Five animals died at 2 weeks, 6 at 3 weeks, and 3 at 4 weeks. All animals had pulmonary metastases. No tumor was seen in the liver of any of these animals. One animal survived 9 weeks. At autopsy no metastases were seen in either the lungs or the liver. Lung metastases were present in 93%, and liver metastases in none.

Test animals.—Twenty-five rats were fed the high fat diet for 3 months and then were inoculated with cancer cells. Five rats died in less than 2 weeks with only pulmonary metastases. Six died at 2 weeks. All had pulmonary metastases, and 1 had liver metastases. Five died at 4 weeks; all had lung metastases, and 1 had liver metastases. Six died at 5 weeks with only pulmonary metastases.

TABLE 3
INCIDENCE OF METASTASES IN LUNG AND LIVER OF CONTROLS AND RATS FED A HIGH FAT DIET

<table>
<thead>
<tr>
<th></th>
<th>Controls (15 rats)</th>
<th>Fed high fat diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total No. of animals</td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td>Pulmonary metastases</td>
<td>14 (93%)</td>
<td>22 (88%)</td>
</tr>
<tr>
<td>Liver metastases</td>
<td>0</td>
<td>2 (8%)</td>
</tr>
</tbody>
</table>

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Brain (10 rats): None of these animals developed a tumor after 9 weeks.

2. Table 5.—The lungs again showed 100% takes even with as little as 0.03 gm/ml. Kidney and liver were very similar, showing 80–90% takes with equivalent weights of the organ, i.e., 0.15 and 0.18 gm/ml, respectively. The brain and testicle now showed a few takes, 20% and 10% with 0.3 and 0.65 gm/ml, respectively. The spleen again failed to show any takes, even with the inoculation of larger doses of homogenate.

**TABLE 5**

<table>
<thead>
<tr>
<th>Organ</th>
<th>Suspension in saline</th>
<th>Volume injected into each rat</th>
<th>Weight of organ (gm/ml)</th>
<th>No. of animals injected</th>
<th>No. of takes</th>
<th>% of takes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lungs</td>
<td>1 lobe in 10 ml</td>
<td>1 ml</td>
<td>0.03</td>
<td>10</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Liver</td>
<td>1 lobe in 10 ml</td>
<td>1 ml</td>
<td>0.18</td>
<td>10</td>
<td>8</td>
<td>80</td>
</tr>
<tr>
<td>Kidney</td>
<td>1 kidney in 3 ml</td>
<td>1 ml</td>
<td>0.15</td>
<td>10</td>
<td>9</td>
<td>90</td>
</tr>
<tr>
<td>Heart</td>
<td>1 heart in 1 ml</td>
<td>1 ml</td>
<td>1.0</td>
<td>10</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Brain</td>
<td>1 brain in 1 ml</td>
<td>1 ml</td>
<td>0.3</td>
<td>10</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>Testicle</td>
<td>1 testicle in 1 ml</td>
<td>1 ml</td>
<td>0.65</td>
<td>10</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Spleen</td>
<td>1 spleen in 3 ml</td>
<td>1 ml</td>
<td>0.3</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The majority of tumor cells present in the systemic venous circulation lodge in the pulmonary capillaries or arterioles and, when in sufficient numbers, produce metastases. Under ordinary circumstances, metastases are not observed in other organs. However, tumor cells are known to pass through the lungs, since they are seen and counted in the aortic blood (7, 22). Furthermore, they are viable, as proved by secondary inoculation of the blood into weanling animals (7, 22). What, then, happen to these cells when they enter the arterial supply of other organs? They might pass through, because all the cells sticky enough or in clumps large enough to lodge, have already been filtered out in the lungs. They might pass through arteriovenous shunts, which are known to be numerous in the liver, spleen, lungs, and kidney and perhaps in other organs (26). They might lodge and be destroyed or become nonviable, or they might lie dormant for an indeterminate period (18). Organ specificity might be a factor (23), except that the liver has already been shown to develop Walker 256 carcinosarcoma when very few cells are injected into the portal vein (18).

The data reported in this paper indicate that cells do lodge in the liver, heart, and kidney, as shown by the incidence of takes from organ homogenates obtained 4 hr after inoculation. Tumor growth was not obtained from homogenates of brain, testicle, or spleen (Series A, Table 4), presumably because there were not enough cells present in these organs. However, the relative mass of these organs was less than that of the liver, heart, or kidney tissue.

The amount of organ/ml was shown to be a significant factor (Series B, Table 5). When the masses of kidney and liver were more nearly equivalent, the organs gave very similar growth rates. The testicle and brain were also shown to contain tumor cells by this second series, but the relative incidence of growth was much less than that from lung, liver, or kidney.

There is no positive evidence from these experiments that tumor cells lodge in the spleen, either by injection of homogenates or by trauma caused by glass fragments. Perhaps if we used still larger relative amounts of spleen homogenate, or very large numbers of animals with glass fragments, we might obtain growth.

The experiment with local trauma using glass fragments supports the evidence that cells do lodge in some organs, at least in the liver, kidney, and mesentery. The trauma may act in some way to localize fixation of the cells by increasing the circulation in the area. The inflammatory reaction also results in increased vascular permeability, which probably facilitates passage of tumor cells into the tissues. Fibrinogen and fibrin formation, which are also increased with trauma, could increase the fixation of cells in the vessels.

Carbon tetrachloride acts primarily on the liver. It causes local tissue destruction, which also induced increased tumor localization and growth, presumably owing to inflammatory reaction since these experiments were made after only 3 days of CCl₄ feeding—too soon for fibrosis to have developed.

A high fat diet is presumed to add local stress on the liver, which results in early fatty degeneration and eventually in cirrhosis. It has also been suggested that a high fat diet would increase the inhibitors of fibrinolysis and cause hypercoagulability (8, 20). Presumably this should increase the incidence of metastases in both liver and lungs (13). The experiment was positive (questionably) for the liver, which had an incidence of metastases of 8%. However the incidence of lung metastases (Table 3) was the lowest of any group in this series of experiments. Several factors must be taken into account to explain these findings. First, we have been unable to increase fibrinolysin inhibitors by chronic high fat feeding although a single high fat meal does result in significant change in fibrinolysin inhibitors and coagulability (8). Second, it is possible that a high fat diet might increase general resistance to tumor growth. This could explain the decrease in incidence of lung metastases; the rare liver metastases might be due to liver damage from the chronic high fat diet.

Stress has been considered to be an important factor in the development of metastases (6, 10). It has been presumed that stress has a general as well as a local effect (10). The experiments reported here, and our study with s.c. inoculation of turpentine (6), support the importance of local trauma and stress, but they give no support to the idea that there is a general effect on metastasis formation. There has been a marked increase in tumor growth in the local area of stress, but none in other areas. Actually, with the turpentine experiment, metastases elsewhere were fewer (6). Where general stress only was present, as with the experiments with anesthesia, the increase in metastases occurred only in the lungs (5).

Some investigators have shown that cortisone reduces the resistance of the host to cancer and increases tumor growth (31). Others implicated blood loss with decrease in resistance (19). Studies in our laboratory (5) and in Sweden (C. M. Rudenstam, personal communications) have shown that during surgical stress there is a consump-
tion of coagulation factors, i.e., of fibrinogen, prothrombin, and Factors VII and VIII. The consumption of these factors probably indicates that an intravascular coagulation process goes on during the first hours after trauma; such a process may explain the increase in metastases seen with trauma by many investigators (10, 18, 29). Subsequently, there is a rapid increase in platelets, fibrinogen, and inhibitors of fibrinolysis, which also may lead to increase in metastases (5). This hypothesis is supported by studies that report a decrease of bilateral leg and liver metastases in animals treated with fibrinolysin or heparin after local turpentine inoculation (6).

The failure of tumor development in the spleen under most experimental conditions warrants additional study. It is possible that reticuloendothelial cells may actually destroy tumor cells, or the circulation in the spleen may be such that tumor cells never lodge. The enlargement of the spleen seen in animals bearing malignant tumors may be related to destruction of circulating tumor cells.

From the results of these studies it is evident that the fate of circulating cancer cells cannot be predicted accurately. It is true that the majority lodge in the first organ they reach. In the case of systemic i.v. infusion, this is the lung, and with portal vein injection, it is the liver. However, many cells apparently pass through the first organ and enter the circulation of other organs, yet rarely seem to induce metastases. This may be due to greater dispersion of the cells, but it could also be due to differences in the vasculature or tissue susceptibility of the organs.

From our experiments, it seems possible to suggest that alterations in the clotting mechanism by local trauma or general reaction and vascular changes induced by local trauma may explain the many apparent eccentricities of tumor cells in producing metastases. This offers promise for controlling the spread of tumor by a relatively simple mechanism.

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Domenico Agostino and Eugene E. Cliffton

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