The Inflammatory Response to a Foreign Body within Transplantable Tumors

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SUMMARY

Several transplantable mouse and rat tumors were injured in a reproducible manner by implanting a length of cotton thread in their substance. Normal tissues, including liver, spleen, kidney, and subcutaneous connective tissue, were treated similarly. At the end of 1 week tissues containing thread were removed for histologic study. Consistently, the inflammatory response to the foreign body within the neoplasms was minimal as compared with the normal tissues. Although the tumors varied considerably in architecture and origin, differences in inflammatory response among the tumor types were not evident. A rapidly growing nonmalignant tissue, regenerating liver after partial hepatectomy, responded like the normal tissues after 7 days' exposure to the thread.

The response of one of the tumors, the mouse gastric Adenocarcinoma 328, was studied at various intervals, from 1 day to 23 days. Through this period the response within the tumor and that within subcutaneous connective tissue followed the usual sequence of inflammatory events but was always minimal in the tumor.

Inflammation is a response common to virtually all organs of the body when injured by a wide variety of means. This response seemingly lies in the stroma and, although mechanisms underlying the inflammatory reaction in normal tissues are not fully understood, the morphologic manifestations have been well described (7). Surprisingly enough, neoplastic tissues have not been studied with regard to their capacity for an inflammatory response. We therefore undertook to study inflammation in tumors by implanting an irritating foreign body, a cotton thread, in several transplantable tumors of mice and rats; normal tissues were investigated in the same way for comparison with the tumors. It was hoped that the response to the presence of a foreign body might also help to elucidate the relationship between parenchyma and stroma in neoplastic tissues.

MATERIALS AND METHODS

Several transplantable tumors of mouse and rat were used in the appropriate inbred strains, as listed in Table 1. The animals were mature adults; the mice weighed 20–30 gm. each, and the rats weighed 200–300 gm. each. The tumors were located subcutaneously.

Tumors and normal organs at 1 week.—A length of black cotton sewing thread (mercerized; average diameter 0.3 mm.) provided the inflammatory stimulus. Two threads were placed in each animal at the same time. One thread was inserted into the normal subcutaneous connective tissue, and the second one was put into the tumor or, for animals without tumor, into the liver, spleen, or kidney. The normal connective tissue site, common to all animals, served as a convenient site for comparison of normal inflammatory activity from animal to animal. Animals were operated on in groups (Table 1). For tumor-bearing animals the thread was implanted when the tumor reached a palpable size of about 1 cm.

For insertion of the thread into the subcutaneous connective tissue or into the tumor, two small skin incisions were made, and a needle with thread was pushed through the tissue. The thread was cut flush with the incisions at each end. The incisions were closed so that neither end of the cotton thread extended into the epidermis; such extension would have permitted epithelialization of the thread track. To place the thread in an abdominal organ,
a ventral incision through the abdominal wall into the peritoneal cavity was made, and a needle with thread passed through the organ.

Routine laboratory cleanliness was followed for most of the surgery; the cotton thread was not sterile. One group of animals, group IX, did have sterile lengths of thread, and for these animals sterile surgical procedures were adhered to.

At the end of 7 days, animals were killed, and the tissues containing thread excised and processed for histologic study. Microscopic sections were cut perpendicular to the long axis of the thread. The hematoxylin and eosin stain was used for all tissues. In addition, toluidine blue, Masson trichrome, and Wilder's silver reticulum stains were also used in most cases.

TABLE 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Tumor or other organs</th>
<th>Strain of animal</th>
<th>No. animals</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Gastric adenocarcinoma 328</td>
<td>C57BL/6 mouse</td>
<td>6</td>
<td>M</td>
</tr>
<tr>
<td>II</td>
<td>Mammary carcinoma H52454</td>
<td>BALB/CAN mouse</td>
<td>9</td>
<td>M</td>
</tr>
<tr>
<td>III</td>
<td>Mammary carcinoma 733</td>
<td>C57BL/6 mouse</td>
<td>6</td>
<td>M</td>
</tr>
<tr>
<td>IV</td>
<td>Mammary carcinoma CSHBA</td>
<td>C3H/Jax mouse</td>
<td>7</td>
<td>M</td>
</tr>
<tr>
<td>V</td>
<td>Uterine adenocarcinoma 94153</td>
<td>C3H mouse</td>
<td>5</td>
<td>F</td>
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<tr>
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<td>Cervical squamous carcinoma D98</td>
<td>C3H/Jax mouse</td>
<td>4</td>
<td>M</td>
</tr>
<tr>
<td>VII</td>
<td>Plasmacytoma 70429</td>
<td>C3H mouse</td>
<td>6</td>
<td>F</td>
</tr>
<tr>
<td>VIII</td>
<td>Osteogenic sarcoma 1904</td>
<td>C3H mouse</td>
<td>6</td>
<td>F</td>
</tr>
<tr>
<td>IX</td>
<td>Gastric adenocarcinoma 328</td>
<td>C57BL/6 mouse</td>
<td>9</td>
<td>M</td>
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<tr>
<td>X</td>
<td>Gastric carcinoma 342</td>
<td>Marshall 520 rat</td>
<td>5</td>
<td>M</td>
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<td>M</td>
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<tr>
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<td>Normal liver, kidney, or spleen</td>
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<td>13</td>
<td>M</td>
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<tr>
<td>XIII</td>
<td>Normal liver, kidney, or spleen</td>
<td>Holtzman rat</td>
<td>12</td>
<td>M</td>
</tr>
</tbody>
</table>

Regenerating liver.—To observe the inflammatory response in a rapidly growing non-neoplastic tissue, partial hepatectomies were performed on eleven rats before implantation of a cotton thread in the liver tissue. The series included both male and female rats; three were Holtzman strain, and eight were animals bred in our laboratory which were descended from the Marshall 520 strain. Through a ventral incision the three large anterior-most lobes of the liver were removed. In three animals a thread was placed in the remaining liver at the time of the hepatectomy; in the other eight the abdomen was re-entered from 40 hours to 7 days after the hepatectomy in order to insert the thread. A second thread was always placed in the subcutaneous connective tissue when the liver thread was inserted. Animals were killed after the threads had been in place for 7 days. Tissue sections were stained with hematoxylin and eosin.

Adenocarcinoma 328 at several stages.—A second line of investigation studied the history of the inflammatory response in a single tumor type. Groups of six or seven male C57BL mice with gastric Adenocarcinoma 328 were operated on in the manner described above. Groups were killed after 1, 2, 3, 4, 5, 6, 7, 14, and 23 days, respectively, and tissues were processed for histologic study. Hematoxylin and eosin, toluidine blue, Masson trichrome, and Wilder's silver stains were used.

OBSERVATIONS

First to be described will be the animals whose tissues were examined after 7 days. All tissues showed evidence of a chronic inflammatory reaction, but there was a consistent quantitative difference in the appearance of the response in the neoplastic tissues as contrasted with the non-neoplastic tissues. The microscopic criteria for evaluating the response were the density of chronic leukocytic infiltration of the area around the thread and of the thread itself, the presence of a granulation tissue with proliferating capillaries and fibrous tissue around the foreign body, and the presence of foreign body giant cells. By this standard the neoplastic tissues had a minimal response, whereas the normal tissues, whether subcutaneous connective tissue, liver, kidney, or spleen, all showed a similar and comparatively intense response. Figures 1 and 2 show thread in tumor, mammary Carcinoma H52454, and in subcutaneous connective tissue sites from the same mouse. Figure 3 shows the thread in normal liver of a rat.

The several neoplasms, although differing widely in tissue origin, cellular density, and stromal architecture, all manifested a minimal response at the end of 1 week which was similar among the tumor types. Rates of growth among the neoplasms differed in that week, consistent
with the natural history of the transplantable tumor. Gross inspection at autopsy, excluding the central nervous system, uncovered no metastases from the transplanted tumor in any of the animals. In some animals the tumor extended into or through the abdominal wall.

Within a given group, quantitative variations in response from animal to animal could sometimes be seen. Some animals had a comparatively limited chronic inflammatory reaction; nonetheless, in such an animal, the response at the normal site was substantially greater than in the neoplasm.

Group IX was made up of nine animals on which sterile surgical technics were used during the operative procedure. Both tumor and subcutaneous connective tissue sites responded as they had in the nonsterile situation.

Since the thread routinely extended beyond the limits of the tumor, the periphery, where the foreign body left the tumor and entered peri-tumor connective tissue, was of interest. Where the thread made partial contact with the connective tissue, an intense reaction was seen outside the tumor which diminished as the thread made contact with the tumor parenchyma. The intensity of reaction in peri-tumor connective tissue was usually of similar magnitude as that in control connective tissue on the opposite side of the animal; occasionally, the response in peri-tumor connective tissue was greater than that in the control tissue, though not markedly so. Where the thread was entirely within the substance of the tumor, even though only a few cell layers from the invading edge, the usual minimal neoplastic tissue response was seen. In some of the tumors there were broad connective tissue septa, continuous with the connective tissue surrounding the tumor. Where the thread lay in one of these areas there was an intense response.

The rat shows an earlier appearance of foreign body giant cells than does the mouse, and this difference was observed at both neoplastic and normal sites (Fig. 3). No other difference between species could be discerned.

In the rats which had undergone partial hepatectomy, liver regeneration occurred normally, and the animals remained active and alert. The response to the thread after 7 days' exposure (Fig. 4) was the same as in the other non-neoplastic tissues investigated earlier. The intensity of response in the regenerating liver was like that in the subcutaneous connective tissue in the same animal, and there was no difference from animal to animal, although the threads had been inserted at different times relative to the hepatectomy.

When it was observed that the inflammatory reaction in tumors examined at the end of 7 days was minimal, we proceeded to study a single tumor at various time intervals to learn whether this minimal response was present throughout the history of the inflammation, or whether it represented a decline following an earlier, intense reaction. The mouse gastric Adenocarcinoma 328 was used for this purpose.

At daily intervals from 24 hours (Figs. 5, 6) to 7 days we continued to find a minimal response within the tumor as compared with the normal subcutaneous connective tissue. During the period of a week the inflammatory picture changed from an acute response to a chronic response. The periods at which the difference between the neoplastic and normal sites was smallest were at 24 and 48 hours—i.e., during the acute phase. The studies at 14 and 23 days (Figs. 7, 8) showed the same kind of difference between the tumor and normal sites as was present at 7 days.

Most of the histologic information reported above was derived from a study of hematoxylin- and eosin-stained slides. Corroborative findings came from toluidine blue, Masson trichrome, and silver reticulum stains, although no new differences could be noted. Metachromatic staining with toluidine blue showed no marked difference in ground substance metachromasia around the thread in any of the sites; mast cells were present within the neoplasms in somewhat fewer numbers than in the non-neoplastic tissues. Collagen staining was most marked around the thread in normal sites, but the silver impregnation technic failed to show a reproducible difference.

DISCUSSION

We have observed a consistent difference between normal tissues and rodent transplantable tumors in their production of an inflammatory response to a foreign body. Using histologic criteria to evaluate the response, we found the difference to be a quantitative one. The reaction was intense in the normal tissues studied—the liver, spleen, kidney, and subcutaneous connective tissue; the reaction was reduced in the several neoplasms studied (Table 1). This was found to be true for all investigated tissues when they were in contact with the cotton thread for 7 days. It was also found to be true for one tumor and for the subcutaneous connective tissue at periods from 1 to 23 days.

In addition, although we looked for differences in inflammatory response among the several tumors after a week, we found none with the histologic technics used. The tumors included both carcinomas and sarcomas, ones that kill their hosts...
from the vicinity of the foreign body, there would be blood-borne elements of the inflammatory reaction, or alternatively might manufactures or concentrate some material that would inhibit the appearance or maintenance of an inflammatory reaction, or alternatively might lack a substrate or enzyme necessary to inflammation. We have no evidence to support or discredit these possibilities.

An area which might offer some insight into our findings is the blood supply to these tumors. If the blood-borne elements of the inflammatory reaction, or oxygen and metabolites necessary for its maintenance, could not be brought to and carried from the vicinity of the foreign body, there would be an effective inhibition of the reaction. Using vital dye uptake studies, Goldacre and Sylven (5) report a diminished blood supply except at the periphery of transplanted tumors after 1–2 weeks of growth. This may apply to our experiments, since tumors had been transplanted at least a week prior to operation. Day (3) offers evidence from several methods that, in the rat, transplantable tumors of the liver have an inefficient blood supply compared with autochthonous tumors of the same organ. However, in the mouse gastric tumor we found acute inflammatory cells to be present in moderate numbers around the thread at 24 and 48 hours. Many of the tumors also showed capillaries containing red blood cells in close proximity to the thread and elsewhere. Thus, although an inadequate blood supply may play a role in our findings, it is probably not a sufficient explanation.

Another possibility is that the potential for a sustained inflammatory reaction is present, but the invasive nature of the parenchymal cells of the tumor is such that the reaction cannot be maintained for more than a day or two. That inflammatory reaction provides a good area for tumor invasion is suggested by Podil’chak’s work with experimental metastases to inflamed sites. He was able to show a high incidence of metastatic tumor in the spleen (8) or stomach (9) of rabbits which received intravenous inoculations of Brown-Pearce carcinoma cells after having inflammation induced in the given organ by chemical injection. In his controls those two organs were never involved by the Brown-Pearce tumor. Fisher and Fisher (4) induced hepatic trauma in a number of ways and found that metastases of Walker Carcinosarcoma 256 were increased in the damaged liver. Clinically, tumor recurrence in incisional wounds (1) or biopsy tracks (2) is a common surgical problem. Not all experiments have indicated that an inflamed site increases metastases to that area. Kinsey (6), using the S-91 Cloudman Melanoma, found that the tumor did not metastasize to the traumatized thigh of mice or to organs transplanted to the thigh except in the case of the lung, the usual site of metastases.

Vasilev (10), in a review article, suggests that epithelial invasion, both malignant and nonmalignant, is preceded by the formation of an immature connective tissue bed. He cites several examples of proliferative invasion being accomplished in the presence of a young connective tissue. Among these are the invasion of mammary gland epithelium and placental villi during pregnancy, the proliferation of epithelium of skin and of other organs in response to inflammatory stimuli, and the invasive growth of transplanted tumor cells. The possibility of tumor cells showing preferential invasion of an inflammatory tissue needs further evaluation; if true, it could account in large part for our findings in these experiments. An inflammatory reaction around the thread might encourage a rapid invasion and replacement by tumor cells. Under these circumstances, morphologic evidence of the inflammatory reaction could be eradicated and the reaction go undetected unless the tissue were examined early. In agreement with such a concept is the finding that the difference in the extent of the inflammatory reaction at normal and neoplastic sites was less marked at 1 or 2 days than at 6 or 7 days after insertion of a thread into a transplant of mouse gastric Adenocarcinoma 328.

The reduction in inflammatory response might be a property of a rapidly growing tissue rather than specifically of a neoplastic tissue. The results in those animals with partial hepatectomy show that the regenerating liver does not lose its capacity to produce a chronic inflammation around a foreign body left in place for a week. We cannot at this time say, however, that there is no alteration in the first day or two post-hepatectomy when mitotic rate reaches a peak.

A question of great interest to us now concerns the extent to which primary cancers and their metastases resemble transplanted tumors in the response to an implanted cotton thread. Also of interest will be the response of benign tumors. We do not, at this time, have sufficient spontaneous tumors in our series to assay their reaction to a foreign body. Conclusions are thus necessarily limited. Nonetheless, our findings may prove fruitful in unraveling the roles of tumor and host stroma in the malignant process.
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REFERENCES


Photomicrographs of cotton thread in mouse or rat tissues after 7 days. All were stained with H. & E. and magnified X175.

Fig. 1.—Mouse mammary carcinoma H52454. Tumor cells are directly adjacent to the thread. A few scattered remnants of inflammatory cells are present within the thread.

Fig. 2.—Subcutaneous connective tissue of same mouse as in Fig. 1. Granulation tissue surrounds the thread, and large numbers of inflammatory cells are present among the fibers of the thread.

Fig. 3.—Normal rat liver. A chronic granulation tissue surrounds the thread, and inflammatory cells are numerous within the thread. Foreign body giant cells are prominent.

Fig. 4.—Regenerated liver of rat. Thread placed in remaining liver immediately after extensive partial hepatectomy and left there 7 days. Inflammatory response is intense as in normal liver of Fig. 3.
Photomicrographs of cotton thread in mouse tissues. All were stained with H. & E. and magnified X175.

Fig. 5.—Mouse gastric adenocarcinoma 328 after 24 hours. A collection of acute inflammatory cells has appeared at the periphery of the thread.

Fig. 6.—Subcutaneous connective tissue after 24 hours from the same mouse as in Fig. 5. A dense collection of acute inflammatory cells is present.

Fig. 7.—Adenocarcinoma 328 after 23 days. Almost no remnants of an inflammatory response remain. Tumor cells surround thread.

Fig. 8.—Subcutaneous connective tissue after 23 days from same mouse as in Fig. 7. Chronic inflammatory reaction continues at periphery of thread. At this time only a few cells remain within the thread.
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