The Vascular System of Two Transplantable Mouse Granulosa-Cell Tumors*

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Successful transplants of neoplastic tissue acquire a blood supply from the host vascular system; the process of this acquisition and the nature of the acquired vascular system are at best poorly understood. Coman and Sheldon (2) observed that the first visible effect of a transplanted tumor was an intense hyperemia in the region around the transplant; however, Molomut et al. (5) reported that a previously induced local inflammatory reaction was of no consequence in the number of successful transplants, latent period, or subsequent growth of tumors transplanted in the area of the reaction. Algire and Legallais (1) investigated and compared the vasculature of rapidly and slowly growing tumors; the former were said to be characterized by the presence of enlarged capillaries, appearing as sinusoids, with little tendency for differentiating into arterioles or venules, while the vascular bed of the slowly growing tumors was composed of smaller capillaries with a greater tendency toward differentiating into arterioles and venules. Williams (6) investigated the blood supply of transplanted neoplasms and concluded that neoplastic cells grow out from the original mass and surround the vessels in the adjacent tissue.

This investigation was undertaken to study the development, organization, and histology of the blood supply of two transplantable mouse granulosa-cell tumors.

MATERIALS AND METHODS

The transplantable neoplasms were two granulosa-cell tumors that arose from intrasplenic ovarian grafts in gonadectomized CHI mice. The tumors have been described previously (4) under the designation of ST 2 and ST 3. The former was a rapidly growing tumor that became palpable 4–6 days after transplantation; hosts to this tumor rarely survived more than 8 weeks. The latter did not become palpable until 2–3 weeks after transplantation and grew at a slow pace thereafter; hosts to this tumor survived 6–8 months.

Transplantation of these tumors was carried out on young adult hosts of the CHI strain. A small piece of tissue (approximately 2 cu. mm.) was cut out of the periphery of the donor tumor and placed in a trocar, which was then inserted into the skin over the last rib. The trocar was gently pushed forward and the plunger depressed. Tumors transplanted in this way were usually found at the level of the fourth or fifth rib in the lateral region of the thorax. From the fast-growing line tumors were taken at 24-hour intervals for 16 days after transplantation; an additional group of 30–40-day-old tumors were also taken from this line. From the more slowly growing line several tumors were taken which were selected on a basis of size rather than age.

For demonstrating the intact vascular bed of tumors, undiluted India ink or liquid latex was injected into the aorta. When the injection of the medium was completed the tumor and surrounding skin and body wall were excised, fixed in 10 per cent formalin, and cleared by the Spalteholz technic (8).

All the tissues taken for histological study were fixed in Bouin’s solution and prepared for paraffin sectioning by routine procedure. Sections were stained in hematoxylin, Gomori’s ammoniacal silver for reticulum, orcinol-New Fuchsin for elastin, and Heidenhains “Azan” for collagen.

OBSERVATIONS

The connective tissue between the cutaneous maximus and the thoracic musculature in the region of the transplant was supplied by two branches from the costocervical trunk of the subclavian artery. These branches extended nearly to the inguinal region and supplied the lateral thoracic and lateral abdominal body wall. The...
venous drainage of the region ran parallel with
the arterial branches along the abdominal and
thoracic wall; just before the venous branches
entered the axilla they joined into a common
trunk that drained into the external jugular vein.
It was from these vessels and their capillary
beds that the vascular system of these transplants
was derived.

The only vascular change observed in the
cleared specimens at 24, 48, and 60 hours post-
transplantation was in the capillary network sur-
rounding the transplant (Fig. 1). The capillaries
were dilated and appeared to be more numerous
than in other areas. Examination of sectioned
material at these times revealed infiltration with
polymorphonuclear leukocytes, fibrin deposition,
and areas of cellular debris. Small pieces of the
transplanted tumor could be found surrounded
by phagocytic cells, and in the 60-hour transplants
a thin rim of tumor cells, eight to ten cells thick,
was observed surrounding a central area composed
of eosinophilic, necrotic material. During this pe-
riod vascular enlargement became progressively
more marked in the vessels of the connective
tissue surrounding the transplant. At 72 hours,
examination of the cleared specimens showed that
the area of the transplant was represented by a
dense network of small vessels (Fig. 2). It appeared
at first that some of these vessels were located
within the tumor, but closer inspection revealed
them to be situated in the sheath of connective
tissue that surrounded the neoplasm. Sections
of a 72-hour transplant showed that the capillaries
of the connective tissue in the vicinity of the
transplant were enlarged and prominent (Fig. 4).
Cleared, ink-injected specimens of transplants 4,
5, 6, and 7 days old showed very little change
from the 72-hour transplant. Sections of trans-
plants 4 and 5 days old showed a rim of tumor
cells around a center of polymorphonuclear leu-
kocytes, fibrin, and cellular debris. Neighboring
vessels were enlarged, and some of the vessels
nearest the transplanted tumor were seen to have
neoplastic cells encircling them as a sheath one
or two cells thick. By 7 days the inflammatory
response was diminished, and a thin layer of
neoplastic cells surrounded a center of nearly
homogeneous, eosinophilic debris. At 8 days, there
was considerable increase in the vascularity of the
connective tissue surrounding the tumor; this in-
creased vascularity extended for some distance
around the tumor. Sections of transplants taken
at 8 days showed vessels in the periphery of the
tumor. At this time the tumor was composed of
a rim of neoplastic cells around a necrotic center;
within the rim of viable tumor cells was a rich,
vascular bed of stellate or oval vessels of naked
endothelium. At 10, 12, or 16 days injection and
clearing showed more completely vascularized tu-
mors, with the neoplastic mass outlined by large
vessels at the periphery (Figs. 3, 5).

In the older tumors the vasculature of the
parenchyma was composed of sinusoidal vessels.
The size of these vessels varied greatly; the small-
est were several times larger than the capillaries
of skeletal muscle of mice, while the largest ap-
proached the size of small veins. India ink injec-
tion revealed a marked tortuosity of the vessels,
with any given vessel winding over and under
its neighboring vessels and frequently doubling
back on its own course. The endothelial cells
appeared normal, and mitotic figures were infre-
dquent among them.

The endothelial cells of these sinusoids lay
directly against the parenchyma of the neoplasm
with few intervening connective tissue fibers (Fig.
6). The vessels just outside the neoplastic mass
which were in contact with the adjacent sub-
cutaneous connective tissue generally had a thin
sheath of collagenous and reticular fibers. When
a vessel was followed into the parenchyma of the
neoplasm the collagenous fibers were found to
completely disappear, and the reticular fibers
diminished rapidly as the vessel approached the
central core of the mass. Furthermore, sinusoids
which were completely devoid of fibers were fre-
quently encountered in the more central regions
of the mass. Arteries, arterioles, veins, and venules
were never encountered within the parenchyma
of these tumors.

The sinusoids of necrotic areas tended to have
a larger diameter than those located among ac-
tively growing tumor cells. It was interesting
to note that in the central necrotic portion of
a tumor occasional sinusoids could be found that
had no surrounding viable cells.

These tumor sinusoids possessed such a mor-
phological similarity to the sinusoids of the liver,
adrenal, and pituitary that it appeared feasible
to investigate whether the endothelium of the
tumor sinusoids was also capable of phagocytosis
of particulate matter.

Seven mice bearing tumor transplants for ap-
proximately 1 month were selected for injection
with particulate matter. Five of these were given
0.15 ml. of a 24-26 per cent solution of stabilized
colloidal thorium dioxide (Thorotrast) through
the tail vein. Three of the mice were sacrificed
1½ hours post-injection and two 20 hours post-
jection. The other two mice were given 0.15
ml. of raw India ink through the tail vein and
sacrificed 1½ and 20 hours later. Portions of the
liver, spleen, and tumors from each of the seven mice were fixed in Bouin’s solution. After paraffin embedding sections were made and stained with Heidenhain’s “Azan.”

The presence of large quantities of particulate matter in the phagocytic endothelial cells of the sinusoids of the liver and spleen of all the mice indicated that the injections were successfully performed, but no particulate matter was found in the endothelial cells of the tumor sinusoids. A few macrophages containing the particulate matter were observed in the parenchyma of the tumors; these macrophages were usually found in the peripheral, actively growing tumor tissue rather than near the necrotic central portion.

It was noted at the onset of the investigation that several of the main vessels in the region of a transplant were always greatly enlarged. Close examination of these revealed that the enlarged vessels were veins that were draining the neoplasm; the arteries supplying the neoplasm seemed to be of normal size. To study this in more detail, three mice bearing 68-day-old transplants and four tumor-free mice of the same age were selected. The mice were sacrificed, and the area of skin over the tumor was shaved. An incision was made and the skin laid back to expose the vessels in question. A 1-inch segment of the vessels was ligated, and this isolated segment was removed with the skin. The tissue obtained was fixed in Bouin’s solution, and sections from it were stained with hematoxylin-eosin, ammoniacal silver for reticulum, resorcin-fuchsin for elastin, and Heidenhain’s “Azan.”

A comparison was made between the vessels of normal and tumor-bearing mice. The connective tissue fibers and smooth muscle of the walls of these vessels and their endothelium were studied in detail. The walls of the vessels supplying the neoplasms were morphologically similar to the walls of their counterparts in the subcutaneous connective tissue of control mice. The one striking difference was that the diameter of the vein draining the neoplasm was 3 times the size of those in the control mice; but the diameter of the artery remained unchanged.

There were no differences noted between the blood supply of the two lines of ovarian neoplasms.

**DISCUSSION**

It appeared that the capillary bed of the connective tissue surrounding these neoplasms was being stimulated toward proliferation. This resulted in a dense bed of vessels available to the neoplasm which had only to include them in its parenchyma when it invaded that connective tissue.

This observation indicated that the tumor, in some unknown fashion, was inducing a proliferation of capillaries. It leads one to speculate that a substance from the tumor was diffusing into the surrounding connective tissue to produce an effect upon its vasculature. This diffusibility is suggested by the localization of this response; if the tumor were actively secreting a substance into the blood stream a systemic response would be expected.

The vascular system of these tumors was of a peculiar type. The vessels were sinusoidal and had very little connective tissue associated with them. The morphology of these sinusoids was similar to that of the sinusoids of the liver and certain endocrine glands, but, unlike these glands, the tumor sinusoids are not phagocytic. There was no tendency toward the differentiation into arterioles and venules that would have been expected in a tissue undergoing vascularization. These large tumor sinusoids were usually distorted by the surrounding connective tissue, and the circulation of blood through the tumor was visualized as being rather sluggish.

Since there were no arterioles or venules within the parenchyma of these neoplasms, the tumor mass was a body of growing tissue with only a capillary circulation. This observation makes the invariant central necrosis of these tumors understandable: as the neoplasm increases in volume the blood reaching the central cells is depleted of the nutrient material necessary for survival because these materials have been extracted by the prolonged contact of the blood with the more peripheral cells.

**Fig. 1.**—A cleared, India ink-injected specimen showing the area of a 24-hour-old transplant. The neoplasm in the darker area outlined by arrows. Approximately X10.

**Fig. 2.**—A cleared, injected specimen 75 hours after transplantation. The transplant is the dark area in the center. Approximately X10.

**Fig. 3.**—A cleared, injected transplant 15 days old. The vessels supplying the neoplasm are enlarged. Note the density of the capillary beds in the whole field. Approximately X10.

**Fig. 4.**—A section of a strip of the body wall containing a transplant 96 hours old. The transplant is the long, dark mass at the arrow. There is no vascularization of the mass. Note the enlarged vessel at the left of the field. X75.

**Fig. 5.**—A section through a transplant 10 days old. The vessels were perfused with India ink and show in black. On the left-hand border of the photo is the developing capsule and vessels can be seen invading the neoplasm from there. X700.

**Fig. 6.**—A section from a 30-day-old transplant. The sinusoid in the center of the field is typical of the vessels found in these neoplasms. X700.
The transplant is the dark area in the center. A darker area outlined by arrows. Approximately X 10.

vessels supplying the neoplasm are enlarged. Note the density remained unchanged.

the area of a ~4-hour-old transplant. The neoplasm in the control mice; but the diameter of the artery was 3 times the size of those in the connective tissue of control mice. The one striking difference was that the diameter of the vein drain-ting the neoplasm was 3 times the size of those in detail. The walls of the vessels supplying the neoplasm were morphologically similar to the tissue fibers and smooth muscle of the walls of Hain's "Azan."

These large tumor sinusoids were usually distorted with hematoxylin-eosin, ammoniacal silver for re-motumors; these macrophages were usually found with the skin. The tissue obtained was fixed in embedding sections were made and stained with Bouin's solution, and sections from it were stained was ligated, and this isolated segment was removed of skin over the tumor was shaved. An incision was made and the skin laid back to expose the vessels in question. A 1-inch segment of the vessels selected. The mice were sacrificed, and the area and four tumor-free mice of the same age were detail, three mice bearing 68-day-old transplants neoplasm; the arteries supplying the neoplasm seemed to be of normal size. To study this in more close examination of these revealed that the en-these vessels and their endothelium were studied in the peripheral, actively growing tumor tissue ing the neoplasm was 3 times the size of those in the parenchyma of the neoplasm which had only to include them in matter were observed in the parenchyma of the tumor sinusoids. X 700.

A few macrophages containing the particulate matter in the phagocytic endothelial cells of the sinusoids of the liver and spleen of all the mice performed, but no particulate matter was found indicated that the injections were successfully suggested by the localization of this response; some unknown fashion, was inducing a prolifera-tion of capillaries. It leads one to speculate that diffusibility is the presence of large quantities of particulate matter in the phagocytic endothelial cells of the tumor sinusoids. X 700.

A comparison was made between the vessels in these neoplasms. X 700.

ume the blood reaching the central cells is depleted because these materials have been extracted by the prolonged contact of the blood with the more nutrient material necessary for survival as being rather sluggish. The morphology of these sinusoids was similar to that of the sinusoids of the liver and spleen from each of the seven tumors; these macrophages were usually found in these neoplasms. X 700.

FIG. 1.--A cleared, India ink-injected specimen showing the invariable central necrosis of these tumors; these macrophages were usually found in these neoplasms. X 700.

By the surrounding neoplastic cells, and the cir-culation of blood through the tumor was visualized as being rather sluggish. The morphology of these sinusoids was similar to that of the sinusoids of the liver and spleen from each of the seven tumors; these macrophages were usually found in these neoplasms. X 700.

A few macrophages containing the particulate matter in the phagocytic endothelial cells of the tumor sinusoids. X 700.

FIG. 2.--A section of a strip of the body wall containing a transplant 96 hours old. The transplant is the long, dark mass at the left-hand border of the photo is the developing capsule and arrow. There is no vascularization of the mass. Note the can be seen invading the neoplasm from there. X700.

FIG. 3.--A cleared, injected transplant 15 days old.

FIG. 4.--A section from a 30-day-old transplant. The left-hand border of the photo is the developing capsule and arrow. There is no vascularization of the mass. Note the can be seen invading the neoplasm from there. X700.

FIG. 5.--A cleared, injected specimen 75 hours after trans-

FIG. 6.--A section through a transplant 10 days old. The...
This type of a circulation also suggests an explanation for the restriction of mitotic activity to the tumor periphery. Neoplastic cells can survive anaerobically but require aerobic conditions for cell division; thus, the sinusoids of the periphery contain sufficient oxygen for cell division, but as they pass centrally the oxygen level probably falls below that necessary for cell division.

The circulatory pattern of neoplasms appears to be a fertile field for investigation. There is no small possibility that more knowledge in this sphere could be applied therapeutically, since checking this acquisition of a blood supply might restrict tumor growth.

SUMMARY

The morphology of the vascular system of two transplantable granulosa-cell tumors of mice was studied throughout the life of the tumor. This was studied by means of histological sections and India ink-injected, cleared, whole mounts.

Immediately after subcutaneous transplantation of the neoplasm the capillary bed surrounding the tumor became engorged. During the first 11 days this capillary bed appeared to become more dense. By 11 days the tumor itself became vascularized.

The vessels of the tumor parenchyma were always sinusoids. These sinusoids were endothelial sheaths with relatively small amounts of connective tissue associated with them.

ACKNOWLEDGMENTS

The authors are grateful to Doctor William Dossel, Mrs. Barbara Roennau, and Mrs. Geraldine Head for their help in the preparation of this manuscript.

REFERENCES

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