Hemodynamic Characteristics in Microcirculatory Blood Channels during Early Tumor Growth

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INTRODUCTION

The growth of malignant tumors in animals and humans is associated with neovascularization and development of microcirculatory blood vessels from preexisting tissue interspaces. It appears that vascularization permits an implant of tumor material to grow exponentially, while the factors that determined avascularization cause an implanted tissue piece to remain dormant at a very small size. A great number of microvascular phenomena occur particularly at the advancing edge of malignant tumors. It has been shown by several investigators that, as tumor transplants begin to grow, the appearance of many blood vessels in tumors passes through different stages (5, 8, 19, 20). These stages are: (a) development of capillary sprouts from the preexisting capillary bed and venular blood vessels; (b) growth of capillary sprouts; and (c) completion of the terminal vascular bed in the tumor which is accomplished by cross-connection between these sprouts.

ABSTRACT

Red blood cell (RBC) velocity was determined in the micrrocirculation of the BA 1112 rat sarcoma and in regions located outside the tumor (repairing tissue), which were used as a control and were assumed to be representative for the inflammatory-reparative reaction of a healing wound. Studies were carried out in modified Algire chambers modified according to the technique of Reinhold (13). Briefly, this design permits an implanted piece of tumor tissue to grow in a sheet-like fashion. The tumor portion grew in such a “sandwich” chamber in 30 days to a size of approximately 4 sq mm, its thickness being limited to about 110 to 150 μm. These “sandwich” tumors were sufficiently translucent to allow the analysis of the circulation in virtually all microcirculatory blood vessels. Observations and measurements were started 14 days after implantation of a 0.1-cu mm tissue piece of a BA 1112 sarcoma. The tumor used in this study is isologous in WaG/Rij rats and arose in December 1962 in the musculature of the jaw of a rat radiation chimera.

A given preparation was followed up by means of intravital observations recorded photographically and by video tape for a period of 18 days at 1-day intervals. The experiments were terminated when tissue growth in the control areas obscured the vascular architecture to the point that measurements were no longer possible. This event roughly coincides with the obliterations of the vascular supply by the tumor.

Measurements. A photographic overview of the entire chamber was made every day. At 1-day intervals selected areas in the chamber were photographed at the beginning of each experiment utilizing an AO3 long-working-distance

MATERIALS AND METHODS

Biological Preparations. The experiments were performed on female Wistar rats of an inbred strain from Rijswijk, The Netherlands (WaG/Rij) fitted with Algire “window” (2, 3) chambers modified according to the technique of Reinhold (13). Briefly, this design permits an implanted piece of tumor tissue to grow in a sheet-like fashion. The tumor portion grew in such a “sandwich” chamber in 30 days to a size of approximately 4 sq mm, its thickness being limited to about 110 to 150 μm. These “sandwich” tumors were sufficiently translucent to allow the analysis of the circulation in virtually all microcirculatory blood vessels. Observations and measurements were started 14 days after implantation of a 0.1-cu mm tissue piece of a BA 1112 sarcoma. The tumor used in this study is isologous in WaG/Rij rats and arose in December 1962 in the musculature of the jaw of a rat radiation chimera.

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objective (UM 10X/0.22) to yield a final magnification of approximately ×100 on high-contrast photopaper (variable contrast; Kodak, Los Angeles, Calif.). The mosaic was assembled in such a fashion that measurements of blood cell velocity, capillary length, and diameter in single microscopic channels could be related to the tumor architecture.

Data were obtained from the selected regions of the growing tumor, evidenced by the change in coloration and size of the area of implantation (see Fig. 1), and from selected regions of the healthy tissue in the chamber far from the tumor which were taken to be representative of granulation or repairing tissue.

The microscopic blood vessels under observation were categorized following, in general, the nomenclature of Rhodin (15, 16); accordingly, the term “terminal arteriole” was used for 2 types of vessels: (a) those having “a single layer of smooth muscle cells and a diameter of 30 to 50 µm”; and (b) those vessels “which begin to distribute capillary side branches.” This includes in our study microscopic blood vessels with a diameter of down to 17 µm. The term “capillary” was used to designate the network of endothelial tubes devoid of smooth muscle that connects the smallest feeding blood vessels and postcapillary venules.

In order to obtain RBC velocity measurements, different areas of the preparation were transilluminated by a tungsten-halogens projector lamp (GTE Sylvania Inc., Winchester, Ky.) and observed microscopically under an AO Microstar focusing unit with a ×10 objective with a ×20 eyepiece (AO). The image of the microcirculatory bed was televised (Model 2810 video camera; Cohu Inc., San Diego, Calif.) and recorded on a video tape recorder (Sony AV 3600; Sony Co., Long Island, N.Y.). The final magnification on the monitor (VM 502 Shibaden, Woodside, N.Y.) was about ×350.

The velocity measurements in the microcirculation of tumors, as well as in control preparations, were carried out by the dual-window video method. This technique consists of obtaining video recordings of RBC flow and in measuring the transit time of erythrocytes between an upstream and downstream location in a given vessel by on-line cross-correlation described previously (11, 12). The velocity data are reported in terms of the mean value over a period of 15 sec.

Video recordings needed for quantitative flow measurements as well as high-resolution photographs used for capillary density determinations were obtained under “slight” pentobarbital anesthesia (Nembutal, 20 mg/kg i.m.; Abbott Laboratories, North Chicago, Ill.). The required level of anesthesia was established on the basis of the degree of immobilization needed for proper functioning of our flow-measuring instrumentation, as well as the requirement of no motion for the high-resolution photography. Systematic comparisons between microscopic observations carried out with no anesthesia and observations at the level of anesthesia needed for our velocity measurements showed no significant differences other than those that are normally attributed to motion.

The “overview” photographs of the entire chamber were made without anesthesia every day by means of an electronic flash.

Control of Temperature. During any given experimental period, rats were housed in single cages under laboratory conditions (23–26°). For the purpose of direct observation, as well as video and photographic recordings, the animal was made to crawl into a transparent plastic tube of approximately the same length and diameter as the rat in its crouched position. The head end of the tube was conical and perforated. The tail end consisted of a cylindrical stop which slid inside the body tube with a central hole for temperature probe and tail and which could be secured gently but firmly against the hindquarters of the animal. The body tube had a slot which ran lengthwise and which allowed the skin flap and the chamber to be outside the tube while providing support which was sufficiently rigid for the placement onto the microscope stage. Special care was taken to avoid any artificial constraint of the preparation and to maintain the animal at a constant temperature.

The body temperature, measured rectally with a small-animal temperature probe (VWR Scientific Div., San Diego, Calif.) was kept constant by means of a heating blanket which was draped around the plastic tube (average ± S.D., 36.2 ± 0.3°). The skin temperature, measured near the preparation by means of a hypodermic thermometer probe (VWR Scientific Div.), remained constant during each experimental procedure (average ± S.D., 29.2 ± 1.3).

RESULTS

Photography and video recordings were carried out through the window in the chamber when the microvascularization was “established” and the sarcoma was growing (Fig. 1). After implantation of the tumor piece, there were no vessels in the neighborhood of the neoplastic cells. However, 5 to 10 days later, the tumor becomes increasingly vascularized, new vessels appear, and the implanted tissue piece grows rapidly thereafter.

It was noted consistently that tumor tissue presents a specialized microscopic vessel configuration in the form of relatively large vessels that are predominantly postcapillary venules which run around the edge of the implanted tumor and leave and reenter the graft. These vessels also communicate with vessels of the repairing tissue and lie at the edge or just outside of the tumor area. The tumor tissue portion was supplied through 2 to 5 arteriolar vessels (diameter range, 17 to 31 µm at the edge of the tumor). The capillaries originating from these blood channels emptied into 3 to 8 collecting venules (diameter range, 30 to 45 µm) which were irregularly dilated and tortuous, particularly at the edge of the tumor. The abundance of postcapillary and collecting venules was also consistently observed as a typical feature in areas of repairing tissue located far from the growing tumor (Fig. 2). In this tissue, comparatively straight and long coursing, narrow capillaries originating from individual terminal arterioles emptied into bulging postcapillary and collecting venules.

Inside the borderline of the tumor granulation tissue, there are short loops of narrow capillaries lying in arcs of small radius. As the tumor advances through these capillary loops, some of the vessels are obliterated, and a small fraction grows into the tumor in the form of long and narrow capillary blood vessels which originate at the tumor...
edge and straighten during the period of observation (Chart 1).

Charts 2 and 3 summarize our flow velocity measurements during the observation period in repairing and tumor tissue, respectively. Blood velocity in terminal arterioles inside the tumor increases during the observation period from an average of 0.7 mm/sec to 0.95 mm/sec. Velocity in venous capillaries and in postcapillary and collecting venules of the BA 1112 sarcoma tends to decrease with progressive tumor growth.

The direction of blood flow in the tumor area in nearly all of the visible vessels was away from the center of the tumor, although in some of the smaller blood channels the flow was either intermittent or regurgitant. Periods of stasis or stasis followed by resumption of blood flow, sometimes in a direction just opposite to the previous one, have also been found to be typical characteristics of the entire terminal vascular bed of the BA 1112 rat sarcoma. In addition, we observed that, with an increase in tumor diameter, a considerable portion of blood was crossing arteriovenous shunts instead of circulating through the microcirculatory network. Besides random variations in RBC velocity observed in individual capillaries, an overall ebb and flow caused by vasoemotion was observed throughout the tumor microcirculation during the initial phase of growth.

The periodicity of this phenomenon was in the order of 8 to 15 sec (Chart 4). Vasoemotion seemed to disappear after approximately 22 days in "tumorous" arteriolar blood vessels, although it was still observed in 15% of the terminal arterioles located in areas void of tumor tissue.

**DISCUSSION**

Endothelial cells in the normal microvasculature have a very low turnover rate and rarely undergo mitosis (1, 4, 7, 17, 18). Pathological stimuli such as wound healing, inflam-
Chart 4. Original tracing of RBC velocity in a 19-μm arteriole supplying the microcirculation of the BA 1112 sarcoma. Periodic changes in flow in the order of 8 to 12 sec are related to vasomotor activity. Arrows, measurement; 16 days after implantation, respiration rate is 42/min, 22 days after implantation, respiration rate is 36/min.

...ation, and tumor growth enhance endothelial replication and induce the formation of new microvascular blood channels. It has been demonstrated by Ausprunk and Folkman (4) that capillaries, proliferating in response to an implanted tumor, develop and elongate by a very similar mechanism as wound-induced blood vessels, namely, mitosis of endothelial cell accompanied by migration of these cells toward the angiogenic stimulus.

Our findings demonstrate that the significant anatomical difference between the microcirculation of "normal" tissue, such as striated muscle, omentum, and mesentery (22), and that of tissues which exhibit permanent, enhanced cellular replication, such as tumor and repairing tissue, is the presence of a significant increase in the number of venous capillaries and the presence of enlarged venular vessels. Our microvascular RBC velocity measurements in postcapillaries and venules in the tumor tissue were found to be lower than was that of the repairing tissue. On the other hand, the RBC velocity in arteriolar blood vessels is slightly increasing in the sarcoma during the observation period. These factors are indicative of the increase in numbers of "short" capillaries and small venular outflow vessels primarily at the edge of the tumor and corroborate our anatomical observation.

The overall difference in hemodynamics between growing tumors and repairing tissue appears to be the greater tissue perfusion at the advancing edge of the tumor, and this corroborates with the presumed greater metabolic demands of this tissue (6). Furthermore, since arterial vessels appear to be preexistent and do not proliferate, the blood supply to malignant tissue is limited to a considerable extent.

The preexistence of supplying arterial vessels should be indicative of the presence of vasomotion that is related to metabolic demands and activity of malignant tissue. In agreement with earlier studies on single-cell oxygenation and flow velocity (10, 14), we demonstrated again the presence of a periodic component of blood flow during early tumor growth. However, flow velocity in supplying arterial blood vessels did not show vasomotion later than 22 days after tumor implantation. Arteriolar blood vessels, which as a consequence of tumor advance through repairing tissue end inside the tumor, occasionally show vasomotion for a brief period after the annexation of an arteriolar blood vessel by the malignant tissue complex.

It is likely that this behavior of flow velocity and blood vessels is related to the enhanced metabolic and nutritional demand during tumor growth, rather than myogenic mechanisms. Nevertheless, the possibility of myogenic regulation exists and cannot be discounted.

In conclusion, the flow velocity patterns in the capillaries and collecting venules of the BA 1112 sarcoma and in healing wounds are very similar. Contrary to healing wounds, blood velocity in arteriolar microvessels of the tumor increases during early growth but decreases slightly in venous capillaries and venular blood vessels as a result of the development of a low-pressure drainage system in the microcirculation of mesenchymal tumors. Moreover, blood vessels in malignant tumors seem to have the ability to regulate blood flow only during very early tumor growth or possibly only at the margin of growing tumors.

It was found that, as a consequence of the rapid changes occurring in the microvasculature, it was not possible to document changes of diameter reliably by studying a single arteriolar vessel during the development of the tumor. However, the observed cessation of vasomotion in vessels inside the tumor, coupled with the increase of arteriolar RBC velocity and decrease of venular flow velocity, is indicative of a permanent maximal dilation of feeding arteriolar blood channels during continuous sarcoma growth.
One of the significant differences during the development of minute blood vessels in healing wounds and mesenchymal tumors is the appearance of short capillary loops at the edge of the tumor and the existence and extension of long capillaries in the center part of the malignant tissue portion, which is expressed through the wide distribution in histograms of capillary length in the BA 1112 sarcoma.

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REFERENCES

Fig. 1. BA 1112 sarcoma during early tumor growth. The tumor is the light, approximately circular area, with black microspheres in its center, which were placed there at the time of implantation to aid in locating the tumor during the early stages of growth; A, 15 days after implantation; B, 18 days after implantation. Chamber diameter, 0.9 cm. × 12.
Fig. 2. Abundance of venous blood channels in the microcirculation of repairing tissue. 1, 2, and 3, arteriolar blood vessels. × 55.
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