Angiogenesis, Microvascular Architecture, Microhemodynamics, and Interstitial Fluid Pressure during Early Growth of Human Adenocarcinoma LS174T in SCID Mice

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ABSTRACT

To date, most quantitative information on tumor angiogenesis, microcirculation, and transport has been derived from rodent tumors grown in transparent chamber preparations. In this paper we present a chamber technique adapted to immunodeficient mice for the study of human tumor xenografts. Microcirculatory parameters in severe combined immunodeficient mice bearing a dorsal skin fold chamber preparation were quantified using intravital microscopy and image analysis. The take rate of the human colon adenocarcinoma LS174T in the chamber preparation was 100%, and the tumor area doubling time was 6.5 days. Three days following implantation of 2 × 10⁶ tumor cells onto the striated skin muscle, capillary sprouts were noted in the tumor cell mass. Microvascular in the tumors was established after 10 days. Capillary density, vessel diameter, red blood cell velocity, and blood flow rates in individual microvessels measured on days 10, 14, 18, and 22 showed no statistical difference in the striated muscle (capillaries) and subcutaneous tissue (arterioles and venules) of the skin of tumor-free animals (N = 6), whereas these parameters increased slightly, but not significantly, in the LS174T tumors (N = 7). Mean interstitial fluid pressure (±SD) in these small tumors was 4.6 ± 1.7 mmHg (N = 4) on day 10 and 5.1 ± 0.9 mmHg (N = 4) on day 22 and significantly elevated compared to that in the subcutaneous and skin tissue (0.9 ± 0.8 mmHg) (N = 4) (P < 0.001). To our knowledge, this is the first model enabling intravital microscopic studies of human tumor xenografts in a transparent chamber preparation in severe combined immunodeficient mice. Studies on angiogenesis, microcirculation, and transport using such a preparation should provide new insights into microcirculation-mediated mechanisms for cancer treatment.

INTRODUCTION

Microcirculatory and transport parameters play an important role in conventional as well as novel methods of cancer treatment such as radiation therapy, chemotherapy, immunotherapy, hyperthermia and photodynamic therapy (1–3). For example, the therapeutic efficiency of monoclonal antibodies, cytokines, lymphokine-activated killer cells, tumor-infiltrating lymphocytes, and other products of genetic engineering is currently limited by their ability to reach their target cells in vivo in adequate quantities. Since these and other agents are delivered to malignant tissue by the bloodstream, it is useful to analyze the microcirculation and the transport parameters of tumors to gain insight into the potential for tumor localization and the tumoricidal effect of these agents.

To date, most of the quantitative studies on microcirculation and transport parameters in tumors have been performed utilizing chamber preparations bearing rodent and not human tumors (for a review, see Refs. 1–3). However, rodent models are limited for intravital studies since they do not allow the investigation of various genetically engineered therapeutic agents developed against human cancer cell lines.

In recent years, nude mice have been used extensively for the transplantation and propagation of human tumor xenografts (4). To further increase the take rate of human tumors, additional immunodeficiencies have been induced in mice. A potentially ideal mouse for heterotransplantation of human tumors, the severe combined immunodeficient mouse, was first described by Bosma et al. (5). These mice, homozygous for the SCID mutation, lack both a functional immune system and mechanisms for DNA repair. The absence of B- and T-lymphocytes is due to a defect in the site-specific recombination pathway that is responsible for the somatic assembly of immunoglobulin and T-cell receptor genes (6). While SCID mice have been used in recent years to study human tumor xenografts (for a review, see Ref. 7), there have been no intravital microscopic studies to date in these mice.

To establish an experimental model for studying angiogenesis, microcirculation, and transport in human tumor xenografts, we have recently adapted a dorsal skin fold chamber preparation to SCID mice. Mice were then used as hosts for the growth of the human colon adenocarcinoma LS174T in this chamber preparation. We describe here the technical procedure for the chamber preparation, the take rate of tumors, the tumor growth kinetics, the microcirculatory parameters, and the interstitial fluid pressure in tumor-free and in tumor-bearing chambers.

MATERIALS AND METHODS

Animal and Tumor Model. The experiments were performed in SCID mice, bred and maintained in a specific pathogen germ-free environment in our laboratory. For the surgical procedure, mice (males, 25–30 g body weight) were anesthetized (7.5 mg ketamine hydrochloride and 2.5 mg xylazine/100 mg body weight, s.c.). Prior to chamber implantation, the entire back of the animals was shaved and depilated, and two symmetrical titanium frames (weight, 3.2 g), which are mirror images of each other (workshop, Department of Experimental Surgery, University of Heidelberg, Germany), were implanted so as to sandwich the extended double layer of skin. One layer of the skin was removed in a circular area of approximately 15 mm in diameter, and the remaining layer, consisting of epidermis, subcutaneous tissue, and the striated skin muscle, was covered with a cover slip incorporated into one of the frames (for additional technical details, see Ref. 8). All surgical procedures were performed under aseptic conditions in a specific germ-free environment.

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4 The abbreviations used are: SCID, severe combined immunodeficiency; FITC, fluorescein isothiocyanate; IFP, interstitial fluid pressure; CEA, carcinoembryonic antigen.


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environment. During surgery the body temperature of the animals was kept constant at 36–37°C by means of a heating pad. Following implantation of the transparent access chamber, animals were allowed to recover for 24 h from microsurgery and anesthesia. Preparations fulfilling the criteria of intact microcirculation (9) were utilized as sites for tumor cell implantation.

For implantation of human adenocarcinoma LS174T cells (ATCC CL 188; American Type Culture Collection, Rockville, MD), the coverslip of the chamber was removed, and 2 μl of a dense tumor cell suspension from the cell culture (2 × 10^6 cells, viability 95–97%, passage 126) were inoculated onto the upper tissue layer of the chamber preparation (striated skin muscle as assessed by histology), and the access chamber was closed again by the coverslip. The small volume of tumor cells was added so as to avoid disseminated growth of the tumors in the whole chamber. LS174T cells are tumorigenic in nude mice and produce large amounts of CEA. The in vitro doubling time of the LS174T tumor cells in Dulbecco’s modified Eagle’s medium was 22.5 h, and the plating efficiency utilizing feeder cells (lethally irradiated cells) was 59.6%.

For blood pressure measurements, a fine indwelling poly ethylene catheter (PE-10; inner diameter, 0.28 mm) was implanted into the right carotid artery 24 h prior to the measurements. This catheter was passed s.c. to the dorsal side of the neck and was sutured to the titanium frame. For mean arterial blood pressure measurements, catheters were filled with physiological saline and connected to a pressure transducer (model P23Gb; Gould, Inc., Cleveland, OH). Amplified pressure signals were sent to a chart recorder (model 595; Omega Engineering, Stamford, CT).

Due to the chamber implantation, the animals lost approximately 15% of their initial body weight within the first 48 h. The body weight remained stable thereafter. The animals tolerated the dorsal skin fold chambers well and showed no signs of discomfort over a period of 23 days. In particular, no modifications of the sleeping and feeding habits were observed. During the experiments the animals were housed one animal per cage with free access to water and standard laboratory chow.

Measurements. Starting at 24 h after the chamber implantation, the entire area of the tumor-free (N = 6) and tumor-bearing chambers (N = 7) was monitored on a daily basis for up to 22 days (Fig. 1). Mice were positioned in a polycarbonate tube of approximately the same diameter (25 mm) as the animal in its crouched position. The tube provides rigid support for holding the chamber on a polycarbonate stage, placed under a microscope. Daily observations were performed employing a ×1.25 objective (×1.25, NA 0.035, Plan Neofluor; Zeiss, Oberkochen, Germany) and a microscope (Axioplan, Zeiss) with a 37-fold magnification on the screen. For these observations, a transilluminator technique (12 V, 100 W halogen lamp, Zeiss) and a green filter for enhancing black/white photomicrography and a conversion filter for converting artificial light of 3200 K into daylight of 5500 K were used. Observations (intensified CCD camera, C2400-88; Hamamatsu Photonics K.K., Hamamatsu, Japan) were recorded on a video cassette recorder (AG-6500; Panasonic, Secaucus, NJ) at a rate of 60 frames/s. Tumor size and growth kinetics were analyzed off-line during playback of the videotapes using a computer-assisted image processing system (Sun Workstation, 3/260; Sun Microsystems, Mountain View, CA; MVP-VME, video digitizer; Matrox International Corp., Québec, Canada).

Intravital microscopic measurements were performed in 7 tumor-bearing and 6 tumor-free chambers in anesthetized SCID mice, on days 10, 14, 18, and 22. To obtain microcirculatory parameters, chosen areas (2–10 locations/animal) of the tumor-free and tumor-bearing chambers were investigated using a ×20 long working distance objective (×20, NA 0.4; LD Achroplan). The primary criterion for selecting these areas in the chambers was high optical quality without edema or hemorrhage. The final magnification on the television screen (PVM-1342Q, Trinitron; Sony, Tokyo, Japan) was about 570-fold.

For contrast enhancement of the intravascular space of small blood vessels, a bolus of 0.1 ml of fluorescein isothiocyanate-labeled dextran (FITC-dextran, M, 150,000; 5 mg/100 μl of 0.9% NaCl; Sigma Chemical Co., St. Louis, MO) was injected into the tail vein of the mice 5 min prior to the recordings. Epillumination was achieved by a 100-W mercury lamp (model 770; Opti-Quip, Inc., Highland Hills, NY) using an excitation filter (485–505 nm), a dichroic mirror (510 nm), and a barrier filter (530 nm). Observations of selected areas were limited to a maximum of 60 s. The whole procedure for each animal lasted 30 min at most (Fig. 2).

For microhemodynamic analysis, microvessels of the preformed tissue in tumor-free chamber preparations were classified into 6 categories. Midcapillaries were utilized as reference, and the venous and the arterial side were divided into two or three categories, respectively (10). Proximal to the capillary network, vessels near capillary diameter were
considered as precapillaries. For vessels feeding these precapillaries, the term "terminal arterioles" was used. On the venous side, a distinction was made between postcapillaries (wide, confluent capillaries), larger collecting venules, and small veins.

Blood vessels within the tumors were not categorized into different subclasses, since the classification of blood vessels developed for normal tissue based on structure (anatomy) and function (physiology) may not be applicable to tumors (3).

All microscopic observations were recorded on videotape and analyzed off-line as follows. Vessel diameters were measured using an image shearing monitor (digital video image shearing monitor, model 908; IPM, San Diego, CA). RBC velocity ($V_{\text{centerline}}$) measurements in the tumor microcirculation, as well as in striated muscle and the subcutaneous tissue of tumor-free animals, were performed by means of a modified four-slit technique (microwave system, model 208C; video photometer version, IPM). Video recordings of blood flow were obtained from randomly selected areas of the microcirculation. The transit times of RBC between upstream and downstream locations in a given vessel were measured by an off-line cross-correlation method (11).

Mean blood flow rates of individual vessels ($Q$) were calculated using the vessel diameter ($d$) and the mean RBC velocity ($V_{\text{mean}}$):

$$Q = d^2 \times V_{\text{mean}} \times \frac{\pi}{4}$$  
(A)

$V_{\text{mean}}$ was estimated from the empirical relationship

$$V_{\text{mean}} = \frac{V_{\text{centerline}}}{\alpha}$$  
(B)

($\alpha = 1.3$, for blood vessels < 10 $\mu$m; linear extrapolation $1.3 < \alpha < 1.6$ for blood vessels between 10 and 15 $\mu$m; and $\alpha = 1.6$ for blood vessels >15 $\mu$m) (12).

Capillary density in the striated skin muscle (direct host tissue for the tumors) and tumor vessel density, defined as the total length of capillaries or tumor vessel per observation area, were analyzed using an image processing system (Sun Workstation, 3/260, Sun Microsystems; MVP-VME, video digitizer, Matrox International Corp.) and are given in cm/cm$^2$.

During the whole experimental procedure, the body temperature of the animals was maintained constant at 36–37°C by warming the holding tube with an electric heating blanket. Between the experiments, animals were kept in single cages with free access to water and standard laboratory chow.

Pilot studies demonstrated that exact measurements of microcirculatory parameters could not be obtained in unanesthetized SCID mice, because of movement artifacts. For quantitative flow measurements, anesthesia (7.5 mg ketamine hydrochloride and 2.5 mg xylazine/100 mg body weight, s.c.) was needed. To evaluate the impact of this level of anesthesia on macrohemodynamic parameters, the mean arterial pressure and the heart beat rate were measured in unanesthetized and anesthetized SCID mice ($N = 6$). Anesthesia with ketamine/xylazine elicited an approximately 20% drop in the mean arterial blood pressure from 104 $\pm$ 13 to 81 $\pm$ 8 mmHg and an approximately 35% drop in the heart beat rate from 370 $\pm$ 35 to 235 $\pm$ 19 beats/min ($P < 0.001$) (mean $\pm$ SD).

In an additional group of animals, the interstitial fluid pressures of the LS174T tumors in the chamber preparation were measured on day 10 ($N = 4$) and 22 ($N = 4$) with micropipettes and a servo-nulling device (model 5; IPM) (for technical details, see Ref. 13). In brief, the animals were anesthetized, and the coverslip of the chambers was removed. During IFP determinations, the chamber area was superfused with warm (32°C) isotonic saline. In 4 tumor-free chambers, IFP in subcutaneous and skin tissue was also measured. For each animal, 3–5 IFP measurements were performed in tumor or subcutaneous tissue, and values were averaged. Following each experiment, the chambers were again sealed with the coverslip.

After the final experiment on day 22, animals were sacrificed and tissue specimens of tumor-free and tumor-bearing chambers were processed for histological studies, including hematoxylin-eosin staining and immunohistochemistry using peroxidase-labeled antibodies against CEA.

Data are given as single values or frequency distribution (%) and mean values $\pm$ SD in the figures and as mean values $\pm$ SD in the tables or text. Mean values of microcirculatory data (6 tumor-free and 7 tumor-bearing chambers) and interstitial fluid pressure measurements (tumor: day 10, 4 animals; day 22, 4 animals; subcutaneous tissue: 4 animals) represent the average of all animals ($N$) calculated from the average values in each animal ($n$). Nonparametric one-way analysis of variance and multiple comparison on ranks of several independent samples were done using the Kruskal-Wallis test. Single comparisons of independent samples were performed using the Wilcoxon test. $P$ values smaller than 5% were considered significant.

RESULTS

Infectious complications due to either mechanical trauma or bacterial contamination, seen microscopically as hyperemia, distortion of venular segments, or edema, occurred in only 15% of the immunodeficient mice in pilot studies.

The take rate of the human adenocarcinoma LS174T in the chamber preparation bearing SCID mice ($N = 7$) was 100%. Within the first 2–3 days after tumor cell implantation, no neovascularization could be visualized within the implanted tumor cell mass. Three days after implantation, the tumor cell mass in the chambers appeared macroscopically as a pink spot, and the first typical thin-walled, sometimes dilated, blood vessels could be visualized; however, due to the dense tumor cell mass overlaying these blood vessels (presumably originating from capillaries of the striated skin muscle), their quality for intravital microscopy was poor. By approximately day 6, a large number of newly formed capillary sprouts surrounded by RBC and blind-ending blood vessels appeared, disseminated at various locations in nearly all tumors. At this stage the blood flow in the newly formed vessels appeared to be stagnant; however, tumors started to grow (Fig. 3). During the subsequent 4 days these blind-ending tubes anastomosed to other newly formed blood vessels, and the static column of RBC began to flow.

Approximately 10 days after implantation of the tumor cells, the optical quality was satisfactory for intravital microscopy. The tumor growth rate ($N = 7$) was linear, and the tumor area doubling time was approximately 6.5 days. Intravital microscopy was performed in the striated muscle and subcutaneous skin tissue of tumor-free mice and in tumors of tumor-bearing
mice on days 10, 14, 18, and 22, to quantify RBC velocity, vessel diameter, and capillary (striated muscle) or tumor vessel density.

RBC velocities and blood flow rates in vessels of the various subclasses in tumor-free mice (N = 6) are given in Figs. 4 and 5. At all observation times, RBC velocities, vessel diameters, and blood flow rates of individual microvessels all decreased from terminal arterioles to the precapillaries. Values were lowest in capillaries and postcapillaries and then increased in the collecting venules and larger veins. No significant differences in RBC velocities, vessel diameter, and blood flow rate in the tumor-free control chambers were found between the chosen observation days (Table 1). In the striated skin muscle, simultaneous changes of RBC velocity in all capillaries which were fed from one arteriolar vessel were frequently observed (95–99%). In addition, random variations were observed in individual vessels. Vasomotion in arteriolar blood vessels, causing this flow motion, was observed less frequently in the preparations.

Since the tumor vasculature could not be categorized in the same manner as normal tissue vessels (for a review, see Ref. 3), RBC velocities in the vasculature of the LS174T adenocarcinoma are not subdivided. Heterogeneity in terms of blood perfusion was more pronounced in tumors compared to that in tumor-free skin tissue. Mean RBC velocity increased slightly but not significantly in the LS174T tumors from day 10 (0.14 ± 0.06 mm/s) to day 22 (0.21 ± 0.09 mm/s). In Fig. 6, only the RBC velocity of tumor vessels showing blood perfusion is given (see below). Similar to the RBC velocity, the mean blood flow rate of individual microvessels increased with tumor growth in the surface vessels from day 10 (16.9 ± 9.5 x 10^-6 cm/s) to day 22 (28.8 ± 10^-6 cm/s) (Fig. 7).

In vascular subclasses of the striated muscle and subcutaneous tissue of the skin, vessel diameter remained constant through all observation days (Table 1), whereas mean vessel diameter in the tumor vessels increased slightly from day 10 (12.2 ± 1.0 μm) to day 22 (17.1 ± 1.9 μm), and the frequency distribution of vessel diameter shifted to larger vessel diameters (Fig. 8). The fraction of small blood vessels (<10 μm) in the LS174T tumors decreased from day 10 (42%) to day 22 (22%) continuously.

Capillary density in the striated muscle was approximately 300 cm/cm² and stable in the tumor-free chamber preparations during the observation period. In the adenocarcinoma, vessel density was significantly lower than the capillary density of the striated skin muscle (P < 0.001). Tumor vessel density increased slightly from day 10 (167 ± 59 cm/cm²) to day 14 (206 ± 24 cm/cm²) and remained stable thereafter. Nearly all parallel running capillaries (97%) of the striated skin muscle that were stained by FITC-dextran were perfused with RBC, whereas only 50% of the tumor vessels on day 10 and approximately 80% on the following observation days (when vessel density was determined) showed RBC flow during the observations, lasting for 1 min (Table 2). Synchronous changes in blood flow, as observed in the striated skin muscle capillaries, were never observed in tumor vessels. Random variations of RBC velocity and blood flow were seen only in individual tumor vessels.

In a separate group of animals, the interstitial fluid pressure of the LS174T adenocarcinoma was measured on day 10 (N = 4) and 22 (N = 4) in the chamber preparation. The removal of the coverslip to perform IFP measurements induced hemorrhage in most tumors (3 of 4) on day 10; however, this procedure was necessary for the exact placement of the micropipettes within the tumors. Measurements in these tumors were done after the hemorrhage had subsided. In contrast, none of the
investigated tumors showed bleeding on day 22. By day 10, IFP was elevated in these tumors (4.6 ± 1.7 mmHg) and was further slightly increased on day 22 (5.1 ± 0.9 mmHg). On both days tumor IFP was significantly higher than in the subcutaneous and skin tissue (N = 4) of the skin fold chambers (−0.9 ± 0.8 mmHg) (P < 0.001). To evaluate the effect of the removal of the coverslip on tumor IFP, interstitial fluid pressure was measured from the back side of the chamber through the skin prior to the removal of the coverslip in one case. Measurements from both sides revealed identical values for tumor IFP.

The histological investigation of the tumors revealed a moderately differentiated adenocarcinoma growing on the striated skin muscle of the mice. Tumors were well circumscribed and showed a large number of glandular formations consistent with the original colon adenocarcinoma. No signs of infiltrative tumor growth into the striated skin muscle and subcutaneous skin tissue were seen, and the LS174T adenocarcinoma seemed to be separated from the host skin tissue by a thin avascular layer of connective tissue. No evidence of tumor necrosis was observed on day 22. Immunohistochemistry revealed that the LS174T adenocarcinoma was positive for the CEA.

DISCUSSION

Following the pioneering work of Algire and Charkley (14), several investigators have analyzed microvascular morphology and microhemodynamic parameters in rodent tumors grown in access chamber preparations (15-25). A limited number of investigators have studied the transport of molecules and cells in these preparations (26-30). Most antitumor agents developed by genetic engineering and other biotechnologies are against human cancer cells and not rodent cells. Therefore, a model enabling studies of microcirculatory and transport parameters in human tumors is required.

Athymic nude mice have been shown to propagate various human tumors after inoculation of cultured cell lines derived from human tumors. However, less than one-third of these tumor lines produce tumor sizes of 1 cm² within 1 month (4). Since dorsal skin fold chamber preparations have a finite lifetime (4-6 weeks), after implantation, due to tilting of the chambers resulting from loss of elasticity of the tissue and/or skin necrosis at those areas where the chambers were sutured to the skin, our investigations were limited to tumor lines growing within that time period. Therefore, we chose SCID mice as host animals for this study because of their enhanced immunodeficiency compared to nude mice, and hence the potential increase in tumor take rate as well as a potential of a decrease in tumor growth time.


Table 1 Mean (± SD) vessel diameter, RBC velocity ($v_{centerline}$), and blood flow rate in successive microvascular segments of the striated skin muscle and the subcutaneous tissue of SCID mice (N = 6)

<table>
<thead>
<tr>
<th>Day</th>
<th>Arterial side</th>
<th>Capillaries</th>
<th>Venous side</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>terminal arterioles</td>
<td>Pre-</td>
<td>Mid-</td>
</tr>
<tr>
<td></td>
<td>Diameter (μm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 10</td>
<td>10.4 ± 1.5</td>
<td>6.5 ± 1.2</td>
<td>5.9 ± 0.9</td>
</tr>
<tr>
<td>Day 14</td>
<td>9.1 ± 2.3</td>
<td>6.9 ± 1.4</td>
<td>6.3 ± 1.1</td>
</tr>
<tr>
<td>Day 18</td>
<td>10.3 ± 2.1</td>
<td>6.7 ± 1.4</td>
<td>6.9 ± 0.7</td>
</tr>
<tr>
<td>Day 22</td>
<td>10.7 ± 1.1</td>
<td>7.1 ± 0.2</td>
<td>6.3 ± 1.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Day</th>
<th>Velocity (mm/s)</th>
<th>Blood flow rate (10^-6 μl/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 10</td>
<td>0.59 ± 0.12</td>
<td>38.9 ± 17.7</td>
</tr>
<tr>
<td>Day 14</td>
<td>0.64 ± 0.27</td>
<td>31.1 ± 16.7</td>
</tr>
<tr>
<td>Day 18</td>
<td>0.63 ± 0.24</td>
<td>39.2 ± 18.3</td>
</tr>
<tr>
<td>Day 22</td>
<td>0.70 ± 0.00</td>
<td>46.1 ± 7.8</td>
</tr>
</tbody>
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Fig. 6. Single measurements of RBC velocities ($v_{centerline}$) in tumor vessels of the human adenocarcinoma LS174T. Arterial side, capillaries; arrowheads, average RBC velocity ($v_{centerline}$) (v = average $v_{centerline}$, N = 7).
The microhemodynamic data obtained in the tumor-free SCID mice in this study correspond well with observations in a similar microcirculatory model in anesthetized rats (20). Intravital microscopic studies in unanesthetized hamsters (8, 22) and nude mice showed slightly higher RBC velocities in microvascular segments of the striated skin muscle and subcutaneous tissue compared to our study.

Unlike previous studies using skin fold chamber preparations in hamsters (8, 22) and nude mice, we were not successful in training the SCID mice to remain still in the polycarbonate tubes during the microscopic investigations. Therefore, it was necessary to anesthetize the animals. The side effects of anesthesia on macro- and microhemodynamics, tumor blood flow, and spontaneous vasomotion were accepted to obtain recordings without movement artifacts. When intravital microscopy in unanesthetized animals was performed, the speed and the direction of flow in the blood vessels was unstable and changed with animal movement or agitated respiration, as observed by others (16). Furthermore, hypertensive stress reactions may influence the actual blood flow in the observation chambers of conscious animals. All of these artifacts in unanesthetized animals are random and beyond our control. Therefore, we chose a combination of ketamine and xylazine for anesthesia. Separate pilot studies showed that this anesthesia compared to pentobarbital (70 mg/kg body weight) induced a less pronounced reduction in mean arterial blood pressure and heart beat rate in these mice within the first 30 min of anesthesia.

The repetitive injections of Mr 150,000 FITC-dextran required for contrast enhancement of small blood vessels were well tolerated by the SCID mice, and no leakage of these macromolecules was observed in the tumor-free tissue, whereas tumor vessels were highly leaky to Mr 150,000 FITC-dextran, as observed previously in a rabbit tumor (27).

The observation time for each area (at most 10 different locations/animal) was limited to 60 s, to minimize phototoxic effects of fluorescein isothiocyanate on microvascular pressure (32). We did not find any evidence of changes in microcirculatory parameters induced by FITC and light; measurements of RBC velocities and vessel diameter did not reveal a systematic reduction in blood flow rate during observations limited to 60 s.

An important finding of this study was that the microcirculatory parameters in the tumor-free tissue of SCID mice were stable up to 22 days and that blood vessels of the striated skin muscle and subcutaneous skin tissue showed physiological characteristics such as vasomotion and flow motion. Therefore, the chamber preparation in SCID mice seems to be an ideal model for chronic studies of the microcirculation of preformed skin tissue, human tumor xenografts, or heterotransplant of nonmalignant tissue.

For our studies on tumor microcirculation, tumor cells of the human adenocarcinoma LS174T were implanted 24 h...
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following chamber implantation. In pilot studies we also grew two additional human tumors (squamous cell carcinoma FaDu, neurofibrosarcoma HSTS26G; their take rate was also 100%) in the chamber preparation. For this study we focused on the LS174T adenocarcinoma, because it expresses CEA antigen. Since more than 70% of human carcinomas express CEA antigen, this tumor would enable us to carry out, in the future, transport studies with monoclonal antibodies against this antigen.

The formation of blood vessels in the LS174T tumor in the chamber preparation occurred in sequential steps, consistent with those described by Folkman (33). After 3 days, capillaries of the host tissue underlying the tumor mass enlarged as reported by Gullino and Grantham (34). While the tumors were nearly avascular, the tumor area did not increase significantly. After the tumor vasculature was established, the tumors began to grow. Compared to most rodent tumors implanted in chamber preparations (14, 16, 20–22), the growth of the human adenocarcinoma was slow. Asaishi et al. (22) reported that 5 days after implantation of the A-Mel-3 amelanotic melanoma, large, avascular, necrotic areas appeared in those tumors. In the human adenocarcinoma LS174T, we did not observe necrotic areas in the tumor within the first 22 days following cell implantation by intravital microscopy and histology.

Vogel (35) quantified the vascular morphology of a mammary adenocarcinoma and found a marked widening of vessel diameter with growth, accompanied by a marked drop in vessel length. The ratio of sinusoidal tumor vessels to capillary tumor vessels increased with tumor growth. Other investigators (15, 16, 36) also reported an increase of vessel diameter in tumors with time. Using photomicroscopy, Endrich et al. (21) and Asaishi et al. (22) measured the frequency distribution of vessel diameter as a function of time in the BA 1112 carcinoma in Wistar rats and in the A-Mel-3 amelanotic melanoma in hamsters, respectively. Both studies showed a slight increase in the diameter of tumor vessels with time. In nearly all of these studies, changes in vascular diameters were not statistically significant, presumably due to the heterogeneity of the tumor growth pattern. In this study we also found a slight but not significant increase in the mean tumor vessel diameter with tumor growth and a shift of vessel diameter to a larger size. Therefore, the findings in the human adenocarcinoma are in good accordance with previous findings in rodent tumors.

The LS174T tumors were spheroidal in shape and did not infiltrate striated skin muscle and the subcutaneous tissue of the SCID mice. Since in this chamber preparation only the tumor surface and not the center of the malignant tissue was accessible for intravital microscopy, we did not divide the tumor into different “zones” as done by Endrich et al. (20, 21), who utilized a “sandwich” chamber preparation and intravital microscopy. We would categorize all surface vessels of the LS174T tumor as the zone of stabilized tumor microcirculation, using their classification scheme. In that zone, these authors reported that tumor tissue perfusion remained constant from day 14 to day 24. In our experiments the surface vessels of the LS174T tumors showed a slight but not significant increase in the blood flow rate during the observation period.

Microrcirculatory parameters in the adenocarcinoma were more heterogeneous compared to the tumor-free tissue, and this heterogeneity increased with time. The number of blood vessels with RBC flow changed with time. On day 10, only 50% of blood vessels were perfused with RBC, whereas nearly all blood vessels were perfused by FITC-dextran, a marker for plasma flow. This number increased on the following days to 80%. The increase of RBC perfused vessels in our study is presumably related to the fact that the vasculature of the LS174T tumors on day 10 is still immature, and therefore a large number of vessels show static RBC columns without flow. In the following days more and more vessels are connected to other tumor vessels and perfused with RBC. This phenomenon is also seen for nonmalignant tissue like pancreatic islets in a chamber preparation in hamsters (37). Trotter et al. (38) reported an overall staining mismatch in tumors of about 7%, indicating intermittent perfusion. However, these mismatch data should be evaluated carefully in light of nonuniform dye delivery, also called streaming phenomenon (39). In the LS174T tumors we observed frequently that blood coming from different small veins does not mix in larger collecting vessels; the interface could be observed as far as 200 µm downstream from the point of confluence.

In addition to microhemodynamic parameters, the interstitial fluid pressure in tumors for this type of preparation was measured for the first time. No significant differences in tumor IFP were observed between days 10 and 22. As observed in several other studies (for a review, see Ref. 2), IFP was significantly higher in tumors compared to tumor-free skin tissue. However, this is the first time that IFP has been measured in such a small tumor mass. A possible explanation for the increase in IFP from nearly 0 to 4–5 mmHg is the enhanced permeability and hydraulic conductivity of tumor blood vessels, resulting in a reduced oncotic contribution (13, 40). Therefore, it seems likely that factors enhancing the vascular permeability and the hydraulic conductivity of tumor vessels are produced and secreted by tumors at this early stage (41).

In this study we defined microrcirculatory baseline parameters of a human tumor xenograft in a chamber preparation, which will form the basis for further studies. However, this experimental model also has some limitations. The tumor investigated in this preparation is a transplanted tumor at an early stage of growth. The LS174T carcinoma does not infiltrate the host tissue and may respond to various treatment modalities differently compared to spontaneous tumors (42). Interstitial fluid pressures as high as 45 mmHg (43), which are seen in large tumors in patients and which influence the transport of various therapeutic agents, cannot be expected in such a model. Furthermore, only vessels of the tumor surface can be observed in this chamber preparation. While the hematopoietic system of these mice can be partly replaced by that of the human (7), the SCID mouse model can not substitute for studies with spontaneous human tumors in situ.

In summary, the dorsal skin fold chamber preparation in SCID mice is a stable model for studying microcirculation in immunodeficient animals. Although Falkvoll et al. (44) already described the successful implantation of a human melanoma into a granulation tissue preparation in nude mice, to our knowledge this is the first quantitative microrcirculatory study of a human tumor xenograft growing in preformed skin tissue. We have characterized the microrcirculatory baseline parameters and demonstrated that the microrcirculation of the human adenocarcinoma LS174T xenograft, similar to that of rodent tumors, does not conform to the standard vascular organization. A further important and novel finding was that the interstitial fluid pressure is already elevated in very small tumors. Therefore, we consider this to be an ideal model, enabling studies on microcirculation, angiogenesis, and transport in human tumor xenografts. Besides studies on tumor microcirculation, this chamber preparation permits implantation of

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