ABSTRACT

Blood flow in six human melanoma xenograft lines grown s.c. in BALB/c-nu/nu mice was studied and analyzed in relation to tumor growth characteristics. Two different methods were used to measure blood flow, i.e., uptake of $^{86}$Rb and clearance of $^{133}$Xe. The percentage of the injected $^{86}$Rb taken up per g of tumor tissue and the $^{133}$Xe clearance rate were used as parameters for blood flow. The results achieved with these two methods were consistent.

Blood flow differed significantly among individual tumors of the same line, even for tumors of similar size. All lines showed a decrease in blood flow with increasing tumor volume. This was due to an increase in necrotic fraction as well as a decrease in blood supply per viable tumor cell. Blood flow also differed significantly among the xenograft lines. All lines showed a lower blood flow than the kidney, spleen, liver, and foot. The blood flow was generally lower in the xenograft lines than in the EMT6 and Lewis lung murine tumor lines. There was no correlation between tumor blood flow and volumetric growth rate.

The xenograft lines could be divided into two distinct groups of three lines each with respect to blood supply per viable tumor cell. The three lines showing a high blood supply also showed a high fraction of cells in S phase (23-31%), whereas the three lines showing a low blood supply had a low fraction of S-phase cells (11-13%). Thus, blood supply per viable tumor cell was probably decisive for the cell proliferation activity in the tumors. Moreover, necrotic fraction increased with increasing tumor volume, and the magnitude of this increase was largest for the three lines showing the lowest blood supply per viable tumor cell. These observations were possibly consequences of basic differences in vascular architecture between the two groups of xenograft lines.

INTRODUCTION

The vascular system of most tumors is poorly developed. The architecture of the capillary network is irregular and coarse, causing great resistance to blood flow (1-3). Blood flow can differ considerably among different tumors, even among tumors of the same histological type (1, 4), but tumors have generally lower blood flow than most normal tissues (2, 5). The blood flow distribution within tumors is often highly heterogeneous (6), and temporary variations in blood flow within tumors may also exist (7). Tumor blood flow is important for the response to treatment as well as the growth and progression of cancer.

Tumor response to radiation therapy, chemotherapy, hyperthermia, and photodynamic therapy depends partly on physiological conditions in the tumor that are mainly determined by blood flow, i.e., oxygen concentration, nutrient supply, pH distribution, and bioenergetic status (8-11). The vasculature serves as a delivery system for chemotherapeutic agents and radiolabeled monoclonal antibodies (12) and as a cooling system that may prevent high temperatures from developing during hyperthermic treatment (13). Moreover, delayed cell death caused by damage to the vasculature is assumed to be a major component of the therapeutic effects of hyperthermia and photodynamic therapy (14, 15).

The cell proliferation and volumetric growth of tumors have also been demonstrated to depend on blood flow. Burton (16) and Summers (17) showed that the commonly observed decrease in tumor growth rate with increasing volume might be due to vascular insufficiency in large tumors. Studies of tumor cell proliferation kinetics have indicated that the growth fraction decreases with increasing distance from blood vessels (18) and that the cell cycle time is shorter for tumor cells adjacent to blood vessels than for cells adjacent to necrosis (19). Relationships between growth parameters and vascular structure across tumor lines were studied by Rofstad (20) using human melanoma xenografts. He found that tumor volume-doubling time and cell loss factor tended to decrease while the fraction of cells in S phase and growth fraction tended to increase with increasing vascular density. Moreover, tumor vascularization may be important for tumor progression; acute hypoxia caused by intermittent blood flow can induce drug resistance and increase the metastatic potential of tumor cells (21).

Six new human melanoma xenograft lines that are metastatic in athymic mice have been established in our laboratory and characterized with respect to tumor growth rate, distribution of cells in the cell cycle, necrotic fraction, histological appearance, chromosome number, and DNA index (22). The growth characteristics were found to differ considerably among the lines. Several important biological features of the donor patients' melanomas were retained during the heterotransplantation. The lines thus represent a valuable experimental model system for human melanomas and will be used to study response to therapy as well as tumor growth and progression. Blood flow characteristics of the xenograft lines are reported in the present communication. The purpose of the work was: (a) to search for possible differences in blood flow among individual tumors of the same size and line; (b) to search for possible differences in blood flow among different tumor lines; and (c) to study relationships between blood flow and tumor growth characteristics. Considerable problems and limitations are associated with the methods used to measure tumor blood flow (23). Two established methods based on totally different principles were therefore used in the present study, i.e., uptake of $^{86}$Rb and clearance of $^{133}$Xe. The validity and consistency of these two methods in our model system were also investigated.

MATERIALS AND METHODS

Mice and Tumor Lines. Male BALB/c-nu/nu mice, 8-10 weeks old, were used. They were bred at the animal department of our institute and kept under specific-pathogen-free conditions at constant temperature (24-26°C) and humidity (30-50%). Sterilized food and tap water were given ad libitum.

The melanoma xenograft lines (BEX-t, COX-t, HUX-t, ROX-t, SAX-t, WIX-t) were established in athymic mice from metastases of patients admitted to The Norwegian Radium Hospital (22). The lines were maintained in the same strain of mice by serial s.c. transplantation of tumor fragments, approximately 2 × 2 × 2 mm. Subcutaneous flank tumors in passages 15-25 were used in the present work. The lines were given ad libitum.
were kinetically stable during the period while the experiments were carried out, as ascertained by flow cytometric measurements of DNA histograms and measurements of volumetric growth rates. Tumor volume was calculated as

\[ V = \pi a b^2 \]  

(A)

where \( a \) and \( b \) are the longer and shorter of two perpendicular diameters, respectively. The diameters were measured with calipers. Tumor volume-doubling time was determined by Gompertzian analysis of volumetric growth data, necrotic fraction by stereological analysis of histological sections, and fraction of cells in \( S \) phase by flow cytometry (22). Cell density was determined histologically using stereological techniques, as described by Brammer and Jung (24). The growth characteristics of the lines have been reported elsewhere (22). Growth parameters of relevance for the present study are summarized in Table 1.

Two well-characterized established murine tumor lines, EMT6 and Lewis lung, were chosen as standards of reference. Tumors of these lines were grown s.c. in the flanks of athymic mice in the same way as the human melanoma xenografts.

\( ^{86}\text{Rb} \) Uptake. When the freely diffusible and water-soluble radioactive ion \(^{86}\text{Rb}^+\) is injected i.v. into animals, the ion distributes in the tissue in proportion to tissue blood flow as a fraction of cardiac output. In experimental tumors and most normal tissues in mice, the concentration of the tracer reaches a pseudostable state within approximately 20 s after the injection (25), and this pseudostable state persists for several minutes (26). The validity of the \(^{86}\text{Rb} \) uptake method for measurement of blood flow in normal tissues and tumors is well documented (23, 27, 28).

\(^{86}\text{RbCl} \) (25 \( \mu \)Ci) dissolved in 0.2 ml 0.9% NaCl (Amersham Int., Amersham, England) was injected i.v. into nonanesthetized mice unless otherwise stated. The mice were killed by cervical dislocation 2 min after the injection, i.e., within the time period when the tracer concentration in the tissue was pseudostable. The tumor, tail, foot (foot and ankle), liver, spleen, and kidney were immediately excised taking precautions to avoid contamination of the excised tissue by blood. Tumors and organs were then weighed and the activity was counted in a well-counter gamma camera (Siemens LFOV, upgraded with a Pho/Gamma ZLC37 inner head) equipped with a pinhole collimator was used to image \( \gamma\)-radiation within the energy range 70–90 keV and store the radiation counts as \( 64 \times 64 \) arrays in a minicomputer. Images were acquired in 20 frames, each of 30 s duration, starting immediately after the injection. Background contribution was subtracted. The clearance curve for each tumor was derived from user-defined regions of interest. \(^{133}\text{Xe} \) clearance rate, which is proportional to blood flow with the tissue-blood partition coefficient, \( \lambda \), as proportionality constant, was determined from the clearance curves. The \( \lambda \) differed insignificantly among the xenograft lines since none of the lines contained detectable amounts of fat tissue (22).

\(^{133}\text{Xe} \) Clearance. When the radioactive tracer \(^{133}\text{Xe} \) is injected directly into tissues, the tracer is washed out at a rate that is related to the blood flow in the labeled tissue. The \(^{133}\text{Xe} \) clearance method for measurement of blood flow has been described by Kallman et al. (29). \(^{133}\text{Xe} \) was supplied in a 0.9% NaCl solution at an activity of 5 mCi/ml (Medegenix Diagnostics, Brussels, Belgium), and 20 \( \mu \)l of this solution were given in a single injection to the tumor center by using a 30-gauge needle (outside diameter, 0.3 mm). The mice, anesthetized with sodium pentobarbital (0.06 mg/g body weight), were kept at normal body core temperature (36–37°C) during the blood flow measurements by using a heating pad with circulating water. A clinical scintillation camera (Siemens LFOV, upgraded with a Pho/Gamma ZLC37 inner head) equipped with a pinhole collimator was used to image \( \gamma\)-radiation within the energy range 70–90 keV and store the radiation counts as \( 64 \times 64 \) arrays in a minicomputer. Images were acquired in 20 frames, each of 30 s duration, starting immediately after the injection. Background contribution was subtracted. The clearance curve for each tumor was derived from user-defined regions of interest. \(^{133}\text{Xe} \) clearance rate, which is proportional to blood flow with the tissue-blood partition coefficient, \( \lambda \), as proportionality constant, was determined from the clearance curves. The \( \lambda \) differed insignificantly among the xenograft lines since none of the lines contained detectable amounts of fat tissue (22).

Statistical Analysis. A two-tailed \( t \) test was applied to investigate whether tumor parameters differed significantly among xenograft lines. Statistically significant correlations between two different parameters measured for the same individual tumors or the same lines were searched for by performing a two-tailed \( t \) test of correlation coefficients determined by linear regression analysis. An \( F \) test was applied to investigate whether the CVs differed significantly between the \(^{86}\text{Rb} \) uptake and the \(^{133}\text{Xe} \) clearance method. A significance level of \( P = 0.05 \) was used throughout.

RESULTS

Methodological Aspects. Comprehensive analysis of \(^{133}\text{Xe} \) clearance curves was performed to find the curve parameter which gave the most reproducible measure of tumor blood flow. For this purpose \(^{133}\text{Xe} \) clearance was recorded twice in each of 36 tumors on 2 consecutive days. The \(^{133}\text{Xe} \) clearance curves for a representative tumor are shown in Fig. 1. A single and a double exponential function, \( A_1 e^{-k_1 t} + A_2 e^{-k_2 t} \), respectively, were fitted to the data. Four parameters were determined for each curve, i.e., the rate constant, \( k_1 \), of the single exponential function; the rate constants, \( k_1 \) and \( k_2 \) of the double exponential function; and the slope, \( k_{init} \), of the initial part of the double exponential function. The \( k_1 \) was governed mainly by the initial part and the \( k_2 \) mainly by the final part of the decay curve, per definition. The \( k_{init} \) was calculated as

\[ k_{init} = (A_1 k_1 + A_2 k_2)/(A_1 + A_2) \]  

(B)

The \( k, k_1, k_2, \) and \( k_{init} \) are listed in the figure panels. Fig. 1a shows that the numerical value of the four rate constants could differ considerably. In Fig. 16 \(^{133}\text{Xe} \) was washed out at an almost constant rate, i.e., the rate constants had approximately the same value. The \( k_1, k_2, k_3, \) and \( k_{init} \) were determined for all 72 \(^{133}\text{Xe} \) clearance curves. A statistically significant correlation between the individual values from the first and the second recording was achieved for \( k_1 \) (\( P = 0.008 \)) but not for \( k_2, k_3, \) and \( k_{init} \) (\( P = 0.16, P = 0.64, \) and \( P = 0.55 \), respectively); i.e., the only reproducible rate constant was \( k_2 \). A double exponential function was therefore fitted to the \(^{133}\text{Xe} \) clearance curves recorded in the subsequent experiments, and \( k_2 \) was used as a parameter for \(^{133}\text{Xe} \) clearance rate and hence tumor blood flow.

Measurement of \(^{133}\text{Xe} \) clearance rate required anesthetized mice, whereas \(^{86}\text{Rb} \) uptake could be measured in nonanesthetized mice. The use of anesthesia may influence tumor blood flow (30). Sodium pentobarbital, however, has been shown to have only minor effects on blood flow in most rodents (31). To investigate whether sodium pentobarbital (0.06 mg/ml body weight) had any effect on tumor blood flow in our model system, \(^{86}\text{Rb} \) uptake as a function of tumor volume was measured in anesthetized and nonanesthetized mice using one xenograft line. No significant difference in \(^{86}\text{Rb} \) uptake was found, provided that the body core temperature of the anesthetized mice was kept at 36–37°C (Fig. 2). Thus, blood flow in our xenograft lines was probably not influenced significantly by the use of sodium pentobarbital anesthesia.

Two types of experiments were performed to investigate whether the \(^{133}\text{Xe} \) clearance and the \(^{86}\text{Rb} \) uptake method gave consistent results: (a) \(^{133}\text{Xe} \) clearance rate and \(^{86}\text{Rb} \) uptake were measured as a function of tumor volume using different tumors from the same xenograft line; and (b) \(^{133}\text{Xe} \) clearance rate and \(^{86}\text{Rb} \) uptake were measured for the same individual tumors on
Consequently, $^{133}$Xe clearance rate differed more among different tumors of similar size than among consecutive measurements in the same tumor; i.e., individual tumors of similar size of the same line could differ significantly in blood flow.

Differences among Xenograft Lines. Possible differences in tumor blood flow among xenograft lines were searched for by measuring blood flow as a function of tumor volume using the $^{86}$Rb uptake method (Fig. 5). Good fits to the data were obtained with power functions, i.e., linear curves in double logarithmic diagrams. All lines showed a decrease in blood flow with increasing tumor volume ($P < 0.001$). The $^{86}$Rb uptake at tumor volumes of 200, 500, and 1000 mm$^3$ and the magnitude of the decrease in $^{86}$Rb uptake with increasing tumor volume were determined from the linear curves and are listed in Table 2. Considerable differences in these parameters were found among the six xenograft lines. The COX-t, HUX-t, ROX-t, and SAX-t lines showed a higher $^{86}$Rb uptake than the BEX-t and WIX-t lines, a difference which was significant at all tumor volumes ($P < 0.05$) no matter which of the COX-t, HUX-t, ROX-t, and SAX-t lines were compared with the BEX-t or the WIX-t line. No significant differences were found among the COX-t, HUX-t, ROX-t, and SAX-t lines at tumor volumes of 200 and 500 mm$^3$. The $^{86}$Rb uptake in the other two lines was not significant in two consecutive days using tumors within a small volume range ($V < 500$ mm$^3$) and two xenograft lines. Both $^{133}$Xe clearance rate and $^{86}$Rb uptake decreased with increasing tumor volume, and the relative decrease was similar for the two methods (Fig. 3a). The scatter in the data, however, was larger for the $^{133}$Xe clearance than for the $^{86}$Rb uptake method. Moreover, most individual tumors showing a high $^{133}$Xe clearance rate also showed a high $^{86}$Rb uptake and vice versa (Fig. 3b). Consistent results were thus achieved with the two methods, showing that they might be used to supplement each other in studies of tumor blood flow.

Differences among Tumors of Similar Size of the Same Xenograft Line. Both $^{86}$Rb uptake and $^{133}$Xe clearance rate differed considerably among individual tumors of similar size of the same xenograft line (Fig. 3a). The differences could be due to experimental uncertainties and temporary variations in blood flow as well as reflect blood flow heterogeneity among the tumors. $^{133}$Xe clearance rate was measured twice for tumors of the same size to investigate whether biological factors might contribute to the differences observed. The measurements were performed on two consecutive days using two xenograft lines and tumors with volume from 100 to 300 mm$^3$. The $^{133}$Xe clearance rate was independent of tumor volume within this volume range. There was a statistically significant correlation between the values achieved in the first and the second measurement for both lines ($P < 0.01$), as illustrated in Fig. 4.
The statistical analysis was based on the data in Table 2. 

86Rb uptake was measured in the kidney, spleen, liver, and foot and in the two murine tumor lines EMT6 and Lewis lung and used as standards of reference for the xenograft lines. Fig. 6 shows the ranges of blood flow (mean ± SE) for the different tissues. The tumors showed lower blood flow than the normal tissues, except that small EMT6 and Lewis lung tumors showed similar blood flow to the foot. Blood flow in the xenografts was within the lower range of or lower than that in the murine tumors.

Blood supply per viable tumor cell was calculated by correcting the 86Rb uptake data for tumor cell density and necrotic fraction. Tumor cell density differed insignificantly among the xenograft lines, with the exception of the COX-t line which showed a cell density of about one-half of that of the other lines (Table 1). Necrotic fraction differed considerably among the lines; the mean value ranged from 3 to 36% in small tumors and from 8 to 41% in large tumors (Table 1). All lines showed a decrease in 86Rb uptake per viable tumor cell with increasing tumor volume (Fig. 7) (P < 0.001). The 86Rb uptake per viable tumor cell at tumor volumes of 200, 500, and 1000 mm3 and the magnitude of the decrease in 86Rb uptake per viable tumor cell with increasing tumor volume are listed in Table 3. The xenograft lines showing a high 86Rb uptake also showed a high 86Rb uptake per viable tumor cell and vice versa, with the exception of the ROX-t line. This line showed a relatively high 86Rb uptake; however, 86Rb uptake per viable tumor cell was relatively low due to a high cell density and a low necrotic fraction. The data in Table 3 were subjected to a statistical analysis similar to that described above for the data in Table 2. The analysis showed that 86Rb uptake per viable tumor cell was significantly higher for the COX-t, HUX-t, and SAX-t lines than for the BEX-t, ROX-t, and WIX-t lines at all tumor volumes (P < 0.05). No significant differences were found among the COX-t, HUX-t, and SAX-t lines at large tumor volumes. The BEX-t, ROX-t, and WIX-t lines were not significantly different at large volumes either (Fig. 7).

133Xe clearance rate was measured for small tumors (V = 100–300 mm3) of all lines in an attempt to confirm the 86Rb uptake data (Table 2). The COX-t, HUX-t, and SAX-t lines showed the highest and the BEX-t, ROX-t, and WIX-t lines showed the lowest 133Xe clearance rate per viable tumor cell (Table 3); i.e., the data for 133Xe clearance rate per viable tumor cell were in agreement with the data for 86Rb uptake per viable tumor cell. However, the differences between the HUX-t and SAX-t lines on the one hand and the BEX-t and WIX-t lines on the other were not statistically significant when 133Xe clearance rate per viable tumor cell was considered. Thus, the distinction between the lines was not so clear with the 133Xe clearance as with the 86Rb uptake method. This was due to the larger experimental uncertainty involved in the 133Xe clearance rate than in the 86Rb uptake measurements, as reflected in the CV which was consistently higher for 133Xe clearance rate than for 86Rb uptake (Table 2). The difference in CV was statistically significant for the BEX-t and SAX-t lines (P < 0.05) but not for the other four lines.

Collectively, the measurements of 86Rb uptake and 133Xe clearance rate showed that the melanoma xenograft lines could be divided into two distinct groups with respect to blood supply per viable tumor cell (Fig. 8, a, b). The COX-t, HUX-t, and SAX-t lines were found to have a high and the BEX-t, ROX-t,
and WIX-t lines to have a significantly lower blood supply per viable tumor cell.

Relationship to Growth Parameters. Tumor volume-doubling time (Td) differed considerably among the xenograft lines (Table 1). However, no relationship was found between this parameter and any blood flow parameter.

The xenograft lines showed different distributions of cells in the cell cycle (22). They could be classified into two distinct groups with respect to the fraction of cells in S phase (Table 1). The COX-t, HUX-t, and SAX-t lines showed high fractions of cells in S phase (23–31%), whereas the BEX-t, ROX-t, and WIX-t lines showed lower fractions of S-phase cells (11–13%). Thus, the xenograft lines showing a high blood supply per viable tumor cell also showed a high fraction of cells in S phase and vice versa (Fig. 8, a–c). The correlation between blood supply per viable tumor cell and fraction of cells in S phase across xenograft lines was highly significant (P < 0.001), no matter which tumor volume was considered.

All xenograft lines developed necrosis and the necrotic fraction increased with increasing tumor volume (22). Necrotic fraction at a given tumor volume, the magnitude of the increase in necrotic fraction with increasing tumor volume, as well as the rate of the increase in necrotic fraction with time differed considerably among the lines (Table 1). There was no relationship between any blood flow parameter and the absolute value of the necrotic fraction or the rate at which the necrotic fraction increased with time. The magnitude of the increase in necrotic fraction with increasing tumor volume (the slopes of the curves in Fig. 4 of Ref. 22), however, was larger for the BEX-t, ROX-t and WIX-t lines than for the COX-t, HUX-t, and SAX-t lines. Thus, the magnitude of the increase in necrotic fraction with increasing tumor volume was large for the lines showing a low blood supply per viable tumor cell and small for the lines showing a high blood supply per viable tumor cell (Fig. 8, a–b, d). The correlation between blood supply per viable tumor cell and the magnitude of the increase in necrotic fraction across xenograft lines was highly significant (P < 0.001), independent of tumor volume.

**DISCUSSION**

Methodological Aspects. Blood flow measurements using the 133Xe clearance technique are based on analysis of clearance curves. The clearance rate is often assumed to be constant (32).
constant $k_2$ was the parameter that correlated best with the $^{86}$Rb uptake when $^{133}$Xe clearance rate and $^{86}$Rb uptake were measured for the same tumors (Fig. 3b). Similarly, a study of blood flow in skeletal muscle showed that the best correlation between blood flow measured by the $^{133}$Xe clearance technique and that measured directly was found when $^{133}$Xe clearance rate was determined from the final part of the clearance curve (35). The use of $k_2$ as a parameter for tumor blood flow is therefore justified.

$^{133}$Xe clearance rate in heterogeneous tumors depends on the site of injection, especially in tumors containing nonvascularized, necrotic areas. Tumors of all xenograft lines studied here developed necrosis, even at volumes of 200 mm$^3$ (Table 1). The necrotic tissue in tumors of this size was always seen as small patches randomly distributed within the viable tissue. Larcombe McDouall et al. (36) estimated the tissue volume labeled with tracer after intratumor injection of 20 µl saline/D$_2$O to be 44 mm$^3$. The tumor volume labeled after injection of 20 µl $^{133}$Xe solution in the present study was thus a number of times larger than the necrotic patches and included both necrotic and viable tissue. The pattern of necrosis in tumors of about 200 mm$^3$ makes the local blood flow, i.e., the $^{133}$Xe clearance rate, comparable to the average tumor blood flow. This justifies calculation of blood supply per viable tumor cell by correcting $^{133}$Xe clearance rate for tumor cell density and necrotic fraction. The results achieved with the $^{133}$Xe clearance method were consistent with those achieved with the $^{86}$Rb uptake method within the entire volume range studied (Fig. 3), indicating that the presence of necrosis had only a minor influence on $^{133}$Xe clearance rate even in large tumors. However, the higher CV for $^{133}$Xe clearance rate than for $^{86}$Rb uptake was probably a consequence of blood flow heterogeneity due to necrosis (Table 2).

The methodological experiments described here showed that the $^{133}$Xe clearance and $^{86}$Rb uptake methods can supplement each other in studies of tumor blood flow in our melanoma xenograft lines. No conclusions were drawn in the present work unless they were supported by results from both techniques.

Biological Aspects. Blood flow in the melanoma xenograft lines was compared with that in four different normal tissues and two murine tumor lines to rank the xenograft lines in relation to well characterized and extensively studied tissues (Fig. 6). The measured normal tissue blood flow values were consistent with results reported elsewhere (37). The tumor lines showed low blood flow compared to the normal tissues, in agreement with the general observation that the vascular system in most tumors is inefficient (1). The blood flow was generally lower in the xenograft lines than in the murine tumor lines, an observation consistent with data showing that the vascular

or is determined from the initial part of the curve (33, 34). However, the clearance rate in tumors may change with time due to heterogeneous and intermittent blood flow. $^{133}$Xe inadvertently injected intravascularly, or impairment and redistribution of the blood flow during the injection. The present work demonstrated that the clearance rate frequently varied with time and that the final part of the clearance curve was more reproducible than the initial part (Fig. 1). Moreover, the rate

### Table 3: Blood supply per viable tumor cell for human melanoma xenograft lines

<table>
<thead>
<tr>
<th>Melanoma xenograft line</th>
<th>$V = 200$ mm$^3$</th>
<th>$V = 500$ mm$^3$</th>
<th>$V = 1000$ mm$^3$</th>
<th>Decrease in $^{86}$Rb uptake per viable tumor cell with tumor volume$^a$</th>
<th>$10^7$ $^{133}$Xe clearance rate per viable tumor cell (mm$^3$/cell)</th>
<th>$10^7$ $^{133}$Xe uptake (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEX-t</td>
<td>1.27 ± 0.13$^a$</td>
<td>1.11 ± 0.06</td>
<td>0.92 ± 0.06</td>
<td>-0.19 ± 0.02</td>
<td>3.76 ± 0.72</td>
<td>1.97 ± 0.08</td>
</tr>
<tr>
<td>COX-t</td>
<td>4.28 ± 0.44</td>
<td>2.93 ± 0.22</td>
<td>2.17 ± 0.15</td>
<td>-0.42 ± 0.04</td>
<td>7.99 ± 0.73</td>
<td>2.25 ± 0.03</td>
</tr>
<tr>
<td>HUX-t</td>
<td>4.19 ± 0.30</td>
<td>2.54 ± 0.15</td>
<td>1.82 ± 0.13</td>
<td>-0.52 ± 0.04</td>
<td>4.72 ± 0.30</td>
<td>3.28 ± 0.03</td>
</tr>
<tr>
<td>ROX-t</td>
<td>2.08 ± 0.27</td>
<td>1.47 ± 0.12</td>
<td>1.14 ± 0.09</td>
<td>-0.37 ± 0.05</td>
<td>2.38 ± 0.32</td>
<td>0.97 ± 0.03</td>
</tr>
<tr>
<td>SAX-t</td>
<td>2.83 ± 0.23</td>
<td>2.23 ± 0.16</td>
<td>1.85 ± 0.17</td>
<td>-0.26 ± 0.02</td>
<td>5.07 ± 1.15</td>
<td>4.07 ± 0.32</td>
</tr>
<tr>
<td>WIX-t</td>
<td>1.64 ± 0.13</td>
<td>1.22 ± 0.08</td>
<td>0.97 ± 0.09</td>
<td>-0.33 ± 0.03</td>
<td>3.77 ± 0.38</td>
<td>1.25 ± 0.06</td>
</tr>
</tbody>
</table>

$^a$ Based on 28-41 tumors.
$^b$ The slope of linear curves fitted to double logarithmic plots of $^{86}$Rb uptake per viable tumor cell (mean ± SE) versus tumor volume (mm$^3$).
$^c$ Mean ± SE.
volume is smaller in human melanoma xenografts than in most rapidly growing, transplantable murine tumors (38).

All melanoma xenograft lines showed a decrease in blood flow with increasing tumor volume (Fig. 5), as observed previously for human tumor xenograft lines of other histological types (39). The decrease in blood flow in the melanoma xenograft lines was a consequence of an increase in necrotic fraction (Table 1) as well as a decrease in blood supply per viable tumor cell (Fig. 7). This latter observation is consistent with data reported by Tozer et al. (40). They studied blood flow in relation to histology in a transplanted rat fibrosarcoma and found that the blood flow in densely cellular, viable tumor regions decreased during tumor growth. Decrease in the blood supply to viable regions in tumors during growth is probably a consequence of a decrease in capillary density (41) as well as an increase in flow resistance (42).

One aim of the present study was to search for possible differences in blood flow among individual tumors of similar size of the same xenograft line. Repetitive measurements of \(^{133}\text{Xe}\) clearance rate demonstrated that such tumors, even though they were implanted at the same site in the flank of athymic mice, indeed could differ significantly in blood flow (Fig. 4), probably as a consequence of differences in the density of the vascular network. However, even though the correlation in Fig. 4 was significant, the two values for \(^{133}\text{Xe}\) clearance rate differed considerably for some tumors, suggesting that temporary variations in blood flow also may have contributed to the differences observed. These conclusions are in agreement with conclusions from studies of blood flow in tissue isolated human tumor xenografts (43, 44).

Another aim was to search for possible differences in blood flow parameters among the xenograft lines. In spite of large experimental uncertainties and differences in blood flow among individual tumors of similar size of the same line, significant differences both in blood flow and blood supply per viable tumor cell were demonstrated. In fact, blood flow differed more among some of the xenograft lines than between the xenograft and the murine tumor lines (Fig. 6). The xenograft lines could be divided into two distinct groups with respect to blood supply per viable tumor cell, i.e., one group with a high and one group with a low blood supply (Fig. 7). The differences in the blood flow parameters might be caused by qualitative and quantitative differences in angiogenesis, leading to differences in vascular architecture among the lines. In accordance with this suggestion, Solesvik et al. (45) studied the vascular architecture in five human melanoma xenograft lines and found that the lines exhibited individual and characteristic microvascular structures. Moreover, Vaupel et al. (44) studied blood flow in human breast cancer xenografts and found that the blood flow in medullary carcinomas was significantly higher than that in squamous cell carcinomas at comparable tumor volumes.

The third aim was to search for possible relationships between blood flow and growth parameters of the xenograft lines. No blood flow parameter showed a significant correlation to tumor volume-doubling time, no matter which tumor volume was considered. Tumor volume-doubling time depends on the rate at which new cells are formed and at which cells are dying. The rate at which cells are formed is mainly determined by the growth fraction but is also influenced by the cell cycle time. The fraction of cells in S phase increases with increasing growth fraction and decreasing cell cycle time, i.e., the rate at which new cells are formed, is reasonably well described by this parameter (46). The xenograft lines showed a high blood supply per viable tumor cell also showed a large fraction of cells in S-phase (COX-I, HUX-I, ROX-I, SAX-I, and WIX-I) (Fig. 8, a-c). The most probable interpretation is that the blood supply per viable tumor cell was decisive for the rate at which new cells were formed. The alternative interpretation that the angiogenesis and hence the blood supply per viable tumor cell was determined by the cell proliferation activity is less likely. Consequently, the proliferation activity of the tumor cells was governed by the supply of oxygen and nutrients rather than by intrinsic properties of the cells. On the other hand, since there was no correlation between blood supply per viable tumor cell and tumor volume-doubling time, blood supply per viable tumor cell was probably not a major determinant of the rate at which cells were dying. Intrinsic properties of the tumor cells, e.g., ability to survive under physiological and oxidative stress in an unfavorable microenvironment, were possibly more important. This latter suggestion is supported by the observation that there was no clear relationship between blood supply per viable tumor cell and necrotic fraction or the rate at which the necrotic fraction increased with time.

Blood supply per viable tumor cell decreased whereas fraction of cells in S phase remained unchanged with increasing tumor volume. Large tumors of the high blood flow xenograft group showed a blood supply per viable tumor cell which overlapped with that in small tumors of the low blood flow group (Fig. 7). The fraction of cells in S phase on the other hand was clearly
different in these two tumor groups. These observations suggest that the rate of uptake (consumption) of oxygen and nutrients per viable tumor cell was (a) similar in small and large tumors of the same line and (b) higher in the high than in the low blood flow group. These suggestions are in accordance with the conclusion that supply conditions rather than cellular properties are decisive for the proliferation activity of the tumor cells, as discussed above. Moreover, studies of human tumor xenografts grown as tissue-isolated tumors in athymic nude rats have given results consistent with these suggestions (43): (a) tumor blood flow as well as the rate of uptake of oxygen and glucose per unit of tumor wet weight were found to decrease with increasing tumor wet weight, but the decrease in tumor blood flow was much more pronounced than the decrease in the uptake rates. The magnitude of the decrease in the uptake rates depended on tumor line and was similar to the magnitude of the increase in necrotic fraction seen in many experimental tumors, suggesting that the uptake rates per viable tumor cell did not decrease significantly with increasing tumor size; (b) the rate of uptake of oxygen and glucose was found to increase with increasing blood supply when tumors of different lines but of similar size were compared.

Blood supply per viable tumor cell decreased and necrotic fraction increased with increasing tumor volume. The magnitude of the decrease in blood supply per viable tumor cell was not significantly different in the high and the low blood flow xenograft groups. However, the xenograft lines showing a high blood supply per viable tumor cell showed a small increase in necrotic fraction (COX-t, HUX-t, SAX-t) and vice versa (BEX-t, ROX-t, WIX-t) (Fig. 8, a, b, d). In other words, the equilibrium between necrotic and viable tissue changed differently with increasing tumor volume in the high and the low blood flow xenograft groups, and this difference was not a consequence of different changes in blood perfusion. It is possible that the high and the low blood flow xenograft groups showed architectural differences in the vascular network and/or the supporting stroma, causing this difference. Rubin and Casarett (47) suggested early that specific patterns of tumor vascular networks might exist. Two main basic patterns were proposed, one with a peripheral and one with a central source. Falk (48) confirmed these observations and showed evidence that oxygenated blood was more homogeneously distributed in centrally than in peripherally supplied tumors, indicating differences in efficiency between the two vascular patterns. Later studies have suggested that vascular density can differ within each basic pattern and that tumors with a peripheral supply can develop central supply with time (49). It is tempting to speculate that the relationships in Fig. 8 are results of differences in basic vascular patterns between the two xenograft groups. Tumors of the high blood flow group may have developed a more central vascular supply at a volume of 200 mm³ than tumors of the low blood flow group, and, if so, this pattern is probably preserved at larger tumor volumes. However, experimental data on vascular architecture supporting this hypothesis are not available.

ACKNOWLEDGMENTS

The technical assistance of Berit Mathiesen and Hanne Stageboe Petersen is gratefully acknowledged.

REFERENCES


Blood Flow in Six Human Melanoma Xenograft Lines with Different Growth Characteristics

Heidi Lyng, Arne Skretting and Einar K. Rofstad


Updated version  Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/52/3/584

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.