Growth Characteristics of Human Colonic Adenocarcinomas Propagated in the Rowett Athymic Rat

James J. Stragand, Benjamin Drewinko, Sheri D. Henderson, Bruce Grossie, L. Clifton Stephens, Barthel Barлогie, and Jose M. Trujillo

INTRODUCTION

Development of the congenitally athymic mouse mutant provided a system which can support the growth of human malignant tissues in vivo (6). These human tumor xenografts maintained most of the biological, morphological, and growth kinetics characteristics of the parent cell lines and/or tissue of origin (9, 10, 16, 17, 21, 22) and have been successfully used in drug-screening studies (15–17). Recently, a congenitally athymic “nude” rat mutant was derived from an outbred colony and maintained most of the biological, morphological, and growth kinetics characteristics of the parent cell lines and/or tissue of origin (2, 4, 5, 18). In addition, the athymic rat reaches 250 g within 3 months of weaning, exhibits a more robust physical character, and when between 125 and 175 g (about 8 weeks). Both male and female animals were used throughout. All animals were housed under laminar flow hoods in rooms maintained at 26.7° with a 12-hr light cycle. All housing materials, as well as food and water, were autoclaved prior to use. Animals were handled under laminar-flow hoods with sterile equipment.

Cell Lines. Both the LoVo and SW 620 lines were established from the lymph nodes of male patients with histologically confirmed adenocarcinoma of the colon, prior to any chemotherapeutic treatment (3, 10, 11). LoVo cells were propagated as monolayer cultures in Ham's F-10 media supplemented with 20% heat-inactivated fetal calf serum, vitamins, glutamine, and antibiotics. Cultures were grown in a humidified incubator with a 5% CO₂ atmosphere and were routinely examined for evidence of Mycoplasma contamination. Under these conditions, line LoVo exhibited many morphological and functional features of a well-differentiated gastrointestinal cancer, including the formation of acinar structures and signet ring cells. Cells were harvested, using hyaluronidase (100 IU/ml) for 5 min at 37°, followed by trypsin (2.5% in HBSS) for 5 min at 37°. These conditions provided for maximum numbers of single viable cells.

SW 620 cells were supplied by Colonel Albert Leibovitz, Scott and White Clinic, Temple, Texas. A clone of this parent line (SW 620-3) with a chromosome number of 55 was used throughout. Cells were propagated in Leibovitz L-15 media, supplemented with a 10% heat-inactivated fetal calf serum, glutamine, vitamins, and antibiotics. Cells were grown in a humidified incubator with a 5% CO₂ atmosphere. Under these conditions, SW 620-3 cells exhibited a poorly differen-

ABSTRACT

Two human colonic adenocarcinoma cell lines were propagated in the Rowett athymic rat. Line LoVo displayed a well-differentiated morphology and exhibited progressive growth over a 70-day observation period with a doubling time of 8.5 days throughout. No metastatic involvement was noted.

Xenografts of SW 620 cells were undifferentiated and highly necrotic. These tumors grew progressively for approximately 30 days with a doubling time of 5.5 days, but over 90% of the animals exhibited a spontaneous regression with a mean time to total regression of 51 ± 3 (S.E.) days. Animals which had rejected the SW 620 xenografts would not support the growth of either the SW 620 or LoVo xenografts when challenged a second time with inocula producing a 100% tumor incidence in control rats. No metastatic involvement was noted for the SW 620 xenografts, but the tumors frequently invaded the underlying musculature and protruded into the peritoneal cavity without producing ascites or tumor nodules at sites distant from the primary.
tiated morphology. Cells were harvested with 0.25% trypsin (1:250; Grand Island Biological Co., Grand Island, N.Y.) for 5 min at 37°.

Tumor Inoculations. After collection and washing, cells were counted with an electronic particle counter (Model ZBI; Coulter Electronics, Hialeah, Florida). Cell aliquots of between 10^6 and 10^8 cells in 0.5 ml HBSS were inoculated s.c. into the right flank of rats at a point midway between the arm and leg. For mice, 10^5 cells were inoculated s.c. into the right flank. Tumor growth was monitored weekly in 2 dimensions [volume = π/6 (length x width^2)]; Volumes were plotted versus time after inoculation and the T0s were determined graphically. Following sacrifice, careful macroscopic examination was performed on all animals for tumor metastasis. Samples of the tumor, lung, liver, spleen, intestines, brain, and lymph nodes were taken, fixed in neutral buffered formalin for 48 hr, and routine histological sections were prepared. Slides were stained with hematoxylin and eosin, periodic acid-Schiff, mucicarmine, and Alcian blue, and sections were examined for microscopic tumor involvement. For i.c. inoculations, anesthetized animals were placed in a small animal stereotaxic apparatus (7). The scalp over the frontal lobe was incised, and a burr hole was made with a 20-gauge needle at a precisely measured distance from the sagittal and coronal sutures. Suspensions of 10 µl of HBSS were injected into the brain at a depth of about 4 mm; the hole was plugged with dental cement, and the incision was sutured.

Cell Kinetic Analysis. Individual cell kinetic profiles were obtained from tumor-bearing animals using the PDP assay to estimate the tumor GF and in vitro labeling to estimate the [3H]dThd LI. The procedures are described in detail elsewhere (1, 19). Briefly, 1 cu cm of tumor tissue was minced in the appropriate media, filtered, washed, and resuspended in fresh media. Aliquots of the sample were taken, and cytocentrifuge preparations were made on air-dried slides for use in the PDP assay. In this assay, sample nuclei were provided with all materials necessary for DNA synthesis, including [3H]dThd. If both α-DNA polymerase and DNA template are present in the sample nuclei, DNA synthesis occurs and can be quantitated autoradiographically. The percentage of labeled nuclei is termed the PDP index and has been shown to provide a reliable estimate of the tumor GF in murine and human tumor xenografts (19, 22). One thousand cells were examined for each PDP index.

The remaining tumor sample was incubated with [3H]dThd (2.5 µCi/ml, 20 Ci/mmol; New England Nuclear, Boston, Mass.) at 37° for 30 min in the shaker bath. The reaction was terminated on ice, and viable cells were isolated on a Ficoll-Hypaque gradient (1). Following washing, cytocentrifuge preparations were made, and 1000 cells were examined for the [3H]dThd LI determination. These slides were also used in the determination of the tumor MI.

Autoradiography. All studies used Kodak NTB2 emulsion diluted 1:1 with triple-distilled water and applied by dipping at 45°. All slides were exposed for a period necessary to produce a mean grain count of 10 per labeled cell. Background counts were typically less than one per cell.

Studies i.v. One goal in the development of the Rowett athymic rat-human colon xenograft model was its potential use in continuous drug infusion and i.v. hyperalimentation studies. However, the athymic nature of these animals and the potential susceptibility to infection raised questions concerning their tolerance to the attendant surgical procedures. In an effort to evaluate this, cannulation studies were performed on the basis of the method of Steiger et al. (20). Briefly, 150-g athymic rats were anesthetized with a ketamine:acepromazine combination (44 and 6 mg/kg i.m., respectively) and the right jugular vein was exteriorized. A small puncture wound was made in the midauscapsular region of the back, and a Silastic tube filled with heparin-0.9% NaCl solution was advanced s.c. to the area of the exteriorized jugular. The tube was inserted through a small puncture wound such that the end was next to the atrium of the heart. The tube was then attached in this position with sutures, and the wound was closed. The infusion tubing was covered with a stainless steel spring to prevent damage by the animal and to allow free movement within the cage.

Animals were given infusions for 17 days with a sterile 0.9% NaCl solution at a rate of 55 ml/day with laboratory chow provided ad libitum. Body weights were monitored throughout the procedure and compared to sham-operated littermate controls.

RESULTS

Incidence of tumor development in athymic mice was similar to that described in previous reports (21, 22). At a concentration of 10^7 cells, the LoVo line gave 100% of tumor takes and the SW 620 line gave about 66%. Incidence of tumor development versus inoculum size for the LoVo and SW 620 lines inoculated s.c. into athymic rats is presented in Table 1. Both cell lines required a minimum inocula of 10^5 cells before tumor development was observed. Histologically, both tumor xenografts maintained the morphological characteristics of the respective parent cell line. LoVo xenografts exhibited a well-differentiated morphology with extensive acinar formations, although without mucin production. These tumors were well vascularized from the murine epidermis and highly cellular, although focal necrosis was evident at all sites examined. No encapsulation by the murine connective tissue was noted. LoVo tumors grew by contiguity with no metastatic involvement in the proximal or distal lymph nodes or in any normal tissues examined. In one case, the LoVo xenograft penetrated the underlying musculature and infiltrated into the peritoneal cavity but did not produce ascites fluid or invade the surrounding normal tissue. SW 620 xenografts appeared as undifferentiated cords of cells surrounding individual murine blood vessels. Immediately surrounding these cords were areas of pyknotic nuclei and acellular material. In general, the SW 620 xenografts were composed of a large amount of fluid and acellular material. No encapsulation by the murine connective tissue was apparent. SW 620 tumors also grew by contiguity, but penetration of underlying musculature was more common, with subsequent growth into the peritoneal cavity. However, ascites fluid was not demonstrable, nor was there i.p. growth of tumor nodules at sites distant from the primary. No metastatic involvement was noted in the proximal or distal lymph nodes or in any normal tissues examined.

The s.c. growth pattern of the LoVo and SW 620 xenografts at 2 inoculum levels are presented in Charts 1 and 2, respectively. The LoVo xenografts at 10^7 and 5 x 10^6 inoculums differ primarily in the onset of exponential growth. Both LoVo inocula exhibited a T0 of 8.5 days throughout the 70-day observation period with no apparent plateau region noted. The animals tolerated the growth of these xenografts quite well, with no mortality observed. Of the 28 animals used in the LoVo growth studies, no spontaneous regressions were noted.

The growth of SW 620 xenografts (Chart 2) indicated an earlier establishment of exponential growth than the LoVo cell

Table 1

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>LoVo</th>
<th>SW 620</th>
</tr>
</thead>
<tbody>
<tr>
<td>10^6</td>
<td>0/8</td>
<td>0/5</td>
</tr>
<tr>
<td>5 x 10^6</td>
<td>0/8</td>
<td>0/5</td>
</tr>
<tr>
<td>10^7</td>
<td>12/16</td>
<td>2/15 (13)</td>
</tr>
<tr>
<td>5 x 10^7</td>
<td>6/6 (100)</td>
<td>27/33 (82)</td>
</tr>
<tr>
<td>10^8</td>
<td>3/3 (100)</td>
<td>4/5 (88)</td>
</tr>
</tbody>
</table>

* Tumors developing/total animals inoculated.
* Numbers in parentheses, percentage.
Growth Characteristics of Human Colonic Adenocarcinoma

In a related series of studies to investigate this regression phenomenon, 6 animals of similar sex, age, and weight, which had rejected the SW 620 cells previously no tumor development was observed.

To evaluate the feasibility of xenograft i.e. implantations, we inoculated $10^6$ cells in a precise location of the frontal lobe of 3 rats each for LoVo and SW 620 cell lines, respectively. All animals inoculated with LoVo cells died showing neurological signs within 60 to 65 days after implantation, while all those that received SW 620 died within 44 to 47 days. Autopsy revealed that all animals had colorectal tumors confined exclusively to the brain. Two animals (one for each cell line) had macro- and microscopic evidence of pneumonia.

**Cell Kinetics.** The $[^{3}H]dThd$ LI, MI, and PDP indices for LoVo and SW 620 rat xenografts of various sizes are presented in Tables 2 and 3, respectively. Of the 2 xenografts, the SW 620 shows a much higher proliferative profile with a mean value for the $[^{3}H]dThd$ LI of 19.6 ± 1.8%, with a PDP index of 28.9 ± 2.2%, and a MI of 0.50 ± 0.14%. This is in comparison to the LoVo xenograft profile with a $[^{3}H]dThd$ LI of 4.5 ± 0.6%, a PDP index of 11.9 ± 1.4%, and a MI of 0.1 ± 0.4%. No relationship could be established between tumor volume and the 3 cell kinetic parameters measured. Mean values of growth kinetics parameters determined for xenografts in athymic mice were similar to those defined in previous reports utilizing a larger number of animals (21, 22): LoVo line, GF = 32%, $[^{3}H]dThd$ LI = 9.7%, and MI = 1.0%; SW 620, GF = 31%, $[^{3}H]dThd$ LI = 25%, and MI = 1.0%.

**Infusion i.v.** Animals tolerated this procedure well. They continued to ingest solid food, and their weight gain per week was similar to that of sham-operated animals over the observation period. No animal mortality was observed, nor was there any evidence of infection following autopsy.

**DISCUSSION**

Previous studies in this laboratory have established the biological and growth kinetics properties of the LoVo and SW 620

animals which had rejected the SW 620 cells previously no

<table>
<thead>
<tr>
<th>Tumor volume (cu cm)</th>
<th>$[^{3}H]dThd$ LI (%)</th>
<th>PDP index (%)</th>
<th>MI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.48</td>
<td>2.5</td>
<td>13.7</td>
<td>0.00</td>
</tr>
<tr>
<td>2.16</td>
<td>3.3</td>
<td>19.0</td>
<td>0.01</td>
</tr>
<tr>
<td>2.45</td>
<td>6.4</td>
<td>9.2</td>
<td>0.30</td>
</tr>
<tr>
<td>2.87</td>
<td>5.3</td>
<td>10.3</td>
<td>0.00</td>
</tr>
<tr>
<td>4.65</td>
<td>4.5</td>
<td>8.2</td>
<td>0.27</td>
</tr>
<tr>
<td>7.85</td>
<td>7.6</td>
<td>18.8</td>
<td>0.10</td>
</tr>
<tr>
<td>9.20</td>
<td>5.1</td>
<td>9.4</td>
<td>0.00</td>
</tr>
<tr>
<td>11.09</td>
<td>3.3</td>
<td>11.6</td>
<td>0.00</td>
</tr>
<tr>
<td>14.31</td>
<td>2.4</td>
<td>7.7</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td><strong>4.5 ± 0.6</strong></td>
<td><strong>11.9 ± 1.4</strong></td>
<td><strong>0.10 ± 0.04</strong></td>
</tr>
</tbody>
</table>

* Mean ± S.E.

**Table 3**

Growth kinetics parameters of SW 620 cells grown in athymic rats

<table>
<thead>
<tr>
<th>Tumor volume (cu cm)</th>
<th>$[^{3}H]dThd$ LI (%)</th>
<th>PDP index (%)</th>
<th>MI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.01</td>
<td>22.1</td>
<td>28.5</td>
<td>0.74</td>
</tr>
<tr>
<td>2.33</td>
<td>21.5</td>
<td>30.2</td>
<td>0.83</td>
</tr>
<tr>
<td>2.63</td>
<td>15.1</td>
<td>21.1</td>
<td>0.20</td>
</tr>
<tr>
<td>3.90</td>
<td>23.4</td>
<td>27.4</td>
<td>0.40</td>
</tr>
<tr>
<td>4.21</td>
<td>22.9</td>
<td>36.1</td>
<td>0.85</td>
</tr>
<tr>
<td>14.09</td>
<td>13.0</td>
<td>26.2</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td><strong>19.6 ± 1.8</strong></td>
<td><strong>28.9 ± 2.2</strong></td>
<td><strong>0.50 ± 0.14</strong></td>
</tr>
</tbody>
</table>

* Mean ± S.E.
cell lines as xenografts in the BALB/c athymic mouse (21, 22). In the present study, the properties of these cell lines were examined as xenografts in the Rowett athymic rat. Results indicated that the athymic rat-colonic xenograft models maintained many of the morphological, biological, and growth kinetics properties of the parent cell line and tissues of origin, suggesting a certain stability of these characteristics during explantation in vitro and reestablishment in vivo. This was particularly noticeable for growth kinetics properties such as $[^{3}H]dThd$ LI determined for both xenografted cell lines, which were well within the ranges of values determined by others directly on fresh specimens of human colorectal cancers (12, 14).

The well-differentiated line LoVo grew at a slower rate ($T_D$, 8.5 days) with respect to the poorly differentiated SW 620 line ($T_D$, 5.5 days). These growth differences were further reflected in the respective cell kinetics profiles. Line LoVo exhibited a mean tumor GF of 12%, a $[^{3}H]dThd$ LI of 4.5%, and a MI of 0.1%. This was in contrast to the SW 620 GF of 29%, $[^{3}H]dThd$ LI of 20%, and a MI of 0.5%.

The growth kinetics profile obtained for line LoVo in the athymic rat exhibited a marked reduction from that observed in the BALB/c athymic mouse. In the latter case, a GF of 32%, $[^{3}H]dThd$ LI of 9.7%, and a MI of 1.0% were observed. In contrast, the SW 620 xenograft growth kinetics profile in the rat shows a marked similarity to that observed in the mouse. In the latter case, a GF of 31%, $[^{3}H]dThd$ LI of 25%, and a MI of 1.0% were observed. It was interesting that the in vivo SW 620 growth kinetics in both the rat and mouse modes were similar to the kinetic profile observed in vitro. This was again in marked contrast to the line LoVo kinetics in vitro, which indicated an increased proliferative potential over that seen in the mouse. This growth kinetics stability of the SW 620 line suggests an inherent property not affected by external control mechanisms. In contrast, the well-differentiated LoVo line appears to adapt to environmental conditions with distinct kinetic profiles.

The progressive growth of line LoVo and the spontaneous regression of the SW 620 xenografts presents an interesting phenomenon. Other reports have indicated the spontaneous regression of several human tumors in the Rowett athymic rat including 2 colonic tumors (2). These observations, as well as those reported herein, are in marked contrast to those in the athymic mouse in which progressive tumor growth appears to be the more common observation. In the more than 200 LoVo and SW 620 tumors grown in BALB/c athymic mice in our laboratories, no spontaneous regressions have been observed. Both the athymic mouse and rat lack functional T-cell activity, as indicated by morphological and biochemical techniques (23, 24). However, the reduced susceptibility of the athymic rat to infection and the ability to reject some human tumors suggest that the rat may possess a non-T-cell immune mechanism. Previous studies have suggested that the NK cell, a prethymic T-cell, may provide a certain degree of immune competence in the athymic mouse (8). It is possible that a higher level of NK cell activity in the rat may be responsible for the increased spontaneous xenograft rejection capacity. In our studies, when animals which had rejected the SW 620 xenografts were challenged with either LoVo or SW 620 cells, no tumor development was observed despite a 100% tumor incidence in the control animals. This suggested that, whatever rejection mechanism was operant in these animals, it was further stimulated by the inoculation of SW 620 cells but not by LoVo cells. These possibilities are supported by the fact that our athymic rats possess significant NK cell activity and that this activity can be further augmented by interferon-inducing agents (13).

Whatever the reason, the athymic rat appears more robust than does the athymic mouse. In the first year of breeding our colony, we have experienced less than 5 animal deaths of weaned animals among the more than 300 animals produced and used in a variety of experimental studies. In the cannulation studies, animals tolerated the surgical procedures well and survived for the 17-day observation period needed for continuous drug infusion and i.v. hyperalimentation studies. Additionally, successful implantation of colonic tumors restricted to brain matter provides for the study of antitumor agents capable of crossing the blood-brain barrier. These characteristics, coupled with the relatively easy breeding capacity, make the athymic rat an excellent system for human tumor xenograft studies at least equivalent to the athymic mouse model.

REFERENCES


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