Analysis of Variations in the Cell Population Kinetics with Tumor Age in the L1210 Ascites Tumor

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SUMMARY

The cell kinetics of the murine L1210 ascites tumor have been investigated on Days 5, 6, and 8 after transplantation of $10^5$ cells.

The experimentally obtained data, growth curve, percentage of labeled mitoses curves, and continuous labeling curves have been analyzed by means of a computer method based on alternative mathematical models for the cell kinetics.

The experimental doubling time determined from the growth curve is 10, 25, and 75 hr in the 5-, 6-, and 8-day tumors, respectively. From the models, growth fractions of 1.0, 0.96, and 0.56, respectively, are calculated for the same tumor ages. The calculated mean cell cycle time increases from 13.0 to 21.9 hr from Day 5 to 6, due to a proportional increase in the mean transit times of all phases in the cell cycle. In the 8-day tumor the mean cell cycle time surprisingly decreases to 15.3 hr and $T_G$ becomes unappreciable.

The cell loss is undetectable in the 5-day tumor, 0.1%/hr in the 6-day tumor, and 2.5%/hr in the 8-day tumor. The analysis makes it probable that the mode of cell loss is an age-specific elimination of noncycling cells with postmitotic DNA content.

INTRODUCTION

The L1210 murine leukemia is one of the tumors that has been used in primary drug screening by the Cancer Chemotherapy National Service Center with the greatest success in predicting clinically useful drugs, and as such it has been of great importance.

In recent years, the chemotherapy of this tumor has been improved by utilizing variations in scheduling of cell cycle-specific drugs (16–18). A further knowledge of all cell kinetic parameters, including the variation with age of tumor, thus becomes important.

Many studies have shown that the growth rate of experimental tumors varies during the course of tumor development. Generally, the increase in cell number is rapid during the early tumor growth and then gradually slows down as the tumor gets older. An increasing fraction stops dividing (3, 5, 11, 20) and the duration of the mitotic cycle increases (3, 5, 11, 20, 22, 23). Increased cell loss due to either accelerated cell death (7, 10) or increased migration of cells away from the site of tumor inoculation are other factors that can contribute to the gradual deceleration of growth in advanced tumors.

It is the purpose of this communication to give a qualitative as well as a quantitative description of the cell kinetics in the L1210 ascites tumor in Swiss X DBA/2 F1 mice on Days 5, 6, and 8 after i.p. transplantation of $10^5$ tumor cells.

The experimental data consist of a growth curve, PLM² curves, and CL curves. The data obtained are analyzed by a computer method based on 3 alternative mathematical models for the cell kinetics. The models are worked out on an analytical basis and developed along principles used earlier (6, 13, 14).

MATERIALS AND METHODS

Experimental Work. The strain of leukemia L1210 used was obtained from Southern Research Institute, Birmingham, Ala., in May 1969, and has since been maintained in DBA/2 mice by weekly i.p. inoculation of $10^5$ cells.

First-generation hybrids of female random-bred Swiss mice and inbred DBA/2 male mice are used in all experiments. All mice are males weighing 18 to 22 g. They are housed 10 to a cage and given a sterilized dry food enriched with vitamins and water ad libitum.

An inoculum of $10^5$ tumor cells in a volume of 0.2 ml is given to each animal in all experiments, as described earlier (4).

In each experiment 20 mice are randomized into a control group to determine the mean survival time after transplantation. The mean survival time is estimated as an average of the survival time of each animal in the group. The animals are examined at the same time each day. Length of survival (host life-span) of each animal after inoculation is estimated as the time interval between inoculation and the mean of the last time it is seen alive and the day it is found dead. The mean survival time after inoculation of $10^5$ tumor cells has earlier been estimated as 7.9 days (4). If the mean survival time of a control group in this study differs from 7.9 days by more than 0.5 day, the experiment is rejected.

Two identical tumor growth experiments are set up and 216 mice are used in all. In each experiment the inoculated mice are randomized to be sacrificed on Day 4, 5, 5.5, 6, 6.5, 7, 8, or 9 after inoculation, when the total number of ascites tumor cells per mouse is determined (4). The result of the 2 experiments is expressed by the growth curve, which is the...
mean number of tumor cells per mouse plotted in a logarithmic scale as a function of time after inoculation.

TDR-3H (specific activity, 5.0 Ci/mmole) (The Radiochemical Centre, Amersham, England), is used for the high-resolution autoradiography. Five μCi of TDR-3H are given i.p. after dilution in 0.9% NaCl solution to 25 μCi/ml. In the PLM experiments a single injection of TDR-3H is given to 70 mice on Days 5 and 6, and to 120 mice on Day 8 after inoculation of tumor cells. During the next 36 hr, less than 0.05 ml ascites fluid is withdrawn from 5 to 7 animals at short intervals. The CL experiments are carried out with 48 mice on the same days after transplantation as the PLM experiments. Injections of 5 μCi TDR-3H are given to the animals every 4 hr over a period of at most 28 hr, and ascitic fluid is withdrawn from 6 animals 1 hr after every injection. Autoradiographs are prepared from the ascitic fluid in the PLM and CL experiments (4).

In the PLM experiments the number of labeled mitoses is determined in 100 mitotic cells per mouse; only metaphases and anaphases are included. In the CL experiments the percentage of labeled cells is determined in 1000 tumor cells per mouse.

The results of the PLM and CL experiments are expressed by the PLM and CL curve, respectively. The experimental PLM curve is the fraction “number of labeled mitoses/number of cells in mitosis” plotted as a function of time elapsed since the short pulse of TDR-3H is given to the population. The experimental CL curve is the fraction “number of labeled cells/total number of cells” plotted as a function of time elapsed since the start of injections with TDR-3H.

The Computer Method. In this study, 3 mathematical models are used in the analysis of the experimental PLM and CL curves obtained from each age stage of the L1210 population. The models are designated as Model I, Model II, and Model III (Chart 1).

Terms and symbols necessary for the theoretical work are defined below.

- G1, S, G2, and m are 4 compartments which constitute the proliferative pool.
- m consists of the mitoses which are scored for deriving the PLM curve. In the following the mitosis is assumed to be divided between G2, m, and G1.
- Q is a quiescent compartment with a transit time which might be considerably longer than the transit time in the other compartments involved.

The transit times in the different compartments are stochastic quantities which are expressed by their PDF’s.

\[ T_{G1}, T_{G2}, T_s, T_m, T_S, T_Q, T_C, \text{and } T_Q \]

are the mean value and standard deviation of the transit time in G1, G2, S, Q, and the proliferative pool, respectively.

\[ T_D \]

is the doubling time.

\[ K_{e,m} \]

and \[ K_{e,m} \]

are influx in S and efflux from m, respectively.

\[ p \]

is the growth fraction, the relative number of cells in the proliferative pool.

\[ a \]

is a parameter equal to the average number of daughter cells entering the proliferative pool after division of a mother cell.

\[ q \]

is a parameter equal to the fraction of \[ K_{e,m} \] which comes from G1 in Model II.

\[ K_{e,m} \]

is a parameter which, when multiplied by \[ K_{e,m} \], equals the cell loss from Q in Model III.

The different models are based on a hypothesis which consists of 6 assumptions. The 1st 4 are general for all models. The assumptions are: (a) no biological variation occurs in the material investigated; (b) the cell population is growing exponentially; (c) the PDF’s of the transit time in the different compartments do not change during the time of study and are independent of each other; (d) the transit time in m is negligible in relation to the other transit times involved; (e) in Model I, \( p = 1 \); in Models II and III, \( p < 1 \) and a quiescent compartment Q is connected to the system parallel with G1; (f) in Model I, there is no cell loss; in Model II, there is no cell loss, and cells that are leaving Q enter the proliferative pool in S. It is furthermore assumed due to calculation of the PLM function that the transit time in Q is greater than the maximum time elapsed since the moment of pulse labeling. For Model III, there is cell loss from the system and the loss occurs from Q.

The PDF’s of the transit times are simulated by normal distributions. Relevant theoretical expressions are obtained and these expressions are, among others, the PLM and CL functions corresponding to the experimental data. The functions are expressed by the cell kinetic parameters. Finally, a computer is used to estimate the parameters in the case of each hypothesis by fitting the PLM and CL functions to the experimental data.

When the PDF’s of the transit times are simulated by normal distributions it is possible to obtain analytical expressions for all desired quantities. Models I and II have...
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been described earlier (6, 13). In the present study the analysis of the experimental CL curve has been improved as far as Model II is concerned. A detailed description of the mathematical work in all 3 models is given elsewhere (N. R. Hartmann and E. E. Scheufens, in manuscript).

When Model I is used in the analysis of the experimental data, all necessary cell kinetic parameters are estimated by fitting the PLM function to the experimental PLM curve. The theoretical CL curve is calculated on the basis of the estimated parameter values and compared to the experimental data as a control of the hypothesis. In the case of Models II and III it is necessary to fit both the PLM function and the CL function to the corresponding experimental data in order to estimate the cell kinetic parameters.

Before a theoretical function is fitted to an experimental curve the experimental data are multiplied by a correction factor in the interval 1.01 to 1.04. This correction is due to the observation that the experimental PLM and CL curves only reach a maximum labeling of approximately 96 to 99% at moments where 100% labeling is expected. The best fit between a theoretical curve and the experimental data is obtained by the maximum likelihood method. The principle is to optimize the probability for obtaining the experimental data available (14).

An IBM 360/75 computer is used for the computer analysis, and the programs are written in FORTRAN IV.

RESULTS

Chart 2 shows the growth curve of the L1210 ascites tumor. Furthermore, the fraction “total number of surviving mice/total number of mice” at the moment of cell counting in the tumor growth experiments, is plotted along the abscissa.

The cell growth is exponential from Day 4 to 6, and the doubling time $T_D$ is estimated as 10.1 hr calculated by the method of least squares. A gradual prolongation occurs with time and $T_D$ is estimated as about 25 hr between Day 6 and 7 and about 75 hr from Day 7 to 9. The last 2 values of $T_D$ are calculated from the tangent to the growth curve.

Charts 3 and 4 show the experimental PLM and CL curves, respectively, obtained from the 5-, 6-, and 8-day tumors. The black circles represent the uncorrected observed data. Standard error of the mean is less than 3.1% as far as all data are concerned in Chart 3 and never exceeds 1.6% in Chart 4.

When the 3 models are applied to these data they all give an almost identical fit in case of the 5-day tumor, but the result from Model II is rejected due to a value of $T_Q$ estimated as 1.9 hr, which is inconsistent with the hypothesis, and the analysis with Model III is unsatisfactory due to a $T_D$ estimated as 852 hr, which is inconsistent with the growth curve.

As far as the 6-day tumor is concerned, all models show a rather good fit to the experimental data, but again the result from Model II is rejected due to a value of $T_Q$ (13.8 hr) which is inconsistent with the hypothesis. Model III gives a slightly better fit to the experimental CL curve than Model I.

In case of the 8-day tumor, the absolutely best fit is obtained with Model III. The other models give very bad fits to the experimental CL curve.

Thus, the best fit is obtained with Model I in the case of the 5-day tumor and Model III in the 6- and 8-day tumor as shown in Charts 3 and 4.

The $T_C$ values, calculated from the mathematical models, are 13.0, 21.0, and 15.3 hr, respectively, in the 5-, 6-, and 8-day tumor. From Days 5 to 6 the increase in $T_C$ is due to an increase in the mean transit times of all phases in the cell cycle, while the decrease in $T_C$ in the 8-day tumor is mainly due to a considerable shortening of $T_G$.

All parameter values which correspond to the theoretical curves in Charts 3 and 4 are recorded in Table 1, and the computed distributions of $T_C$ for the 5-, 6-, and 8-day tumors are shown in Chart 5.

DISCUSSION

Thus, the important finding in our study is the surprising decrease in $T_C$ from the 6-day to the 8-day tumor, whereas this parameter increases from the 5-day to the 6-day tumor and $T_D$ increases with tumor age. In view of these findings, it is necessary to discuss a number of factors on which the conclusions are drawn.

The shape of the growth curve, an initial exponential phase followed by a continuous decline in the growth, is well established in ascites tumors (5, 7, 8, 11, 17, 20) and within the limits of the standard error can be fitted equally well to a Gompertz function (9, 15). The growth curve, and therefore also $T_D$, is quite accurately estimated in ascites tumors, where
Kinetic Analysis of L1210 Ascites Tumor

the total number of cells can be counted direct, except during the 1st 2 or 3 days of tumor development, where massive infiltration of host leukocytes makes it difficult to obtain reliable cell counts (7, 11).

The differences in $T_D$ values between our results and other estimations in the L1210 tumor (3, 17, 22) are probably due to the use of different L1210 sublines, strains of mice, and tumor inocula.

The increase in $T_D$ with increasing tumor age may result from a decrease in $p$, a prolongation of $T_C$, or an increasing cell loss either by cell death or migration from the abdominal cavity.

In our experiment we observe a gradual decrease in $p$ from Day 5 to Day 8, a result corresponding with observations in other ascites tumors (3, 5, 11, 20). Using repeated TdR-$^3$H injections, Yankee (22) finds that nearly all tumor cells are labeled after 20 hr and evaluates this as a $p$ equal to 1.0. In our 8-day tumor we reach a labeling of 100% after 28 hr and calculate a $p$ of 56%. It is obvious from our results that a CL value of 100% obtained after a period of time, does not always imply a $p$ equal to 1.0.

According to Table 1, $T_C$ increases from 13.0 to 21.9 hr from Day 5 to Day 6 and decreases again to 15.3 hr on Day 8 after inoculation. In the development of a prolonged $T_C$, a primary effect is a prolongation of $T_{G1}$, $T_{G2}$ and $T_B$ from Day 5 to Day 6. As far as the 8-day tumor is concerned, the
Table I
Growth parameters of L1210 ascites tumor in different stages of tumor development

<table>
<thead>
<tr>
<th></th>
<th>5-day tumor (Model I)</th>
<th>6-day tumor (Model III)</th>
<th>8-day tumor (Model III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell population (millions)</td>
<td>7.5</td>
<td>210</td>
<td>490</td>
</tr>
<tr>
<td>$T_D^a_\text{10}$ (hr)</td>
<td>10</td>
<td>23</td>
<td>75</td>
</tr>
<tr>
<td>$T_D^b_\text{10}$ (hr)</td>
<td>12.4</td>
<td>22.8</td>
<td>77.7</td>
</tr>
<tr>
<td>$T_{G1}$, $\alpha_{G1}$ (hr)</td>
<td>3.8, 3.4</td>
<td>5.4, 2.6</td>
<td>0.4, 0.01</td>
</tr>
<tr>
<td>$T_{G2}$, $\alpha_{G2}$ (hr)</td>
<td>0.8, 0.4</td>
<td>1.6, 0.8</td>
<td>2.4, 0.9</td>
</tr>
<tr>
<td>$T_{S}$, $\alpha_{S}$ (hr)</td>
<td>8.4, 2.9</td>
<td>14.8, 3.5</td>
<td>12.6, 3.4</td>
</tr>
<tr>
<td>$T_Q$, $\sigma_Q$ (hr)</td>
<td>13.0, 4.4</td>
<td>21.9, 4.4</td>
<td>15.3, 3.5</td>
</tr>
<tr>
<td>$p$ (%)</td>
<td>2.00</td>
<td>1.93</td>
<td>1.15</td>
</tr>
<tr>
<td>Cell production (%/hr)</td>
<td>5.6</td>
<td>3.2</td>
<td>3.4</td>
</tr>
<tr>
<td>Cell loss (%/hr)</td>
<td>0.00</td>
<td>0.13</td>
<td>2.5</td>
</tr>
</tbody>
</table>

$^a$ Calculated from the growth curve.  
$^b$ Calculated from the PLM and CL curves.

decrease in $T_C$ seems to be due to a disappearance of $T_{G1}$ and a decrease of $T_S$, while $T_{G2}$ increases. This observation has been confirmed in 2 later experiments and in advanced stages of the JB-I ascites tumor (P. Bichel, personal communication). The shortening of $T_C$ in the advanced tumor and the changes in the $T_{G1}$ values are not consistent with other observations in ascites tumors. An increase in $T_C$ at the later stages of growth, mainly by prolongation of $T_{G1}$, has been described in the L1210 ascites tumor (22, 23). Most other authors (3, 5, 20) find prolongation of $T_{G1}$, $T_{G2}$ and $T_S$ in L1210 and other ascites tumors. Lala (11), in an Ehrlich ascites tumor with no appreciable $T_{G1}$, finds a prolongation of the other phases of the cell cycle. The difference between our observations and the results of others cannot be explained. A possible factor is the condition of the tumor-bearing mice, which in our 8-day tumor have very advanced disease and are nearly terminal. Another contributing factor is our mathematical models, which have made possible a more precise analysis of the experimental data.

Cell loss cannot be detected in the 5-day tumor, it is negligible in the 6-day tumor, and is of the magnitude 2.5 %/hr in the 8-day tumor, compared with a cell production of 3.4%. In mice with Ehrlich ascites tumor, Lala (10) has calculated cell loss in the same range during the terminal period of growth, while cell loss in earlier and advanced stages is moderate. In the L1210 ascites tumor, Hofer and Hofer (7) have calculated a cell loss of 5%/24 hr in advanced but not terminal mice, an observation comparable with the cell loss in our 6-day tumor.

In Model III, cell loss due to metastases or migration is not included. With iododeoxyuridine-125I-labeled L1210 cells it has been demonstrated that the fractional metastases of the actual L1210 ascites population is very high during the 1st day of growth but minimal in more advanced tumors (7). In the 6-day and to a much higher degree in the 8-day tumor, there can be a progressive transition of a small but fast-growing population into a nonproliferating state, from which the cell loss takes place after a mean transit time of 22 and 16 hr respectively. This hypothesis is in agreement with observations in mice with Ehrlich ascites tumor in advanced stages, where it has been shown that the mode of cell death is an age-specific elimination of noncycling cells (with postmitotic DNA content) and a small amount of mitotic death (10).

The results discussed above are based mainly on the computer analysis of the considerable amount of data from the experimental PLM and CL curves, and one can question whether the mathematical models are plausible. The 1st part of the hypothesis assumes no biological variation, exponential growth, and PDF's that do not change during the time, assumptions that are necessary for the theoretical work. These assumptions are to some extent fulfilled within the period of each experiment. However, there is a critical time interval between Day 6 and Day 7, where the decrease in growth rate is most pronounced. It is here assumed that the growth follows a tangent to the growth curve. The rest of the hypothesis, including a quiescent compartment and the mode of cell loss, is specially set up for the cell kinetics in a type of system which is here investigated.

It is satisfactory that a good agreement is established between $T_D$ determined from the growth curve and $T_D$.

chart 5. Computed distributions of cell cycle time for 5-, 6-, and 8-day L1210 ascites tumor: - - - - , 5-day tumor; ----- , 6-day tumor; ---, 8-day tumor.
calculated from the PLM curves. There also seems to be a rather good fit between the experimental and theoretical curves in Chart 3 and 4. One exception, however, is the agreement between the experimental and theoretical PLM curve obtained for the 6-day tumor. The experimental 2nd wave seems to be slightly out of phase with the theoretical curve; this phenomenon might be due to the decreasing growth rate of the tumor. The discrepancy can be explained by a decreasing $T_{G1}$ within the period of the experiment.

There are 4 sources of error in the experimental work: tumor aspiration, radiation effects, reutilization of TdR-3H, and false negatives in the autoradiographs.

After aspiration of most of the ascites tumor cells, reentry of quiescent cells into the cell cycle can be demonstrated by a prompt rise in mitotic index and labeling index (1, 2, 12, 21), and one can question whether the necessary tumor aspirations will influence the kinetics of remaining cells. However, in the 6- and 8-day tumors only 2 to 3 aspirations of less than 0.05 ml tumor are used to obtain enough data for the PLM and CL curves. With this gentle technique, control experiments show no change in the mitotic index in an 8-day tumor over a 24-hr period after aspiration (P. Dombernowsky, unpublished observation).

With the TdR-3H dose used in the present experiments, no change has been observed in doubling time or host life-span of mice with L1210 ascites tumor (4).

In fast-growing tumors, reutilization of TdR-3H occurs from dying labeled tumor cells or host cells. Reutilization to some extent will give a diffuse increase in the percentage of labeled mitoses, and the minima between the tops of the PLM curves will therefore increase. $T_{S}$ will then be calculated too long and $T_{C}$ too short, while $T_{C}$ is not expected to change. High standard deviations relative to the transit times in the different compartments of the proliferative pool will also contribute to high minima values in the PLM curves. Comparison between experiments in this work and other PLM curves (11) with lower minima show that reutilization cannot quite be ruled out. Reutilization from host cells might occur in all our tumors, but this phenomenon is not expected to occur to a considerable degree until at least 24 hr after pulse labeling (19). In our study the possibility of reutilization from dying cells is most pronounced in the 8-day tumor, where the cell loss is considerable. An argument against reutilization in all our tumors is, however, that high minima values are present in the 5-day PLM curves at moments when both types of reutilization are expected to be negligible. The use of the same small amount of TdR-3H in all tumors, and the use of the same fixed grain limit of 4 grains used in the evaluation of the autoradiographs, will to some degree neutralize the effect of the possible reutilization in the 8-day tumor, where the number of tumor cells is 60 times greater than in the 5-day tumor.

The introduction of a correction factor is necessary due to false-negative labeled cells, which give a slight decrease in all experimental PLM and CL values.

Thus the finding of decreasing $T_{C}$ and disappearance of $T_{G1}$ in the 8-day tumor is surprising. It is possible that this phenomenon is more common than is generally believed, but other experimental techniques and improvement in the computer methods have to be introduced in order to confirm our findings.

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REFERENCES


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