Mathematical Model of Granulocytopoiesis and Chronic Myelogenous Leukemia

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ABSTRACT

We present a mathematical model of granulocytopoiesis that depends on certain physiologically meaningful parameters. By choosing different values of these parameters, the model describes both the normal process and that in chronic myelogenous leukemia (CML). The model fits all the available experimental data tested. Furthermore, it shows how the CML cells can ultimately outnumber the normal cells and how this process can be very slow. The model provides a quantitative approach to the relationship between proliferation and maturation and resolves the apparent contradiction between decreased proliferation and increased production, by assuming that a greater fraction of CML cells is produced by division rather than by maturation. The model should be helpful in designing experiments to better define the abnormalities of proliferation and maturation in CML and in seeking to define the specific alterations in the cell regulatory networks resulting from the production of the chimeric p210bcr-abl protein characteristic of CML.

INTRODUCTION

Granulocytopoiesis, the production of white blood cells, occurs in the bone marrow where cells evolve through a sequence of cell types beginning with committed stem cells (see Fig. 1). Quantitative data have been obtained on various aspects of this process, such as the number of cells of each type, the time they remain of that type, the time they spend in mitosis, the time they spend in DNA synthesis, etc. Both normal subjects and those with CML have been studied. Recently, supplementary data have been obtained on the growth of granulocytes in vitro (1), and progress has been made in identifying the pluripotent stem cell (2, 3).

To organize and correlate the current data, as well as those forthcoming from new experimental procedures, we have devised a mathematical model of granulocytopoiesis. It describes both the normal process and that in CML, with different parameter values for the 2 cases. The model is formulated in terms of physiologically meaningful measurable quantities. It is more complete than previous models (4–6), and although it incorporates many simplifications, it is reasonably compatible with available data, insofar as we have been able to resolve inconsistencies and input missing values in the data. As a consequence, the model can provide quantitative information about granulocytopoiesis and it can be used to make predictions.

The model deals with the number of cells of the 7 types indicated in Fig. 1, but primarily with those of the 3 proliferative types—the myeloblasts, promyelocytes, and myelocytes. It is assumed that cells of each of these 3 types go through a number of stages either by dividing a certain number of times, or else by maturing without further dividing. In Fig. 2, the route via cell division is represented by the upper line, and that via maturation is represented by the lower line. For each cell type, there is a specific number $n$ of stages, a certain time that a cell remains in each stage, and a number $f$, which is the fraction of cells that continues dividing as opposed to maturing without further dividing. In Fig. 2, myeloblasts pass through 3 stages ($n_b = 3$), promyelocytes go through 2 stages ($n_p = 2$), myelocytes go through one stage ($n_m = 1$), and cells of subsequent types have one stage each, which involve maturing but no dividing. The subscripts $b$, $p$, and $m$ refer to myeloblasts, promyelocytes, and myelocytes, respectively.

The data indicate that the $n$s and/or the $f$s are slightly greater for CML cells than for normal cells. We fit all the data for CML by taking greater $f$s but by keeping the $n$s the same for both CML and normal cells. This is in accord with the claim of Strife et al. (7, 8) that CML involves discordant maturation rather than uncontrolled proliferation.

The model for the proliferative cells and its consequences is presented in “Materials and Methods.” It involves 8 parameters, $n_b$, $f_b$, $n_p$, $f_p$, $n_m$, $f_m$, $q$, and $T$, where $q$ denotes the rate at which myeloblasts are produced by stem cells and $T$ is the time a cell spends in each stage. The experimental data are then described under “Results,” and it is shown that the model fits the data when the parameters in it are given appropriate values.

We also present a model for the committed stem cells somewhat similar to others discussed in the literature (see for example Refs. 9 and 10). It hypothesizes that a stem cell differs from an ordinary cell in having the ability to gain proliferative advantage by resting for a sufficiently long time. This hypothesis distinguishes stem cells from ordinary cells, and gives physiological meaning to the notion of “recycling” of cells used in previous models. The same model, but with different parameter values, is assumed to apply in CML. It shows how the CML cells can ultimately outnumber the normal cells, and how this process can be very slow.

MATERIALS AND METHODS

Formulation of the Model. A list of the symbols used in formulating the model is shown in Table 1.

In granulocytopoiesis, a cell passes through a succession of stages before it reaches the bloodstream. Stem cells produce myeloblasts in Stage I at the rate $q$ cells per unit time per kilogram of body weight. A fraction $f_1$ of these cells is active. After a time $T_1$, the active cells divide remaining myeloblasts but in Stage II, while the remaining cells enter Stage II by maturing. We assume for simplicity that this also happens in time $T_1$.

These processes of dividing and maturing continue as is indicated in Fig. 2. At each division, a fraction of the daughter cells remains active and the others become inactive. After a certain number $n_b$ of divisions or equivalent maturation, the myeloblasts become promyelocytes. After $n_p$ more divisions or equivalent maturation, the promyelocytes become myelocytes and after $n_m$ additional divisions or equivalent maturation, the myelocytes become metamyelocytes. Then the metamyelocytes mature to become bands that mature into segmented granulocytes before entering the bloodstream.
We want to determine the number \( A_j(t) \) of active cells and the number \( M_j(t) \) of maturing cells in stage \( j \) at time \( t \). To do so, we translate the preceding verbal description of the process into differential equations (see Fig. 3). The rate of change of the \( A_j \)th compartment, i.e., \( \frac{dA_j}{dt} \), equals the rate at which cells leave this compartment, i.e., \( -\frac{A_j}{T_j} \), plus the rate at which cells enter this compartment, i.e., \( 2f_j A_{j-1} \). Similarly, the rate of change of the \( M_j \)th compartment, i.e., \( \frac{dM_j}{dt} \), equals the rate at which cells leave this compartment, i.e., \( -\frac{M_j}{T_m} \), plus the rate at which cells enter this compartment, i.e., \( 2(1 - f_j) A_{j-1} \). Using a dot to denote a time derivative, and multiplying by \( T_j \) we obtain:

\[
\begin{align*}
T_j \frac{dA_j}{dt} &= -A_j + 2f_j \frac{T_j}{T_{j-1}} A_{j-1}, \\
&T_j \frac{dM_j}{dt} = -M_j + 2(1 - f_j) \frac{T_j}{T_{j-1}} A_{j-1} + \frac{T_j}{T_{j-1}} M_{j-1},
\end{align*}
\]

(2.1, 2.2)

Cells in stage \( j \) are slightly more mature than cells in the stage \( j - 1 \), so that \( T_j \) is slightly different than \( T_{j-1} \). To simplify the mathematics and limit the number of parameters entering the model, we shall use some average values of the \( T_j \), which we denote by \( T_o, T_p, T_m \), for the blasts, promyelocytes, and myelocytes, respectively. Also we expect that \( f_j \) is a function of \( j \), slowing decreasing from a value near 1 for a first-stage myeloblast to a value near zero for a last-stage myelocyte. However, for simplicity we assume that \( f_j \) is piecewise constant with values \( f_b, f_p, \) and \( f_m \) for the relevant types.

Equations 2.1 and 2.2 admit steady-state solutions for \( A_j \) and \( M_j \). Let \( \lambda \) denote the generation time of a cell, \( T \) the time spent in mitosis, \( h \) the number of divisions, and \( M_0 \) the total number of cells. Then:

\[
\begin{align*}
A_j &= \left( 1 - f_j \right)^h A_{j-1}, \\
M_j &= \left( 1 - f_j \right)^h A_{j-1} M_{j-1},
\end{align*}
\]

(2.3)

In order to reduce the number of parameters in the model, we assume that \( T_b = T_p = T_m \). Then the model in general and \( Q, N_b, N_p, N_m \) in particular depend on 8 parameters: \( n_b, n_p, n_m, f_b, f_p, f_m, q, \) and \( T \).

**RESULTS**

**Experimental Data for Normal Humans**

Measured values of the ratios \( N_b/N_b \) and \( N_m/N_p \) are given (4, 11-14). Except for the 5 terminally ill patients studied in Ref. 4, these ratios lie in the ranges given in Table 2. Measured values of the \( (M)_{1} \), i.e., the fraction of cells of type \( i \) undergoing mitosis at some fixed time, are given (11, 12); \( (M)_{1} = 0.025 \), \( (M)_{1} = 0.014 \), and \( (M)_{1} = 0.010 \). Values for the \( (T_{1})_{1} \), the time spent in mitosis by a cell of type \( i \), are given (11, 15); \( (T_{1})_{1} = 0.75 \), \( (T_{1})_{1} = 0.9 \), and \( (T_{1})_{1} = 1.07 \pm 0.23 \). The \( (L_{1})_{1} \) is the fraction of cells of type \( i \) that have acquired a label 1 h after being first exposed to labeled thymidine. The results for in vivo and in vitro studies are similar, but the variability of these results is considerable, ranging from 0.45 to 0.65 for myeloblasts and from 0.15 to 0.38 for myelocytes (16). Mean values, computed from the published studies (17), are

<table>
<thead>
<tr>
<th>Table 1 List of symbols used in formulating the model</th>
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<tbody>
<tr>
<td>( f_b, f_p, f_m ): Fractions of myeloblasts, promyelocytes, myelocytes, respectively, which will continue dividing</td>
</tr>
<tr>
<td>( n_b, n_p, n_m ): No. of divisions of myeloblasts, promyelocytes, myelocytes, respectively</td>
</tr>
<tr>
<td>( T_b, T_p, T_m ): Generation time of proliferative cells</td>
</tr>
<tr>
<td>( q ) (h(^{-1}) kg(^{-1})): Rate at which myeloblasts are produced by stem cells</td>
</tr>
<tr>
<td>( Q ) (h(^{-1}) kg(^{-1})): Flux from myeloblasts to promyelocytes</td>
</tr>
<tr>
<td>( N_b, N_p, N_m ): Total no. of myeloblasts, promyelocytes, and myelocytes, respectively</td>
</tr>
<tr>
<td>( T_b, T_p, T_m ): Mitotic time</td>
</tr>
<tr>
<td>( B ) (kg(^{-1})): Total blood granulocytes</td>
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<tr>
<td>( T_{1/2} ): In 2/half-life of a granulocyte</td>
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</table>

where \( n_b = 2(1 - f_b)/(2f_b - 1) \) and \( f_1 = f_2 = f_m \), and \( f_3 = f_n \).

Let \( Q \) denote the flux per kilogram of body weight from myeloblasts to promyelocytes and let \( N_b, N_p, \) and \( N_m \) denote the total number of myeloblasts, promyelocytes, and myelocytes per kilogram of body weight, respectively. Then:

\[
Q = \frac{24n_b + 24n_p + 24n_m}{T_m}, \quad N_i = \sum_{j=1}^{n_i} (A_j + M_j), \quad 1 \leq j \leq n_m
\]

(2.4)

\[ a = b, \text{ or } p, \text{ or } m \]

In order to reduce the number of parameters in the model, we assume that \( T_b = T_p = T_m = T \). Then the model in general and \( Q, N_b, N_p, N_m \) in particular depend on 8 parameters: \( n_b, n_p, n_m, f_b, f_p, f_m, q, \) and \( T \).
ever, it is known that during cinematography, the light slows cinematography experiments described previously (15). How 22, and 23). It appears that if the white blood cell count is 8 = 0.56, and (Tm)m= 0.79. The only discrepancy is with respect to promyelocytes. Indeed judging from the low mitotic index = 0.46, (LI)P = 0.38, and (LI)m = 0.19. The DNA synthesis time, T, is the time spent in DNA synthesis; values both in vivo and in vitro range from 13 to 16 h (16). The total number of myelocytes per kilogram of body weight, Nm, is 26 x 10^8/kg (18).

The estimated value of Q given in Table 2 is calculated as follows. The total number of blood granulocytes B equals the sum of circulating and marginal granulocytes, i.e., B = (3.1 x 10^8 + 3.9 x 10^8) = 7 x 10^8 (19). At steady state, Q = A/B, where λ = ln 2/(half-life of a granulocyte); given that the half-life of a granulocyte is 7 h (19), it follows that Q = 0.103, hence Q = 0.103 x 7 x 10^8. Let G denote the ratio T/LI = Tm/MI; it follows from the model that these ratios are equal. The estimated values of the Gs given in Table 2 are computed as follows: Assuming that (Tb)b = 14, (Tb)b = (Tm)m = 15, and the values of LIs given above, we find Gb = 30, Gp = 40, and Gm = 79. Using these Gs and the MIs given above, we find (Tm)b = 0.75, (Tm)p = 0.56, and (Tm)m = 0.79. The only discrepancy is with respect to promyelocytes. Indeed judging from the low mitotic index we would expect a (LI)p around 0.28. Thus, a better value for Gp is 47. We point out that the data on promyelocytes appearing in the literature are the most disparate as compared with those of myeloblasts and myelocytes. A reasonable option is to assume the experimental values for myeloblasts and myelocytes and then extrapolate the value for the promyelocytes. This also implies that Gb = 47 is preferable to Gp = 40.

The generation time T was computed by using T = 0.75T. It is consistent with the values of T, given previously (20). A measured value of T of about 30 h can be inferred from the cinematography experiments described previously (15). However, it is known that during cinematography, the light slows down the mitotic process.

Experimental Data for CML Patients

Values for Nf/Ns and Nm/Np are given (21). The data regarding labeling index are quite variable (see for example Refs. 20, 22, and 23). It appears that if the white blood cell count is greater than 4 x 10^4/(mm)^3, (LI)_b for CML cells is slightly larger than one-half of the normal value. The labeling index for myelocytes seems to be independent of the white blood cell count and is approximately equal to the value for normal cells (23). The DNA synthesis time of CML myeloblasts is similar to that of normal myeloblasts (24).

Using these data for myeloblasts and myelocytes, we find the Gcmls given in Table 2, where the value of (Gcml)p was interpolated from the values of (Gcml)b and of (Gcml)m. The total number of blood granulocytes and the half-life of granulocytes are also influenced by the white blood cell count. The half-life in CML is 5 to 10 times longer than for normal granulocytes (25) and B is increased by 10 to 100 times (26).

It is well-established that there is traffic of immature CML cells between bone marrow, spleen, and peripheral blood. The cytokinetics of cells in bone marrow and spleen are similar (20). The immature CML cells in the periphery have the striking feature that, although they synthesize DNA, they divide extremely rarely. In what follows, we will consider a functional pool of proliferative cells. This pool contains the immature cells existing in bone marrow, spleen, and blood. For the cytokinetic data of this pool, we will use the relevant data in bone marrow, since it contains the majority of the typical CML immature cells. Using the typical values λcml/λ = 0.25 and Bcml/B = 50, we find Qcml = 12.5Q.

One of the most important developments in recent years is the discovery of growth factors that allow investigators to culture and follow granulocytes in vitro. In a typical such study, an aspirate of cells is obtained from the bone marrow, and after it is appropriately treated, so that only certain types of cells survive, it is cloned and followed for a certain period of time. Usually both the number of colonies and the number of cells in each colony are recorded at 3, 7, and 14 days. A number of such studies for CML are reviewed by Strife and Clarkson (8), from which we extract the following data. Let a denote the number of colonies produced, divided by the number of cells cloned. Then αcml/α = 2.5, where αcml and α refer to CML and normal, respectively. If only immature cells are cloned (mature promyelocytes and essentially all myelocytes are excluded), then αcml/α = 1. The proliferative model presented here provides a convenient basis for discussing the culture data. Since only the active compartment produces colonies, it follows that α = A/N, where A is the total number of active cells, M is the total number of maturing cells, and N = A + M. Furthermore, A, N, and M are the values at steady state. Recall that G = T/LI, thus G = TN/A = T/α. Assuming that the value of G for the entire proliferative compartment is approximately the same for normal and CML cells, then the culture data and T = Ga imply that the generation time for CML cells is approximately twice the value of that for normal cells. This is consistent with older cytokinetic data (20).

The culture data also indicate that the number of divisions in the proliferative compartment is in the range of 5 to 10 (21).

A Fit to the Model

Recall that G = T/LI, LI = AT/NT, and N = M + A. Hence G/T

### Table 2 Experimental data

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>CML</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nf</td>
<td>2.4-3.5</td>
<td>2.1</td>
<td>(11-14, 21)</td>
</tr>
<tr>
<td>Nm</td>
<td>3-4.5</td>
<td>5.5</td>
<td>(11-14, 21)</td>
</tr>
<tr>
<td>Gc</td>
<td>30</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Gp</td>
<td>47</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Gm</td>
<td>79</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>Nf/Ns</td>
<td>26 x 10^8</td>
<td>90 x 10^7</td>
<td>(18, 26)</td>
</tr>
<tr>
<td>Q</td>
<td>72 x 10^8</td>
<td></td>
<td>(19, 26)</td>
</tr>
<tr>
<td>Nm/Np</td>
<td>0.028</td>
<td>39</td>
<td>(20)</td>
</tr>
</tbody>
</table>

Fig. 3. Elaboration of one compartment of granulocytopoiesis.
A steady-state solution of these equations exists only if \( f \) equals one-half, and then they yield:

\[
A_0 = kA_{-1}; \quad A_j = 2^{-j}kA_{-1}, \quad j = 1, \ldots, m
\]  

Active stem cells from any Stage \( j \) equals 0, 1, \( \cdots \), \( m \) (see Fig. 4) could in principle go to a resting state and then recycle. However, since this more complicated situation does not alter our conclusions in a significant way, we assume for simplicity that recycling happens only from the active stage denoted by 0 in Fig. 4. Since we are concerned with granulocytopoiesis, we have treated granulocytopoiesis progenitors as if they were the fundamental stem cells. Although this is a mathematical idealization, it is straightforward to incorporate the pluripotent stem cell in our model: (a) The granulocytopoiesis progenitors possess the property of “stemness,” but they also get an input from the pluripotent stem cells. Thus a term \( \alpha_{GQ} \) should be added to Equation 3.1a, where \( q \) is the flux from the pluripotent stem cell compartment, and \( \alpha_{G} \) is the fraction of these cells committed to granulocytopoiesis. (Similarly, \( \alpha_{E} \) and \( \alpha_{T} \) are the fraction of cells committed to erythropoiesis and thrombopoiesis, respectively, and \( \alpha_{G} + \alpha_{E} + \alpha_{T} = 1 \).) (b) The pluripotent stem cells should be described by equations identical to Equations 3.1 and 3.2.

Several stem cell multiplication mechanisms have been proposed in the literature. The most popular is the clonal succession mechanism, which suggests that normal hematopoiesis is maintained by sequential activation of different stem cell cones (27–31). In this system, there is always a large resting stem cell reserve. An alternative system suggests that the entire stem cell pool contributes to maintaining hematopoiesis (32–34). The stem cell models are not necessarily mutually exclusive; one or another system may predominate in different circumstances depending on the need for replacement or expansion of the mature cell compartments or for replenishment of the stem cell compartment (35–39). Pluripotent stem cells can presumably commit to produce cells of multiple lineages or exclusively of one lineage via a stochastic mechanism (27, 40, 41). The model depicted in Fig. 4 incorporates both the views mentioned above. Indeed, if \( T_{-1} \gg T_0 \), most of the sem cells are resting for most of the time. At the same time, the stem cell pool is continuously rejuvenated through the production of new cells. This model admits a steady-state solution even with zero external input. This is a consequence of the postulate that cells “recuperate” after resting. The loop between Stages –1 and 0 may be interpreted as a concrete physiological realization of recycling.

Recall that \( q \) denotes the flux from stem cells to myeloblasts. Thus, \( q \) equals \( 2A_n/T_0 \) equals \( 2^n A_{-1}/T_{-1} \), i.e.:

\[
2^n A_{-1} = qT_{-1}
\]  

The model of §2 calculates \( Q/q \) in terms of the \( f_s \) and the \( n_s \), and \( Q \) is known experimentally. Thus, \( q \) can be computed in terms of \( f_s, n_s, T_0, n_p, n_m \). Then Equation 3.4 gives \( A_{-1} \) in terms of \( T_{-1} \) and \( m \). As a numerical example, consider the case with \( n_s = 3, n_p = n_m = 2, i.e., Q/q = 16 \) assume that \( T_{-1} = 8 \) years and write \( m = 20 + \alpha \). Then Equation 3.4 yields \( 2^n A_{-1} = 2.6 \times 10^3 \). Thus, if \( m \) (the number of times a stem cell can divide without resting) is of the order of 20, and \( T_{-1} \) (the resting time of stem cells) is 8 years, then \( A_{-1} \) (the total number of resting stem cells) is of the order of \( 10^4 \). For a life of 70 years, the additional number of division that a stem cell performs as a consequence of resting is \( 70/8 \), or 8.7.

We postulated that the steady state of the CML stem cell model is also characterized by Equation 3.2 or Equation 3.3.

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### Table 3 A fit to the model

<table>
<thead>
<tr>
<th>( n_s = 3, n_p = n_m = 2 )</th>
<th>( f_s )</th>
<th>( f_p )</th>
<th>( f_m )</th>
<th>( N_s )</th>
<th>( N_p )</th>
<th>( N_m )</th>
<th>( Q/N_s )</th>
<th>( Q/N_p )</th>
<th>( Q/N_m )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.8</td>
<td>0.6</td>
<td>0.5</td>
<td>2.2</td>
<td>2.3</td>
<td>0.03</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CML</td>
<td>0.8</td>
<td>0.8</td>
<td>0.7</td>
<td>2.3</td>
<td>2.4</td>
<td>0.023</td>
<td>29.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>( n_s = 5, n_p = n_m = 3 )</th>
<th>( f_s )</th>
<th>( f_p )</th>
<th>( f_m )</th>
<th>( N_s )</th>
<th>( N_p )</th>
<th>( N_m )</th>
<th>( Q/N_s )</th>
<th>( Q/N_p )</th>
<th>( Q/N_m )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.8</td>
<td>0.7</td>
<td>0.6</td>
<td>2.8</td>
<td>2.9</td>
<td>0.024</td>
<td>75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CML</td>
<td>0.8</td>
<td>0.8</td>
<td>0.7</td>
<td>4.0</td>
<td>3.8</td>
<td>0.017</td>
<td>217</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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The model of §2 calculates \( Q/q \) in terms of the \( f_s \) and the \( n_s \), and \( Q \) is known experimentally. Thus, \( q \) can be computed in terms of \( f_s, f_p, f_m, n_s, n_p, n_m \). Then Equation 3.4 gives \( A_{-1} \) in terms of \( T_{-1} \) and \( m \). As a numerical example, consider the case with \( n_s = 3, n_p = n_m = 2, i.e., Q/q = 16 \) assume that \( T_{-1} = 8 \) years and write \( m = 20 + \alpha \). Then Equation 3.4 yields \( 2^n A_{-1} = 2.6 \times 10^3 \). Thus, if \( m \) (the number of times a stem cell can divide without resting) is of the order of 20, and \( T_{-1} \) (the resting time of stem cells) is 8 years, then \( A_{-1} \) (the total number of resting stem cells) is of the order of \( 10^4 \). For a life of 70 years, the additional number of division that a stem cell performs as a consequence of resting is \( 70/8 \), or 8.7.

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**Fig. 4. A mathematical model for stem cells.**
but perhaps with different parameters. As an example, assume 
\( n_{CML} = m \), \((T_{-1})_{CML} = T_{-1}\). Then for the 2 numerical examples discussed in Table 1, Equation 3.4 yields \((A_{-1})_{CML}/A_{-1}\) 6.8 and 4.3, respectively.

The steady state described above characterizes granulocytopoiesis after the CML clone has dominated. In order to understand how a single defective cell leads to the domination of the leukemic over the normal cells, one needs to study the transient state. This state is still characterized by Equation 3.1. To simplify the mathematics, we take the relevant parameters \( f, T_{-1}\), and \( T_o\), to be piecewise linear. We assume that when a defective stem cell appears, then a process similar to what happens in the steady state takes place. Namely, the fraction of defective stem cells recycling is higher, and their resting period is shorter. In a certain time period \( \tau, A_{-1}\) grows from 1 to \((A_{-1})_{CML}\), changes from some value \( F \) to \( 1/2\), and \( T_{-1}\) changes from \((T_o)_{CML}\) to \((T_{-1})_{CML}\). Where \((A_{-1})_{CML}\), \((T_o)_{CML}\), and \((T_{-1})_{CML}\) denote the steady-state values of \( T_o\), \( T_{-1}\), and \( A_{-1}\) for CML stem cells. By choosing \( F \) to be close to \( 1/2\) it is possible to make \( \tau \) quite long. This is consistent with the fact that CML cells could take a long time [on the order of several years (42-45)] to dominate.

Discussion and Extension of the Model

Discussion of the Model. The simplest and best-known model of granulocytopoiesis is that of Cronkite and Vincent (4). It postulates that each myeloblast divides once to produce 2 promyelocytes, each promyelocyte divides once to produce 2 myelocytes, and each myelocyte divides twice to produce 4 metamyelocytes. Rubinow’s (5) model is similar with one important difference: it postulates that a fraction of the cells produced by division of cells of any type remain of that type, while the rest become cells of the next type. Rubinow and Lebowitz (6) proposed a time-dependent model based on the concepts of maturity and aging. It assumes that the stem cell and all the proliferative cell types form 1 compartment in which cells are either resting or dividing. As they note, none of the preceding models matches the experimental data. (Detailed numerical values are given in Ref. 6.) A stem cell model describing both erythropoiesis and granulopoiesis has been presented (9). This model has been used to simulate several mouse experiments including acute, chronic, and postchronic irradiation, hypoxia, various anemias, and hypertransfusion. Several other stem cell models have been discussed in the literature (see for example Ref. 10).

We postulate that a cell can exist in 1 of 3 states: it can be resting, active, or maturing. A new cell can be produced through 2 processes, via division or via maturation. Furthermore, we assume that a stem cell can gain some proliferative advantage after resting. Thus, the most general model should include 3 states (resting, active, and maturing), and it should allow stem cells to recycle. We expect that in the stem cell compartment the maturing component can be neglected, whereas in the proliferative compartment the resting component can be neglected. Thus, we assume a resting and an active state in the stem cell compartment, and an active and a maturing state in the proliferative compartments.

Since there are no experimental data available yet for stem cells, we have concentrated on the non-stem proliferative cells. Most data concern the steady state, so we have investigated mainly the steady state of our model. There are several arguments supporting the concept that proliferative cells can be produced via the 2 mechanisms of division and of maturation: (a) Bands are produced from metamyelocytes through maturation, i.e., metamyelocytes mature but do not divide. Since myelocytes are not that different from metamyelocytes, one would expect from continuity that myelocytes are also capable of maturing. (b) If one assumes only 2 compartments, resting and active, then it can be shown that \( G = T + (1 - f)\sigma\), where \( \sigma \) is the time a cell spends resting. It has been well established experimentally that \( G_m > G_p > G_n\). Thus, according to this model, increasing time is spent in resting as cells evolve toward myelocytes. But this is in conflict with the concept that myelocytes were dividing more times than myeloblasts (see the Cronkite-Vincent model). Also it is difficult to justify why proliferative cells spend increasingly longer times resting. In our model, the increasing \( G_s\) reflect the fact that an increasing fraction of cells is produced via maturation the closer they are to myelocytes. That is, a greater fraction of myelocytes follows the road to maturation than myeloblasts. This is also consistent, using a continuity argument, with our assumption that stem cells are produced only via division. (c) There is direct experimental support for the concept above: Boll and Kuhn (15), during one of their cinematography experiments, happened to find a promyelocyte at mitosis and followed its history. One of its daughter cells entered mitosis after 29.5 h and the other after 28 h. The mitosis lasted for about 1 h and each of them gave birth to 2 myelocytes. One of the 4 newly born myelocytes could not be followed. One entered mitosis after 29.5 h and gave birth to 2 metamyelocytes after 1 h. These 2 metamyelocytes became bands after 7 h. The other 2 myelocytes matured to bands after 37 h. Although these cells could not clearly be assigned to either myelocytes or myeloblasts, in the process of maturation they had to become metamyelocytes before maturing to bands. Thus at least 2 metamyelocytes were created, not through the process of division, but through the maturation of 2 myelocytes. The arguments above suggest that when a cell divides, a fraction \( f\) divides again and a fraction \( 1 - f\) enters a maturation state. A cell in a maturation state has a probability \( g\) of entering the next maturation state and a probability \( 1 - g\) of entering a dividing state. The cinematographic data mentioned earlier indicate that, at least for myelocytes, \( 0 < f < 1\), \( g \neq 0\). Until a suitable experiment is performed [similar to the experiment of Boll and Kuhn (15) but more complete], we assume for simplicity that \( g\) equals 1. By choosing suitable \( f_s\), we can fit all the experimental data with any values of \( g_s\) between 0 and 1.

The advantage of our proliferative model as compared with previous models is that it is more flexible. In particular, the parameters \( f\) can be chosen so that a large number of divisions is possible. Since myelocytes are “close” to metamyelocytes which only mature but do not divide, and since the myeloblasts are more immature than myelocytes, we expect \( f_m > f_m\) and \( n_m \geq n_p \geq n_m\). Table 3 shows 2 numerical examples in which we are able to fit all the experimental data satisfying these constraints. It is interesting that we can fit the leukemic data by allowing slightly greater values of \( f_s\), without having to postulate larger numbers of divisions for CML than for normal cells.

We note that \( f\) can be interpreted as the probability that when a cell divides it will go on to divide again, and \( 1 - f\) as the probability that it will enter a maturation state.

Regarding the stem cells, we assume as it was mentioned earlier that the maturing component can be neglected. To simplify the situation even further, let us assume that there is only one resting compartment. Then a normal cell would satisfy the model of Fig. 5. It is clear that this model cannot admit a steady-state solution unless there exists an external input. But
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then how can the stem cells reproduce themselves? We have proposed the following concrete physiological mechanism. Consider a stem cell at the resting Stage I. We postulate that, as a result of resting for a sufficiently long time, when this cell becomes active it moves to Stage 0 as opposed to Stage I, i.e., a stem cell satisfies the model of Fig. 6. In other words, as a result of resting, a stem cell gains some proliferative advantage. Although it could divide \( m \) times without resting, it can now divide \( m + 1 \) times. The longer the resting period, the fewer divisions a stem cell undergoes, therefore lessening its exposure to the risks of genetic errors that might occur during DNA replication or mitosis. A long resting period also allows the cell ample opportunity to repair any DNA damage it may have incurred previously.

Biological Implications. CML is among the best understood of human neoplasms (21). In the great majority of CML patients, there exists a translocation of the distal segment of the long arm of chromosome 22 to the distal portion of the long arm of chromosome 9 (46–48). This results in the transposition of the c-abl oncogene to a specific region (bcr) on chromosome 22 and in the production of a novel chimeric phosphoprotein p210\(^{bcr-abl}\) (49–52). This chimeric protein is qualitatively different than the protein products of the bcr and abl genes in normal cells, and has altered protein tyrosine kinase activity. It is likely that the t(9;22) translocation is the primary causative genetic lesion in CML and that the p210\(^{bcr-abl}\) gene product is responsible for inducing subtle distortion of critical regulatory messages in the signal transduction pathways (8, 21). The transduction regulatory networks are highly complex and as yet incompletely understood (53). The protein tyrosine kinases that phosphorylate proteins exclusively on the hydroxyl group of tyrosine have key roles in signal transduction and regulation of cell growth and differentiation. There is increasing evidence that aberrant tyrosine phosphorylation is a common mechanism of malignant transformation; many of the known oncoproteins that act outside the nucleus are protein tyrosine kinases (54). It is suspected that mutations that convert normal protein kinase genes into oncogenic genes somehow interfere with negative regulatory domains, thus allowing the kinases to escape from their normal regulation and act constitutively to phosphorylate their substrates. Whereas there is as yet very little information about what specific proteins in the regulatory networks may be abnormally phosphorylated by oncoproteins with increased tyrosine kinase activity (21), the transforming potency of p210\(^{bcr-abl}\) and other bcr-abl oncogene products appear to be correlated with their kinase activities (55–58). It has been proposed that the aberrant phosphorylation of key regulatory proteins may cause faulty programming of the genes whose expression is necessary for controlling the normal orderly sequence of proliferation, differentiation, and maturation of hematopoietic cells, and that the consequence of this misregulation is asynchronous development of the nucleus and cytoplasm (8, 21). It was further proposed that the lag in nuclear maturation produces the following 3 effects in CML cells: (a) They manifest the kinetic changes mentioned earlier as well as various dysplastic, biochemical, and functional abnormalities; indeed a number of investigators have emphasized that many if not all of the biochemical and functional abnormalities of CML granulocytes, including impaired adhesiveness and phagocytic and bacteriocidal activities, are related to the degree of nuclear maturation (59–61). (b) They have decreased proliferative activity and live longer. (c) They perform 1 or 2 more divisions in the process of maturation than do comparable normal cells. The increased longevity and the additional divisions are used to explain the increased CML cellularity.

The "discordant maturation" hypothesis described earlier (8, 21) for CML is similar to the "blocked ontogeny" theory for experimental hepatomas (62, 63), and similar theories have been suggested for other tumors (64–66). Therefore, models used to elucidate the relationship between maturation and proliferation for CML cells should also be useful for analyzing a variety of other neoplasms. The model for the proliferative component of granulopoiesis presented here provides a quantitative approach to the relationship between proliferation and maturation. It resolves the apparent contradiction between decreased proliferation and increased production, by assuming that a greater fraction of CML cells is produced by division than by maturation. It suggests specific experiments to define better the relative roles of proliferation and maturation. It also implies that in trying to uncover the specific abnormalities in transduction pathways resulting from the production of p210\(^{bcr-abl}\), the specific biological consequences of altered tyrosine phosphorylation must be clearly understood. For example, if one assumes that simple unregulated cell proliferation instead of discordant maturation is the end result of aberrant phosphorylation (21), experiments trying to correlate the molecular and biological abnormalities may be improperly designed. The model presented here should aid in designing specific experiments to better define the relative roles of proliferation and maturation. It also implies that in trying to uncover the specific abnormalities in transduction pathways resulting from the production of p210\(^{bcr-abl}\), it would be advisable to concentrate on how a cell committed to differentiation along a particular linkage makes the decision whether to continue proliferating in a given maturation compartment or else to proceed directly to the next stage of maturation without further division. The time-dependent solution of the model presented here should aid in designing specific experiments to better define the relative roles of proliferation and maturation. It also implies that in trying to uncover the specific abnormalities in transduction pathways resulting from the production of p210\(^{bcr-abl}\), it would be advisable to concentrate on how a cell committed to differentiation along a particular linkage makes the decision whether to continue proliferating in a given maturation compartment or else to proceed directly to the next stage of maturation without further division. The time-dependent solution of the model presented here should aid in designing specific experiments to better define the relative roles of proliferation and maturation. It also implies that in trying to uncover the specific abnormalities in transduction pathways resulting from the production of p210\(^{bcr-abl}\), it would be advisable to concentrate on how a cell committed to differentiation along a particular linkage makes the decision whether to continue proliferating in a given maturation compartment or else to proceed directly to the next stage of maturation without further division. The time-dependent solution of the model discussed here should also be of interest in analyzing the effect of hematopoietic growth factors on granulocytopoiesis and the competition between regenerating normal and leukemic cells after bone marrow transplantation. An analysis of the time-dependent model should provide a better understanding of the mechanisms of regulation of granulopoiesis by hematopoietic growth factors (67–72), and it should aid in defining the optimal dosage and temporal intervention of stimulatory factors. Allogeneic bone marrow transplantation is the only consistently curative treatment for CML. Transplantation is performed after lethal marrow ablative doses of irradiation and/or cytotoxic drugs have been given to try to cure the leukemia (73). When successful, all leukemic progenitor cells are eradicated and
hematopoietic reconstruction occurs as a result of sustained proliferation of normal stem cells from the marrow graft (74–77). In some instances, it appears that it is possible to achieve hematopoietic regeneration from only a few donor stem cells or possibly even a single stem cell (76) as was previously demonstrated in mice (29–31). In unsuccessful cases, the donor marrow fails to engraft or is later rejected, or if any leukemic stem cells have survived the ablative treatment, they may regenerate and cause a recurrence of the disease. Bone marrow transplantation provides a unique opportunity to study regenerating normal hematopoietic progenitors as well as the competition between repopulating normal and leukemic progenitors (75, 77). When CML cells do reappear after transplantation, they often proliferate more slowly, and it is much easier to control the recurrent disease with antiproliferative drugs such as hydroxyurea than was the case prior to transplantation (74–77).

Several possible theories have been proposed to explain why the residual CML stem cells have survived the ablative treatment, they may regenerate and cause a recurrence of the disease. One possible explanation is that the residual CML stem cells may have a selective advantage, such as a mutation that confers a growth advantage. Another possible explanation is that the residual CML stem cells may have a lower sensitivity to the antiproliferative drugs used after transplantation. A third possible explanation is that the residual CML stem cells may have a delayed response to the antiproliferative drugs used after transplantation.

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Mathematical Model of Granulocytopenesis and Chronic Myelogenous Leukemia

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