A Comprehensive Modeling Procedure for the Human Granulopoietic System: Over-all View and Summary of Data

By Leslie E. Blumenson

Understanding of the granulopoietic system in both normal and diseased states might be assisted by the use of a quantitative modeling procedure that relates the underlying cellular events of granulopoiesis studied in the laboratory to the marrow and peripheral blood picture as it might be seen in the clinic. In this way, the modeling procedure could become both an adjunct to ongoing laboratory research, as well as a means for rationally deciding on modes of treatment for pathologic conditions. The depth and rapid pace of modern granulopoietic research require that the modeling procedure be, on the one hand, detailed enough to permit the inclusion of the pertinent events at the cellular level as they are known today, while on the other hand, remain flexible enough to permit both the modification of any part and the possibility of seeing the predicted consequences of this modification for both the normal and diseased states. A detailed procedure was developed for an hour-by-hour description of the interrelationship of the kinetics of the various marrow cell types with the events in the peripheral blood and tissue spaces. The model was extended to include the toxic effects of a drug (5-fluorouracil) administered according to the protocol of an actual trial with cancer patients, and the temporal pattern of the predicted effects of the drug on the peripheral blood count was compared with that found in the clinic.

Quantitative investigations of the various phases of granulocyte kinetics are increasing at a rapid pace. It now seems appropriate to develop procedures for organizing the available data with a view to obtaining a dynamic picture of what is known about the various phases of granulopoiesis, how these phases interact with each other, and the implications of this knowledge for clinical practice. The most convenient tool for this purpose is the mathematical model. Mathematical models have been developed for separate phases of granulocyte kinetics, such as stem cell kinetics, and models have also been developed to analyze particular experimental procedures. The modeling procedure introduced in this paper is a synthesis in that several previous mathematical models for the separate phases of granulopoiesis are joined into an interconnecting whole with the behavior of the submodel for each phase affecting, and at the same time being affected by, the behavior of the submodels for the other phases.

The main purpose of this paper is to give a brief overview of the modeling procedure and to present a review of the experimental work that was used in the initial construction of the model. The comprehensive nature of the model requires quantitative data involving all phases of granulocyte kinetics: from the
proliferation of stem cells to the accumulation of segmented cells in regions of infection. The review is organized with the modeling procedure in mind. It is hoped that this will delineate for investigators the type of information that is most valuable in the construction of mathematical models. The review also includes a discussion of concepts and terminology that form the underlying structure of the model. The last section of the paper is a brief reference to an application of the modeling procedure in the analysis of events in the marrow that lead to the leukopenia commonly seen in cancer patients receiving chemotherapy.

The model is divided into blocks, each of which is concerned with a different aspect of granulopoiesis. The user has many options available with regard to these blocks. The interconnections or paths among these blocks can be changed to describe various quantitative realizations of the feedback mechanisms of granulocyte kinetics. In this way the quantitative effect of a change in one feedback loop on the temporal pattern of the peripheral granulocyte count can be examined on the computer. Furthermore, except for the input from, or loss to, other blocks of cellular or chemical materials, each block is a separate part of the model, which itself can be described (modeled) as simply or as elaborately as may be desired. Thus, for example, the stem cell block could be considered simply as a constant source of cells that are released into the granulocyte development track at a rate determined by a simple feedback process, or an elaborate structure involving the total cell cycle and the feedback triggering of \( G_0 \) stem cells into \( G_1 \) could be (and has been) programmed for this block. The remainder of the over-all model can be held fixed while these structures internal to the stem cell block are varied, and the quantitative implications of these changes can be conveniently examined on the computer every step of the way.

A somewhat simplified block representation of the model of King-Smith et al.\(^7,8\) is shown in Fig. 1A. They divided the over-all granulocyte kinetics system into five blocks: stem cell, proliferating marrow, maturating marrow, peripheral blood, and tissue spaces. The details of their model internal to each block will not be discussed here. The dashed lines represent a feedback of the peripheral blood granulocyte concentration to both the maturating marrow and the stem cell blocks. Now it is possible that additional experimental data suggest that the feedback that initiates stem cell activity comes not directly from a depletion in the peripheral granulocyte concentration, but rather as a secondary effect of the ensuing depletion of the marrow cellularity itself. In this case, the block diagram might appear as in Fig. 1B, with feedback to the stem cell block coming directly from the proliferating and maturating marrow blocks. With the modeling procedure discussed in this paper, it becomes possible to examine the implications of such changes with minimal effort.

In addition to these types of changes and the expanding or contracting of the modeling internal to each block, the procedure has further advantages. It is always possible to add new blocks. For example, it is technically possible with this method to join up a model of erythropoiesis with a model for granulopoiesis. The interface between these two models can occur at the stem cell block where they are both demanding stem cells, as well as feeding back stimulants to stem cell production. Thrombopoiesis could also be added in this way. In the procedure presently being used we have not gone this far; erythropoiesis
and thrombopoiesis are represented only as constant sinks for the available stem cells at the stem cell interface with granulopoiesis.

The eventual goal of this procedure is not only to use it as a tool in hematopoietic research, but also to direct it toward clinical applications. For example, it is a simple matter with this procedure to add on to the model effects of radiation or chemotherapy on the stem cell and proliferating marrow systems. In this way, the quantitative relationship between marrow cellularity, drug dosage schedule, and the temporal pattern of the peripheral granulocyte count can be examined in detail. This was done for the model discussed here. The effects of
infection on the granulopoietic system could be modeled with this method. In particular, the cause of death in some cancer patients may be due to the inability to fight infection because of the depletion of the granulopoietic system by "anticancer" drugs. This problem can be examined quantitatively on the computer by using this method. It is also possible to use this method to simulate marrow transplant experiments. Finally, the breakdown of the feedback mechanisms, as well as the mechanisms that control release of cells from the marrow into the peripheral blood, could be studied quantitatively with this procedure. In this way, it may be possible to perform "experiments" on the computer to examine the implications of the several theories concerning the breakdown of these control mechanisms and the etiology of chronic granulocytic leukemia.

BIRD'S-EYE VIEW OF MODEL

A block diagram of the model is shown in Fig. 1C (feedback is not shown in this figure). Except for the addition of a block, corresponding to the development of other hemic cell lines, that is draining stem cells from the stem cell block, the block diagram is similar to that used by King-Smith and Morley. The utility of the new procedure becomes apparent when the description of the model is extended beyond the block diagram to events at the cellular level. This detailed description includes the modeling internal to each block and the feedbacks among the different blocks.

The unit of time chosen here is 1 hr. With this time unit the model can be used to distinguish among the different phases (G1, S, G2, M) of the cell cycle, which is useful in analyzing the hematopoietic toxicity of cancer chemotherapeutic drugs such as 5-fluorouracil, whose cytotoxic activity may be limited to cells in only certain phases of their cycle.

The over-all bird's-eye view of the model is shown in Fig. 2. The upper part of the figure above the double line is the block diagram of the system during two consecutive unit time intervals. Solid lines denote sequence of granulopoietic development. Dashed line denotes that there is a feedback of the state of a block during time \( t-1 \) to the activity of a receptor block during time \( t \). In C the states of three blocks during time \( t-1 \) are jointly involved in the feedback, since here the total (granulopoietic) marrow cellularity during this time affects stem cell activity during time \( t \).
the time \( t-1 \) hr, while the lower part of the figure is the same block diagram but during the next time \( t \). The dashed lines denote that the state of some blocks during time \( t-1 \) affect the activities in other blocks during time \( t \). Only the three main feedbacks of the model are shown in Fig. 2. These are: A, feedback of the granulocyte requirements of the tissue spaces to the peripheral blood; B, feedback of the peripheral blood granulocyte concentration to the maturing marrow; and C, feedback of the marrow cellularity to the inactive stem cell pool. The mode of operation of these three interblock feedback circuits, as well as others internal to the individual blocks (e.g., there is one to replenish the stem cell pool), and the detailed description of the internal construction of each block form the underlying structure of the model. The following review of experimental data is organized with this underlying structure in mind.

REVIEW OF CONCEPTS, TERMINOLOGY, AND DATA

This section will review the various concepts and terminology that have been used in previous theoretical models of granulopoiesis, as well as present some experimental data concerning these concepts. Many of these concepts and the corresponding measured quantities were originally used to describe granulocyte kinetics under steady-state conditions. However, the new modeling procedure is used to calculate hour-by-hour transient changes, and it was necessary to extend the definition of some of these measured quantities to permit the calculation of their variation with time.

The granulocyte concentration in the peripheral blood at a given time is, in part, a reflection of the rate of entry of granulocytes vs. the rate of departure of granulocytes from the blood at this time. If these two rates are equal, the granulocyte concentration remains unchanged (steady state) and the two rates are then identified with the granulocyte turnover rate. It is known that granulocytes spend some time adhering to the walls of capillaries and venules. These sticking cells are called the marginated granulocyte pool (MGP), while the free granulocytes in the peripheral blood are called the circulating granulocyte pool (CGP). The total blood granulocyte pool (TBGP) is the sum of these two: TBGP = MGP + CGP. Under normal conditions there is a dynamic equilibrium between the MGP and the CGP. Estimates have been obtained of the absolute sizes of these granulocyte pools for healthy individuals under normal steady-state conditions. For a 70 kg man, these estimates are 2.2, 2.7, and \( 4.9 \times 10^{10} \) for the CGP, MGP, and TBGP, respectively; i.e., 56% of the peripheral blood granulocytes are marginated. The total blood volume of a 70 kg man is about 5600 cc, and the granulocyte concentration in the circulating blood is, therefore, estimated as \( 2.2 \times 10^{10} / 5600 \times 10^3 = 3929 \) granulocytes/cu mm. This is in good agreement with actual counts from 19 healthy males where the average was 4067/cu mm.

Two additional quantities are used to describe the kinetics of granulocytes in the peripheral blood. These are the granulocyte turnover rate (GTR) mentioned above and the half time of residence of granulocytes in the blood \( (T) \). The half time is obtained from the disappearance curve of autotransfused granulocytes labeled with diisopropylfluorophosphate, and two critical assumptions are made when \( T \) is calculated from the curve: (1) this method measures...
the rate of loss from the TBGP and not just the CGP, and (2) the mature granulocytes are leaving the TBGP in a random fashion, i.e., they all have an equal chance of leaving.\textsuperscript{14,15} For 56 normal men the mean half time of residence in the blood was found to be $T = 6.7$ hr, with 95\% confidence limits of 4–10 hr.

The GTR is calculated from $T$ under the assumption that the number of cells in the TBGP is constant. The GTR is by definition the hourly rate at which cells are entering, or (since it is steady state) leaving the TBGP. For the healthy individual with $\text{TBGP} = 4.9 \times 10^{10}$ and $T = 6.7$ hr, the GTR is calculated as $\text{GTR} = 4.8 \times 10^9$ granulocytes/hr. Besides the half life due to random loss, there is also a senescent loss of granulocytes. The time of senescence for mature granulocytes is about 30 hr after they enter the blood, but for healthy individuals most cells leave the TBGP by this time.

In the steady state the GTR is also the rate at which granulocytes are required in the tissue spaces. If, as was mentioned above, all cells in the TBGP have an equal chance of leaving the blood during a 1-hr period, then the hourly tissue space requirements can also be represented by the numerical value of this chance. In the steady state, this hourly chance is equal to the ratio GTR/TBGP, which is about 0.1 under normal conditions. Transient changes in the tissue requirements (e.g., due to acute infection) for granulocytes can then be represented by changes in this hourly chance. This concept could be useful if an increasing chance could be related to the increasing concentration of a chemical mediator entering the peripheral blood from the tissue requiring an increased number of granulocytes.

The majority of granulocytes are to be found in the bone marrow where they are produced and where they mature before being released into the blood. For the 70 kg man, it has been estimated that the mass of the granulocytic cells in the marrow is 900 g, in the circulating blood 10 g, and in other tissues 600 g,\textsuperscript{16} while the total number of marrow cells of the granulocytic series has been estimated as $79.8 \times 10^{10}$.\textsuperscript{17} Under steady-state conditions, the rate at which cells are released from the marrow into the peripheral blood is equal to the GTR. Granulocytes that are about to be released from the marrow are at the end of a developmental sequence that involves several microscopically identifiable forms in a pipelinelike process of proliferation and maturation. The later stages in the sequence involve maturation without further proliferation, and cells in these later stages are referred to as being in the maturing marrow. Cells in the earlier proliferating stages are in the proliferating marrow. These terms are for descriptive purposes only and should not be construed as distinguishing between distinct locations in the bone marrow.

The three main identifiable types in the maturing marrow are, in order of increasing maturation, the metamyelocyte, the band, and the segmented cell. The approximate number of each kind in the marrow of a normal 70 kg man is 1.9, 2.5, and $1.8 \times 10^1$, respectively.\textsuperscript{17} The compartment transit time (CTT) is the time a cell spends in a given stage of maturation. In the steady state the CTT is obtained by dividing the size of the compartment by the GTR, since the process of maturation is assumed to be a pipeline process, i.e., a cell passes through the metamyelocyte stage for a certain number of hours, then it passes through the band stage, and then through the segmented stage. Thus, the CTT
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is calculated as 40, 53, and 37 hr, respectively, for these three stages of maturation.

Under normal conditions only segmented cells leave the marrow for the peripheral blood, although more immature cells are seen on occasion. However, these immature forms probably circulate in the blood longer than mature forms, and they do not seem to enter the exudates associated with fatal infections in patients with leukemia. Release of cells from the marrow is probably related to the concentration of granulocytes in the peripheral blood. It has been suggested that there is a leukocytosis-inducing factor (LIF) in the plasma of leukocyte-poor blood, and LIF affects the rate of cellular release from the marrow. Endotoxin also seems to stimulate the release of marrow granulocytes, and it has been suggested that stimulation with endotoxin be used as a standard method for determining a patient's ability to mobilize granulocytes to respond to infection.

The three distinguishable types of dividing cells in the proliferating marrow are the myeloblast, promyelocyte, and myelocyte, whose approximate number in a 70 kg man are given as 0.04, 0.11, and $1.8 \times 10^{11}$, respectively. There is scanty data on the generation times of these three types of cells. Present estimates are given as 18, 24, and 52 hr, respectively. An estimate for the DNA synthesis time, i.e., S-phase of the cells, is given as 12 hr for all the cells in the proliferating marrow.

The sequence of events in the proliferating marrow remains unknown. It is not clear that a simple pipeline process is operating here, and several schemes have been suggested to account for the limited amount of quantitative data available. The model uses a deterministic (nonrandom) scheme suggested by Cronkite and Vincent. They found that the best fit to the measured relative numbers of the three kinds of cells would result if they assumed that myelocytes recycle once before they enter the maturating marrow (i.e., mature to metamyelocytes). Specifically, it is assumed that after 18 hr a myeloblast divides to become two promyelocytes; after 24 hr a promyelocyte divides to become two myelocytes. Each of these new myelocytes then generates four metamyelocytes in the next 104 hr, two myelocytes at the end of the first 52 hr, and each of these two generate two metamyelocytes at the end of the last 52 hr.

Stem cells are the earliest precursors of the blood cells in the marrow, and they are capable of taking alternative pathways: either self-reproduction to produce more stem cells, or differentiation to the earliest cell type in the sequence of granulocyte development. Although it is still a subject of much controversy, there is much evidence that stem cells are pluripotent and can differentiate to any one of the blood cell lines. Stem cells are presently being intensively studied by quantitative investigations of the blood cell colony-forming ability of bone marrow cell suspensions under various conditions both in vivo and in vitro. In these experiments, stem cells have been tentatively identified as early forms of the colony-forming unit (CFU) or colony-forming cell (CFC). There is now also some evidence for a diffusible factor, colony-stimulating factor (CSF), which regulates the growth of these colonies. The origin of CSF remains unknown, although its production in mice seems to be associated with a decreasing peripheral neutrophil count, as well as with
a depletion of the marrow of polymorphonuclear cells. Recently, evidence has been presented that suggests that the cellularity of the stem cell block may be a major factor in the determination of the rate of replication of the stem cells. Self-replication seems to increase almost immediately after the block size (as measured by the number of CFUs) is reduced, and there is replication only without any differentiation when the block size is reduced below 10%.32

From counts of the number of colonies formed in the mouse spleen after injecting bone marrow cell suspensions, it has been estimated that there is approximately 1 CFC per 1000 bone marrow cells.27 The ratio of erythrocyte to granulocyte colonies in the spleen was found to be 3/1, while in the marrow the E/G colony ratio was 1/2.25 Estimates of the total number of marrow stem cells feeding granulopoiesis in the 70 kg man have been in the range 2.9–350 x 10^6.15 Since about 56% of all the hemic cells in the adult human marrow are of the granulocytic series (Table 3 in reference 16), then an approximate upper estimate of the total number of stem cells in the marrow of a healthy 70 kg man under steady-state conditions is 350 x 10^6/0.56 = 6.25 x 10^6. In the initial calculations for the model discussed in this paper, another estimate of 5 x 10^6 33 for the total number of stem cells was used. The doubling time of stem cells has been given as 16–31 hr.32

**POTENTIAL APPLICATION OF MODELING PROCEDURE**

The concepts and data presented in the previous section have been incorporated into a comprehensive mathematical model for granulopoiesis. Because of the limitations in space, it is not possible to present the details of the modeling procedure at this time. However, the above review of the literature distinguishes the quantitative data that were particularly important for the development.

<table>
<thead>
<tr>
<th>Table 1. Steady-State Values for a Normal 70 kg Man*</th>
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<tbody>
<tr>
<td>Number Cells X 10^10</td>
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<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Total stem cells</td>
</tr>
<tr>
<td>No. stem cells cycling</td>
</tr>
<tr>
<td>Proliferating marrow</td>
</tr>
<tr>
<td>Myeloblasts</td>
</tr>
<tr>
<td>Promyelocytes</td>
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<tr>
<td>Early myelocytes</td>
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<tr>
<td>Late myelocytes</td>
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<tr>
<td>Maturating marrow</td>
</tr>
<tr>
<td>Metamyelocytes</td>
</tr>
<tr>
<td>Band</td>
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<tr>
<td>Segmented</td>
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<td>TBGP</td>
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<td>GTR</td>
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Granulocyte count = 3850/cu mm.
Half time of residence in blood = 7.13 hr.
Chance granulocyte leaves for tissue spaces during hour = 0.093.
*These were the initial values used in computer calculations of toxic effects of 5-FU on the granulopoietic system.
The model was extended with a view to analyzing the effects of 5-fluorouracil (5-FU) on granulocyte kinetics in humans. The computer was programmed to simulate the dosage schedule and protocol of an actual study of the effects of 5-FU in patients with several types of advanced cancer. The computer program for the drug schedule was joined with the computer program for the model of granulopoiesis, so that it was possible to calculate a prediction for the hour-by-hour response of an ideal patient to the drug. This combination is an example of one of the many options previously discussed that are available when this modeling procedure is used. The initial values used in the calculations are listed in Table 1.

The daily WBC for the ideal patient is shown by the solid curve in Fig. 3. This calculated curve seems to be extremely erratic, and yet the predicted pattern, including the dip at 10 days and the overshoot at 20 days, is consistent with the pattern of WBC that has been reported for many of these patients. The data for a male, age 68 with cancer of the rectum are shown by the crosses in Fig. 3A, while the data for a female, age 47 with cancer of the large intestine, are shown in Fig. 3B. It should be emphasized that the curve for the ideal patient was not fitted to these data. The ideal curve is simply a consequence of the underlying cell kinetics as described by the comprehensive mathematical model. The hour-by-hour response to the drug of the various marrow phases of granulocyte kinetics was also analyzed with this model. Details will be presented in a future publication.

Fig. 3. Daily white blood count for a patient receiving 5-FU according to protocol of a clinical study. Solid curve is the same in both (A) and (B) and represents the predicted WBC every 24th hr as calculated from model. Crosses are the data for (A) a male, age 68, with cancer of the rectum, and (B) a female, age 47, with cancer of the large intestine.
ACKNOWLEDGMENT

I thank Dr. Irwin D. J. Bross for stimulating discussions during the formulation and development of the modeling procedure.

REFERENCES


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