Periodic Oscillation of Blood Leukocytes, Platelets, and Reticulocytes in a Patient With Chronic Myelocytic Leukemia

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A patient with chronic myelocytic leukemia had a cyclic oscillation of blood neutrophils, eosinophils, basophils, monocytes, platelets, normoblasts, and reticulocytes but not of lymphocytes. The cycle interval was 53–69 days. Except for reticulocytes, all other cells cycled with neutrophils. Plasma colony-stimulating factor (CSF) oscillated out of phase with neutrophils, suggesting that granulopoiesis is regulated through CSF by a feedback mechanism. Plasma erythropoiesis-stimulating factor (ESF) also oscillated. ESF crests preceded or coincided with reticulocyte crests, indicating that the ESF elevation may have been responsible for the reticulocyte peaks. The relationship between neutrophils and reticulocytes and their oscillations with plasma CSF and ESF suggests that there is a common stem cell which differentiates along one cell line or the other depending upon the balance of regulatory stimuli. The fraction of blood neutrophilic precursors (myeloblasts, promyelocytes, and myelocytes) in DNA synthesis fluctuated with neutrophil level. The calculated generation time was shorter at the crests than at the troughs of the neutrophil cycles. This finding suggested that the rate of proliferation of the neutrophils changed periodically. This observation, along with a periodic increase in differentiation of the stem cell toward the neutrophilic cells, is the probable explanation of oscillation of the neutrophil count in the blood.

The human pluripotent hematopoietic stem cell (PHSC) is generally believed to be the common precursor for erythrocytic, granulocytic, and megakaryocytic cell lines.1,2 In a mouse system, during periods of increased demand for erythrocytes, augmented differentiation of PHSC along the erythrocytic pathway is accompanied by a diminished differentiation along the granulocytic cell line.2 An erythropoiesis-stimulating factor (erythropoietin or ESF) in part regulates erythropoiesis.3 The in vitro agar colony-stimulating factor (CSF) is asserted to regulate granulocytopoiesis.3 Similarly, thrombopoietin is considered to regulate thrombocytopoiesis.3 The precise in vivo role of these putative humoral agents remains to be elucidated.

It might be assumed that the degree of differentiation of the PHSC along the erythrocytic, granulocytic, and thrombocytic pathways is at least partly governed by the relative intensity of these humoral agents. Certain observations concerning the cyclic oscillations of blood cells, ESF, and CSF in a patient with
Fig. 1. Total blood leukocyte (WBC), platelet, and reticulocyte (RETIC) counts, and hemoglobin (Hb) concentration are shown. Hb values are the moving averages of three consecutive points. R stands for initiation of a course of busulfan treatment during the study and T for transfusion of 2 units of packed red cells. In this and following figures, day 0 on the abscissa represents the time of discontinuation of busulfan.

chronic myelocytic leukemia (CML) have lent some credence to such a hypothesis and are the subject of this report.

CASE REPORT

A 73-yr-old black male was diagnosed as having CML at age 66. Philadelphia chromosomes were found in his bone marrow cells. Blood neutrophil alkaline phosphatase score was <20 (normal range, 40–120).

Initially he was treated with radiation to the enlarged spleen and subsequently with courses of busulfan. Hematologic remission was achieved with each course of busulfan.

Commencing in January 1972 and continuing for 348 days, weekly blood counts were done while he was off antileukemic therapy. He was found to have cyclic oscillations of WBC, platelets, and reticulocytes (Fig. 1) without detectable change in the spleen size. Following another course of busulfan in January 1973 (349-370 days of study), cyclic oscillations of his blood cells were again noted. He died of a “heart attack” elsewhere.

MATERIALS AND METHODS

Routine Hematologic Studies

Standard techniques were employed for routine hematologic studies. Blood nucleated cell differentials were done by counting 1000 cells for each blood sample point.

Cell Cycle Measurements

The fraction of blood myeloblasts ($M_1$) + promyelocytes ($M_2$) + myelocytes (large, $M_3$, and small, $M_4$) in DNA synthesis ($LI$) was determined at 1-3-wk intervals (covering two WBC cycles from days 172 to 266) by in vitro tritiated-thymidine labeling. The generation time ($T_g$) of proliferative neutrophilic precursors ($M_1$-$M_4$) was calculated from the equation: $T_g = DNA\ synthesis\ time (T_d)/LI$. $T_d$ of this patient's neutrophilic precursors ($M_1$-$M_4$) has been reported to be 14.2 hr. Due to the relative constancy of $T_g$, the previously reported $T_g$ value has been used in the current studies.
Assay for CSF and ESF

Blood samples were collected at 1–2-wk intervals in sterile heparinized tubes; plasma was separated and frozen at –20°C until tested for CSF and ESF.

CSF activity of all plasma samples was assayed simultaneously by an in vitro method using canine marrow cells as target cells.6 Three × 10⁵ marrow cells per plate and three to five plates per plasma sample (0.15 ml/plate) were used. The cultures were evaluated on day 10 and a group of > 50 cells was scored as a colony. In vitro colony-inhibitory activity was not tested in these plasma samples.

Plasma ESF was assayed in exsphyxic polycythemic mice.7 ESF assay of the plasma samples collected during days 185–234 was done separately from those collected between days 241 and 290 and between days 355 and 397. Plasma samples were injected on posthypoxic days 5 and 6 (1 ml intraperitoneally per injection per day). ⁵⁹Fe was injected via a tail vein on posthypoxic day 7, and 72 hr later uptake in red cells determined.⁷ Each assay group consisted of six to seven mice. A saline control and at least three concentrations of standard ESF (sheep erythropoietin, Connaught Laboratories, Toronto, Ont., Canada) were included in each assay. Exsphyxic mice with hematocrits of less than 55% were not included for ESF assay. ESF greater than 0.03 U/ml of plasma could be detected by this technique.

Curve Fitting and Correlation

This procedure was done to test if there was any regularity of the periodicity of oscillations of the various parameters evaluated, especially the CSF, ESF, and reticulocyte levels. A computer model was developed involving the use of an iterative least squares method, and this was applied for the various parameters tested.

Total leukocyte count (WBC) = \( A + Bt + Ce^{Et} \cos[(t + E) \times F] \) (Eq. 1)

where \( t \) = time in days; \( e \approx 2.718 \cdots; A-F \) = adjustable parameters; \( \cos(\cdot) \) = cosine for argument in degrees.

The numerical values found by the computer are indicated in Eq. 2, and the curve is plotted with the experimental data in Fig. 2B:

\[ \text{WBC} = 31377 + 464t + 37696e^{0.00165(t + 3)} \cos(5.941) \] (Eq. 2)

A similar function was fitted to the reticulocyte count, but assuming \( B \) and \( D \) to be zero, with the result given by Eq. 3 (Fig. 2A):

\[ \text{Reticulocyte count} = 65.43 + 16.59 \cos[(t - 23) \times 5.916] \] (Eq. 3)

Fig. 2. Reticulocyte (A) and WBC (B) data of the patient are fitted to curves obtained by a computer model. For details see Methods section. Patient’s experimental data points are shown by open circles (○○) and the computed points by solid circles (●●).
The hemoglobin (Hb), reticulocyte, WBC, and platelet counts are shown in Fig. 1. The WBC level oscillated with the platelet level at a crest-to-crest interval of 53-69 days. The reticulocyte level oscillated out of phase with WBC at a cycle time similar to WBC (Fig. 1). Computer-generated curves indicated that WBC (Fig. 2B) and reticulocyte level (Fig. 2A) were oscillating with a regular periodicity. The correlation between experimental and computed values for CSF, however, was not as good as for the other parameters (Fig. 3A).

The reticulocyte level oscillated with no recognizable oscillation in the Hb level except for a slow steady reduction until 2 units of packed red cells were given simultaneously with the initiation of busulfan treatment (Fig. 1). In addition, the Hb level per se did not appear to influence the magnitude of individual reticulocyte peaks or troughs (Fig. 1).
Fig. 4. (A) Reticulocyte counts in absolute numbers are shown. (B) Plasma erythropoiesis-stimulating factor (ESF) in units per ml (- - or - -) is fitted to a curve obtained by a computer model (- - -). See Methods section for details. The ESF activity is expressed in two scales: ESF activity from the plasma samples obtained during the first cycle studies (- -) was assayed separately from the samples of the other two cycles studied (- - -).

Absolute counts of the various nucleated blood cells are shown in Fig. 5. With the exception of lymphocytes, all nucleated cells appeared to cycle with WBC. The major fraction of WBC was represented by the neutrophilic cells (75%-95%). Since sampling was done weekly, a precise sequential maturation pattern of the neutrophilic series could not be established. Nonetheless, there seemed to be a simultaneous or sequential rise and fall in various stages of neutrophilic cells. The average ratio in the blood of $M_{2}+M_{3}:M_{4}$ was 1:8.5 at the troughs and 1:13.4 at the crests. This ratio is 1:12.3 in bone marrow of normal man.

CSF oscillated generally out of phase with WBC. The in vitro LI of the neutrophilic cells paralleled the WBC oscillation (Fig. 3). The calculated $T_g$ of proliferative neutrophils (essentially $M_3$ and $M_4$ since $M_1$ and $M_2$ constituted a very small fraction of the total) during the crests of WBC on days 185 and 241 was 91 and 65 hr, respectively. The corresponding values for the succeeding troughs on days 213 and 266 were 177 and 175 hr, respectively.

For the three cycles investigated, ESF peaks occurred simultaneously with the second and third reticulocyte peaks but somewhat earlier than the first reticulocyte peak (Fig. 4).

**DISCUSSION**

Cycling of the blood WBC in CML patients has been shown to be due to periodic contraction and expansion of the total blood granulocyte pool and not due to any selective shifting between the circulating and marginalizing pools.
A similar periodic fluctuation of the total blood neutrophil pool was probably occurring in this patient. WBC cycle length was 53–69 days in this patient; in other reported cases it has ranged from as short as 30–35 days to as long as 100–120 days. The survival time of neutrophilic cells is known to vary in CML patients. However its variation is in hours and is not likely to be a significant cause of the variation of the cycle time.

Cyclic blood WBC changes in this patient were primarily related to the periodic variations in the proliferation of neutrophilic cells. Increased rate of proliferation during the WBC crests was demonstrated by a reduction in the $T_g$; and the decreased rate of proliferation during the troughs by a prolongation in the $T_g$. The increased proliferation of the cells during the WBC crests (as compared to WBC troughs) occurred to a relatively greater extent in the $M_{1+4}$ cell compartment because of the shift in the ratio of $M_{1+2} : M_{1+4}$ cells in favor of $M_{1+4}$. Since there was some increase in $M_{1+2}$ cells in addition to the $M_{1+4}$ cells at the WBC crests, there was a contribution to the crests formation by the $M_{1+2}$ cells. Whether the $M_{1+2}$ cells increased during WBC crests due to additional division within the compartment or increased input from PHSC was not clear.

This patient had markedly increased blood neutrophil counts at the times of estimation of longer than normal $T_g$ for neutrophilic precursors (Fig. 3B). The values in this patient ranged from 65 to 177 hr in contrast to 52 hr for myelocytes in normal man. This increased number of neutrophilic cells was probably derived from the expanded myelopoietic areas in the peripheral parts of long
bones as demonstrated by the \(^{99m}\)Tc sulfur colloid marrow scanning. Prolonged survival of blood neutrophils has been reported for CML patients\(^1\), which may have contributed to the increased blood neutrophil level.

Granulocytopoiesis in normal man is believed to be controlled in part by a negative feedback mechanism between blood and marrow through the humoral factor CSF\(^3\). Decreased blood neutrophils below a critical level result in increased CSF activity which, in turn, induces proliferation and differentiation of the PHSC along the neutrophilic cell line. With the restoration of “normal” blood neutrophil level, CSF activity diminishes with consequent reduction in the neutrophil production. Negative feedback regulation of granulocytopoiesis was probably operating through CSF in this patient, as suggested for normal man\(^3\), since the neutrophil count and CSF level oscillated out of phase with one another. However, there were quantitative deviations from normal in the regulatory system especially in cycle time, which ranged from 53 to 69 days in this patient as opposed to 14–23 days in normal man\(^3\), and in the magnitude of neutrophil changes, which ranged from normal to \(250.1 \times 10^3/\text{cu mm}\) in this patient as opposed to \(1.3–7.4 \times 10^3/\text{cu mm}\) in normal man\(^4\).

Stimulation of differentiation of the PHSC by the CSF into granulocytic pathways might be responsible for the oscillation of eosinophils and basophils along with neutrophilic cells (Fig. 5). This observation suggests a common immediate parent cell (committed stem cell) for granulocytes.

WBC counts were inversely related to reticulocyte level (Fig. 2). This inverse pattern of proliferation could be ascribed to: (1) a competition for the PHSC to proliferate along the neutrophilic or erythrocytic cell line depending upon the relative intensity of CSF and ESF, and/or (2) an accelerated proliferation of one cell line induced by its appropriate humoral factor resulting in overcrowding of the available marrow space leading to inhibition of proliferation of the other cell line. The available information in the literature favors the former notion\(^2\).

The relationship between the ESF and reticulocyte count (Fig. 4) indicates that the elevated ESF may be responsible for the reticulocyte peaks. The cause of ESF fluctuation is not known.

The reason for platelet and normoblast oscillation with the neutrophilic cells is not clear. It is possible that the overcrowded marrow was pushing the normoblasts and platelets mechanically into the circulation, although other alternatives have also been proposed\(^9\). Monocytes have been proposed as one of the sources of CSF\(^1\). In cyclic neutropenia, monocytes oscillate with CSF and out of phase with neutrophils\(^1\). In this patient, blood monocyte oscillations neither corresponded with the CSF level nor were they inversely correlated with the WBC level. Further discussion of this aspect is not appropriate, as the total body monocyte mass is not known.

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REFERENCES

3. Stohlman F Jr: Stem cell regulation, in


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