Cyclic Oscillation of Blood Neutrophils in a Patient With Multiple Myeloma

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A patient with multiple myeloma developed periodic blood neutropenia (periodicity of 15–25 days) after 3 yr of intermittent treatment with cytotoxic agents. Peaks of serum colony-stimulating activity (CSA) level coincided with valleys of blood neutrophils. Fraction of marrow neutrophils in the multiplicative pool was high during blood neutrophil valleys and low during neutrophil peaks. In contrast, the maturation storage pool exhibited the reverse pattern. An increased fraction of marrow neutrophilic cells in the multiplicative pool was active proliferation during a blood neutrophil valley and a decreased fraction during a blood neutrophil peak. These findings suggest that the marrow granulopoiesis was regulated through CSA. The defect causing the periodicity was probably related to the reduced number of neutrophils in the marrow maturation storage pool, which in turn may be related to a reduced and/or defective granulocytic stem cell pool size consequent to the long-term administration of cytotoxic drugs and/or infiltration of the marrow by myeloma cells.

Neutrophil mass in normal man can be divided into (1) committed granulocyte stem cell pool (CFU-C); (2) multiplicative pool, consisting of myeloblasts (M0), promyelocytes (M1), and large (M3) and small (M7) myelocytes; (3) maturation storage pool, consisting of metamyelocytes (M4), band forms (M5), and segmented forms (M6); and (4) blood pool, in which most of the cells are M3 with small numbers of M4 and CFU-C and rarely M1, M4, and M7. The blood neutrophil level is thought to be regulated by two feedback loops mediated by the neutrophil-releasing factor (NRF); [also known as leukocyte-tosis-inducing factor] and colony-stimulating factor (CSF).12 The concept states that when the blood neutrophils decrease to a low normal level, plasma NRF increases, which mobilizes mature cells from the marrow maturation storage pool into the blood. Plasma CSF also increases in response to the decreased blood neutrophil level. The CSF is believed to induce an increased rate of proliferation and differentiation of CFU-C and of cells in the multiplicative pool.24 Thus, cyclic oscillations of the bone neutrophil level are expected in normal individuals but not seen or seen infrequently5 because of the damping effect of the large reserve of mature neutrophils in the marrow maturation storage pool. Cyclic oscillation of the cells in the blood has been demonstrated after reduction of the marrow maturation storage pool in dogs by cyclophosphamide treatment.6 A patient with multiple myeloma developed cyclic neutrophil oscillation after 3 yr of intermittent treatment with cytotoxic agents. This case may represent a human equivalent of the dog model and thus suggests dangerous depletion of marrow neutrophil resources.

CASE REPORT

A white male physician was 60 yr old in 1975. A diagnosis of multiple myeloma was accidentally made in July 1972 when his plasma proteins were determined in a laboratory control study. He was in good health, playing tennis daily, and accordingly, therapy was not initiated immediately following the diagnosis. Hemograms during the 6 mo after diagnosis and before treatment with cytotoxic drugs revealed mild anemia (Hb 11.9–12.9 g/dl) with anisopoikilocytosis of the RBC. The total leukocyte count ranged from 2.7 x 10³/cu mm to 3.5 x 10³/cu mm. The differential count showed a normal distribution. Platelet counts were normal. Bone marrow aspirates showed an increased fraction of abnormal plasma cells (24%–30%) with immature nuclei, one or more nucleoli, multinuclearity, and large bizarre forms. Dyserythropoiesis was present as reflected by macronormoblasts, ringed sideroblasts, and nuclear fragmentation in the absence of folic acid, vitamin B12, or B6 deficiency. Red cell precursors were 19% of the total marrow nucleated cells. Maturation in the granulocytic series was normal, with 0.5% M1, 9.5% M3, and 25% M4 and M5 at their lower normal limit.7 Megakaryocytes appeared normal in number and morphology.

Serum IgG (kappa type) was markedly elevated (7–8 g/dl) with a reduction in other immunoglobulins. Twenty-four-hour urine collections contained up to 0.5 g kappa-type light chain.* Multiple osteolytic lesions were demonstrated in skeletal roentgenographs. The patient was treated intermittently with cytotoxic agents. t-Phenylalanine mustard (t-PAM) and prednisone were given from January 1973 to November 1973, cyclophosphamide and prednisone from December 1973 to July 1975, two doses of i.v. t-PAM in August 1975, and one dose of i.v. 1,3 bis (2-chloroethyl)-1-nitrosourea (BCNU) in September 1975 and another in October 1975 (Fig. 1). Anemia unresponsive to Halotestin (fluoxymesterone), folic acid, vitamin B12, and B6 required red cell transfusion. The patient died of Escherichia coli sepsis in November 1975, 40 mo after diagnosis. Autopsy revealed the presence of extensive multiple myeloma.

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From September 1975 until death in November 1975, frequent blood samples were obtained, and sera were separated and frozen at 0°C. All samples were assayed simultaneously for in vitro colony-stimulating activity (CSA) with canine marrow nucleated cells (2 x 10³/plate) as target cells, according to the method of Marsh et al.1 For each serum sample, 3–5 plates with 0.1 ml of serum in each plate were prepared, and they were evaluated on day 10, when the colony counts were maximum. Granulocytic colony inhibitory activity (CIA) of these samples was not assayed.

Bone marrow aspirates were obtained at approximately 1-wk intervals during the last 2 mo of life to correlate marrow cellularity with blood neutrophil levels.

Fig. 1. Blood neutrophilic band and segmented forms are shown along the vertical axis and the dates along the horizontal axis. Treatments given are noted at the top. See text for definitions of abbreviations.

With each course of chemotherapy, the blood neutrophil and platelet levels decreased, and following discontinuation of the drugs, returned toward normal. From August 1975 until death the level of neutrophils, but not the platelets, oscillated relatively independently of the administration of cytotoxic agents. Monocytes and reticulocytes did not oscillate.

MATERIALS AND METHODS

From September 1975 until death in November 1975, frequent blood samples were obtained, and sera were separated and frozen at 20°C.
Marrow $M_1$, and plasma cell deoxyribonucleic acid (DNA) content was determined with a Zeiss automatic scanning microcytometer attached to a PDP-12 computer. DNA content data were expressed as fraction of cells with 2N DNA content and with >2N DNA content. The former includes most cells in pre-DNA synthesis (G1 and G0), and the latter includes most cells in DNA synthesis (S), post-S (G2), and mitosis (M). The in vitro incorporation of tritiated thymidine ($^3$HTdr) into DNA of $M_{1}$ and plasma cells (labeling index, LI) was also evaluated in the same preparations in which DNA content was measured. The DNA content and LI were determined on two marrow aspirates, one obtained at a peak blood neutrophil level (10/31/75) and the other at a valley (11/12/75). The aspirates were mixed with $^3$HTdr (specific activity, 1.9 $\mu$Ci/ml) and incubated at 37°C for 30 min. Smears were made, fixed in absolute methanol, and stained by the Wright-Giemsa method. Clearly recognizable $M_1$ and plasma cells not touching other cells were identified and the coordinates recorded on magnetic tapes for later recall and determination of DNA content after removal of Wright-Giemsa stain and staining by Feulgen. Photographic emulsion was then applied and slides exposed as described earlier. The mapped cells were recalled and those having >3 silver grains overlaying the cell were considered labeled.

**Curve Fitting**

The blood neutrophil and serum CSA levels were fitted independently over time to a nonlinear cyclical model to determine if a regular oscillation existed. The relationship between the two variables was also studied by correlating the experimental points of blood neutrophil level with those of CSA level shifting 10 days, or half the determined length of each cycle. The equations used were as follows:

$$ Y = A + C \cos [(t - E) F] $$

where $Y$ = variable (CSA or blood neutrophil level), $t$ = time in days on horizontal axis (time at which $Y$ is measured), $A$ = mid-value of $Y$ (time between peak and valley), $C$ = half height in units of $Y$ (between peak and valley), $E$ = starting time of lowest valley, and $F$ = parameter related to cycle length; that is, $t = E + i (360/F)$ where $i = 0, 1, 2, \ldots$ yields the times of successive valleys, $360/F =$ cycle length in days, and $C =$ cosine length for argument in degrees.

Least squares estimates of the parameters are given as:

- Blood neutrophil level
  $$ Y = 2522 - 1483 \cos [(t - 12.82) \times 18] $$

- CSA level
  $$ Y = 10.85 - 4.99 \cos [(t - 3.89) \times 18] $$

**RESULTS**

Blood neutrophil level, time, and dose of administration of chemotherapeutic agents from August 2 to November 26, 1975, are shown in Fig. 1. The neutrophil level oscillated at intervals of 15–25 days. Both the blood neutrophil and serum CSA values obtained during the last 2 months of the patient's life are shown in Fig. 2. The peaks of one parameter coincided with the valleys of the other. The correlation coefficient between the blood neutrophil and serum CSA values, when tested by shifting one parameter by 10 days and keeping the other parameter fixed, is 0.667 with a $p$ value of <0.001.

Bone marrow differentials revealed that the percentage of $M_{1.1}$ was maximal and that of $M_{5.5}$ was minimal at the time of the valley of the blood neutrophil level; whereas the percentage of $M_{1.4}$ was minimal and that of $M_{5.1}$ was maximal at the peak (Table 1). No definite pattern was seen in the fluctuations of the marrow erythroid cells, monocytes, and plasma cells.

### Table 1. Marrow Neutrophilic Cell Differential Counts Expressed in Percent

<table>
<thead>
<tr>
<th>Date</th>
<th>9/26/75*</th>
<th>10/8/75</th>
<th>10/15/75</th>
<th>10/21/75</th>
<th>10/31/75</th>
<th>11/12/75</th>
<th>11/17/75 Ascending Limb</th>
<th>11/24/75 Peak</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>M1.4</strong></td>
<td>14.6</td>
<td>21.6</td>
<td>15.0</td>
<td>36.5</td>
<td>16.3</td>
<td>49.0</td>
<td>19.0</td>
<td>7.9</td>
</tr>
<tr>
<td><strong>M5.1</strong></td>
<td>34.4</td>
<td>7.2</td>
<td>35.2</td>
<td>6.0</td>
<td>30.9</td>
<td>7.0</td>
<td>31.0</td>
<td>61.3</td>
</tr>
</tbody>
</table>

*The marrow aspirates for differential counting were obtained approximately at the peaks and valleys of the blood neutrophil cycle. The peak represents the maximum neutrophil level, and valley, the minimum level during each blood neutrophil cycle.

†See text for descriptions.
DNA content and LI data are shown in Table 2. M$_{1.4}$ with >2N DNA content comprised 5.1% at the peak and 32.8% at the valley of the blood neutrophil level; the LI of the M$_{1.4}$ was low (7.1) at the peak and high (41.3) at the valley. In contrast, plasma cells showed no remarkable change in DNA content and LI values between the two time points examined.

**DISCUSSION**

The computer model assisted in demonstrating that the periodicity of the blood neutrophil and serum CSA level oscillations was regular. The values given in equations 2 and 3 were useful as nonsubjective estimates of the biologic parameters of the model for the two variables. The oscillations of these two variables were out of phase with one another. The blood neutrophil level oscillated from August 1975 until death in November 1975 in this patient. The neutrophil periodicity was similar to that reported in patients with idiopathic cyclic neutropenia. Although in many reported cases and in grey collie dogs with cyclic neutropenia, the levels of blood reticulocytes, platelets, and/or monocytes also oscillated with a regular periodicity, such an oscillation was not observed in this patient. The mechanism of blood neutrophil oscillation in idiopathic neutropenia involves the periodic reduced formation of granulocytic progenitors, CFU-C, and consequently, of their progeny. When the marrow neutrophil maturation storage pool is contracted due to a reduced formation, the cell entry into the blood is decreased, and consequently, the blood neutrophil cycle valley appears. Conversely, when the marrow neutrophil production is recovered, the maturation storage pool is replenished, and the cell entry into the blood is reestablished, resulting in the blood neutrophil cycle peak. A similar mechanism could be incriminated for cyclic oscillation of blood neutrophil level in our patient, and a periodic variation in the formation of neutrophilic cells in the marrow occurred, as reflected by the marrow differential counts, DNA measurements, and in vitro label with $^3$HTdr.

The average duration from valley to peak of the blood neutrophil cycle was about 10 days in our patient, the time estimated by Cronkite and Vincent for a myeloblast to proliferate, mature through each stage, and emerge into the blood as a segmented neutrophil in normal man. In this case, increased numbers of cells were forming during the valleys of the blood neutrophil cycle, and after differentiation and maturation, they were entering the blood with normal transit time and producing the peak of the cell cycle about 10 days later.

The serum CSA oscillated out of phase with the blood neutrophil level, as has been documented in serum or urine of many patients and dogs with idiopathic cyclic neutropenia as well as in patients with chronic myelocytic leukemia with cyclic oscillations of blood neutrophil levels. The findings of simultaneously elevated serum CSA and increased marrow granulocytosis in this patient supports the concept that the CSA may be the in vivo regulator of granulocytosis. The concept assumes that the CSA is a soluble inhibitor of neutrophil production and that its reduced level results in appropriately reduced stimulation of the CFU-C and M$_{1.4}$ proliferation. An alternate possibility is that the serum CSA level oscillates because of the oscillations in the total body neutrophil level. In this case, the neutrophil liberates the CIA and the quantity liberated is proportional to the body neutrophil mass, and the CIA suppresses the release of CSA from the CSA-producing cells. Although serum CIA was not assayed in this study, the knowledge that it interferes with the release of CSA rather than acting on the target cell makes the interpretation that the CSA influences in vivo granulopoiesis a reasonable one. Another explanation for periodic variation in granulocytosis is that increased concentration of mature cells in the marrow during the blood neutrophil cycle peak may inhibit granulocytosis by cell-to-cell interaction, and the lower density of mature cells during the blood neutrophil cycle valley may permit...
increased granulocytopoiesis in the marrow. Diffusion chamber studies have shown that granulocytopoiesis is higher when the marrow cell inoculum is less, and vice versa.24 If a cell-to-cell interaction is controlling marrow granulocytopoiesis, then the finding of cyclic oscillation of serum CSA out of phase with blood neutrophil level is difficult to explain.

In our patient, the cyclic oscillation of blood neutrophil level does not appear to have been due to the immediate intermittent myelosuppressive effect of periodic administration of cytotoxic agents. Maximum blood neutropenia is expected, with pharmacologic doses, at about 10 days to 3 wk and 4-6 wk after administration of l-PAM and BCNU, respectively.25,26 The low points of blood neutrophil level did not occur at the expected time points after administration of the drugs, although the peak of the blood neutrophil level in early September 1975 may have been somewhat decreased by the administration of l-PAM on August 26 (Fig. 1). Further, the last two cycles occurred when no cytotoxic agents were administered.

Neutrophil oscillation continued in our patient in spite of daily administration of prednisolone, as opposed to a case reported recently in which the prednisolone administration obliterated the oscillation,27 and the reason(s) for the difference is not clear.

The basic defect causing intermittent variation in marrow granulocytopoiesis in this patient seems to have been a reduction in total marrow granulocyto- poietic tissue (and consequently, in the marrow maturation storage pool). The reduction may have been related to the long-term administration of drugs, as in dogs treated with cyclophosphamide,6 and/or to infiltration of the marrow by plasma cells. In two patients with lymphoproliferative disorders, the development of cyclic oscillations of blood neutrophil levels has been reported,28,29 accompanied in one patient by infiltration of abnormal cells into the marrow.28 The oscillation in these two cases, with no family history of cyclic neutropenia, may have been due to reduced formation of granulocytes, as in our case. Alternatively, the CFU-C or its earlier forms may be defective due to the disease and/or the administration of the chemotherapeutic agents, resulting in periodic failure to form neutrophils. Irrespective of the explanation, the appearance of cycling neutrophils may be a good indicator of severe marrow depletion, suggesting that chemotherapeutic drugs should be discontinued to allow normal marrow function to resume. Further careful studies of the CSA and CIA are required to answer the role of each of these factors more definitively in the regulation of marrow neutrophil production.

REFERENCES

7. Kall K: Differential cell count in normal bone marrow aspirate, in Bone Marrow Interpretation. Springfield, Ill, Charles C Thomas, 1973, p xii


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G Chikkappa, AD Chanana, P Chandra, EP Cronkite and KH Thompson