Cyclic Neutropenia: Amplification of Granulocyte Oscillations by Lithium and Long-Term Suppression of Cycling by Plasmapheresis

By Gustav K. von Schulthess, Jörg Fehr, and Clemens Dahinden

A patient with well documented cyclic neutropenia (CN) was given chronic lithium therapy as well as a short course of plasmapheresis with therapeutic intention. While on lithium therapy, an increase in the amplitude of the granulocyte oscillations was observed, but recurrent agranulocytotic periods persisted. A 2-wk course of plasmapheresis (total exchange 17 liters) resulted in a gradual decrease of granulocyte oscillations, with the granulocyte count remaining above 500/cu mm at all times, and the patient became asymptomatic. The improvements in the clinical state of the patient have now persisted for more than 9 mo.

Quantitative analysis of the oscillation period and the phase lags between peak counts of the hematologic cell lines revealed a 20-day cycle for granulocytes, monocytes, reticulocytes, and platelets, but not for lymphocytes. The observed phase lags, together with the literature values for the intramarrow maturation times of the hematologic cell lines, suggest that the feedback regulation abnormality, believed to be at the basis of CN, primarily affects the stem cell that is still capable of differentiating into granulocytes and monocytes, but that the oscillations of reticulocytes and platelets are the result of a stem cell competition for uncommitted precursor cells by the primarily oscillating granulocyte and monocyte production.

Human cyclic neutropenia (CN) is a rare disorder that has been thoroughly studied over the past decade because it promises to offer considerable insight into the mechanism by which granulocytopenia is regulated. The disorder is characterized by a periodic disappearance of neutrophils from the circulation with a period of about 20 days. It appears that this is the result of a cyclic interruption of myelopoiesis at an early precursor level, as the numbers of myeloid precursors in the bone marrow sequentially peak before the maximum number of neutrophils appear in the periphery. Oscillations of other hematologic cell lines (monocytes, reticulocytes, and platelets) occur with the same period, but with various phase lags relative to the neutrophil oscillations. The essential abnormality of this disorder can be understood quantitatively within the framework of a simple model that has been proposed recently.

The clinical course of CN is usually fairly benign, the major symptoms being fever, aphthous stomatitis, and occasional cutaneous and subcutaneous infections. Although the disease is congenital in the animal model of the grey collie dog, there may exist acquired forms of this disease in humans. Therapeutic interventions to cure CN have had only limited success. Most notably prednisolone has cured one patient, but in others, this therapy has failed. Lithium was found to diminish neutrophil oscillations in grey collies and in one or two cases in humans. Similarly, endotoxin diminishes granulocyte oscillations in grey collies, but for obvious reasons it has not been used in man.

Encouraged by the reports on lithium therapy in grey collies, and based on the hypothesis that CN in man may be due to the presence of an abnormal humoral factor, we treated a patient with well documented CN first with lithium and secondly with a short course of sequential plasmaphereses. Furthermore, we have carefully analyzed the phase relations between the various oscillating hematologic cell lines, as there exists some controversy regarding their mutual phase shifts, with these phase shifts being an important clue to the pathophysiology of the disease.

CASE DESCRIPTION AND METHODS

A 70-yr-old woman was admitted to the Medical Clinics of the Zurich University Hospital in September 1980 with fever and localized skin infections. For the 11 yr previously, she had suffered from occasional polyarthralgias, recurrent localized infections, mainly of ears, nose and throat, aphthous stomatitis, as well as skin infections. On several occasions she was found to have a transient agranulocytosis. The rest of her previous medical history and her family history were unremarkable. Her physical exam on admission showed necrotizing lesions on two of her fingers and the left elbow. The laboratory workup showed granulocytosis; Staphylococcus aureus was cultured from her skin lesions, and therapy was instituted first with cefoxitin and later with trimethoprim-sulfamethoxazole. The patient made an uneventful recovery. From that time on, the patient was studied by repeated differential blood counts, usually taken three times per week for much of the ensuing 1.5 yr. While on lithium, a level of 0.8–5 mmole/liter was maintained by administering lithium carbonate (750 mg/day). A 14-day course of plasmapheresis was carried out using venous access through a subclavian catheter. In 6 sessions, a total volume of 17 liters was exchanged. Five bone marrow aspirates were obtained over 35 days when the patient received no treatment. A total of 200 cells were differentiated per smear.

The oscillation period as well as the phase relations between the various cycling blood counts were obtained by computing the granulocyte-granulocyte autocorrelation function and the crosscor-

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relation functions between monocyte, reticulocyte, platelet, and lymphocyte counts on one hand and the granulocyte count on the other hand. The intercepts of these curves with the time axis or their maxima or minima provide the oscillation period, as well as the various phase lags between the appearance of the different cell lines, with a great degree of statistical accuracy.

RESULTS

Initially, the patient showed the oscillations in her peripheral neutrophil count, which are typical for CN. This can be seen in Fig. 1A, where the neutrophil counts for cycles 2–4 postadmission are shown. In addition, the monocyte count is also plotted in Fig. 1A, its maximum typically preceding the neutrophil peak by several days. Also in Fig. 1A are represented three of a total of six cycles on lithium therapy (750 mg lithium carbonate). As can be seen, the amplitudes of the neutrophil count were increased by approximately a factor of 2 compared to the previous cycles. However, no change was observed in the number of agranulocytic days per cycle. No statistically significant increase in the average monocyte level was observed.

In Fig. 1B are the reticulocyte and platelet counts during the same observation period as in Fig. 1A. Although some fluctuations in the cell counts of these two cell lines were apparent, cyclic fluctuations were by no means obvious. Under lithium therapy, there

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Fig. 1.  (A) Blood counts of the granulocyte and monocyte series for a total of 6 CN cycles. The first 3 were obtained in the untreated patient; the second 3 while the patient was on lithium carbonate (750 mg/day). (○—○) Granulocytes. (■—■) monocytes. Ordinate: $10^9$ cells/cu mm. The typical 20-day oscillations in both series are observed. (B) Blood counts of the reticulocyte and platelet series for the same 6 CN cycles as in (A). (○—○) Reticulocytes. (■—■) platelets. Ordinate: $10^9$ cells/cu mm. The fluctuations in these cell counts are much less apparent than those in the granulocyte and monocyte series.
appeared to be an increase in the average platelet level from approximately 400,000 cells/cu mm to approximately 450,000 cells/cu mm; no change in the reticulocyte count was observed.

In addition, five bone marrow differential counts were obtained at various times during the CN cycle. The data are not represented here, but we find, in agreement with previous authors, that the earliest granulocyte precursors peak when the mature bone marrow and the peripheral granulocytes are at their nadir, and vice versa.3,5,7

A graphic representation of the short-term effects of a series of plasmaphereses is presented in Fig. 2 (days indicated by arrows on the figure). The first plasmapheresis was done just when the granulocytes started to appear in the periphery again. It is noted that (1) the regular cycling subsides after plasmapheresis, the peak neutrophil counts appearing at intervals longer than 20 days; (2) there is a decrease in the neutrophil amplitude due to a decrease in the peak absolute neutrophil count, but also, we no longer observe days without granulocytes in the peripheral circulation (200 PMN/cu mm); and (3) the monocyte oscillation pattern has disappeared altogether.

In Fig. 3, serial PMN and monocyte counts of the patient, taken approximately 8 mo after plasmapheresis, are represented over a period of 38 days. There are 2 PMN peaks about 20 days apart, but even at the nadirs, the PMN counts remain around 500 cells/cu mm; hence, on no occasion was there total absence of PMN 8 mo after plasmapheresis. This contrasts with the regular agranulocytic periods of several days in the untreated patient (see Fig. 1A). Finally, in Fig. 3, no regular oscillations are apparent in the monocyte count. The clinical state of the patient has improved dramatically, and she no longer suffers from recurrent infections since her admission for plasmapheresis.

Using the mathematical constructs of auto- and crosscorrelation functions (see Appendix) we determined the exact oscillation periods and phase lags between the oscillations of the granulocytes, monocytes, reticulocytes, platelets, and lymphocytes based on four off-therapy cycles (80 days) in our patient. The results are presented in Table I. The oscillation period for granulocytes, monocytes, reticulocytes, and platelets are all very close to 20 days. No statistically significant periodic oscillations were found in the lymphocyte counts. Assuming that the maximum granulocyte count occurs at day zero, the maximum monocyte, reticulocyte, and platelet counts occur at -6.5 days, (i.e., -0.33 of a 20-day cycle), at -8.5 days (i.e., -0.43 of a 20-day cycle), and at +2.0 days (i.e., 0.10 of a 20-day cycle), respectively. The mean cell counts are found to be within the normal range except for the
granulocyte count, which is markedly below normal. Also, the platelet count is at the upper limit of normal.

**DISCUSSION**

Of the various treatment modalities tested in cyclic neutropenia so far, the only successful long-term therapies have been achieved by chronic administration of prednisolone in one human and by lithium in one or two cases. In other patients, the steroid treatment has apparently failed. Based on our results, we conclude that a short course of plasmapheresis represents a third possible treatment modality for CN that can yield remissions over time spans of many months. The remission in our patient has now lasted for about 9 mo and is characterized by the absence of clinical symptoms and strongly reduced granulocyte oscillations. Periodic oscillations in other hematologic cell counts are no longer evident. It is unlikely that the remission could be a delayed effect of the lithium therapy, as plasmapheresis was started more than 8 mo after starting and 3 mo after discontinuing the lithium therapy. Even though a spontaneous remission of cycling in our patient cannot be excluded altogether, it is extremely unlikely, as the granulocyte counts in CN patients are known to cycle consistently over very long time spans. In fact, our patient had been followed for almost 1 yr before plasmapheresis was started, and she showed agranulocytoses of several days during all cycles.

Lithium therapy itself had an immediate effect, in that it increased the amplitude of the granulocyte and platelet counts without altering the lengths of the agranulocytotic periods. Thus, lithium had a similar effect on the peripheral blood count of our patient as it has in normals. Although our case report suggests that lithium therapy, like prednisolone therapy, is not a universal treatment for CN, the lithium levels maintained in our case were somewhat lower than those reached in the treatment of grey collies, and the administered dose was 750 mg lithium carbonate per day, rather than 900 mg as in the patient reported to respond to lithium treatment. Nevertheless, the serum levels maintained in our patient have been shown to be adequate to induce granulocytosis in normals (≥0.55 mmole/liter).

The etiology of CN is not known, but all evidence points to a periodic failure of granulopoiesis at an early stage of hematologic cell differentiation. Recently, a quantitative model has been proposed that emphasizes that the disease may be caused by an abnormality in the feedback regulation mechanism of granulopoiesis itself, rather than the result of a reduction in the pool of granulocyte precursors available for differentiation. As plasmapheresis can apparently have long-term effects on various pathologic processes, it is possible for it to interfere with such feedback regulation mechanisms. Such interference must be the result of a plasmapheretic removal or the addition of some factors that affect long-lived hematologic or lymphoid cells involved in the regulation of granulocyte production. If plasmapheresis would only result in an alteration of the plasma protein composition without effects on some long-lived cell populations, it would be diffi-

<table>
<thead>
<tr>
<th>Oscillation Period (Days)</th>
<th>Phase Relation to Granulocyte (Days)</th>
<th>Mean Cell Count (Cells/cu mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulocytes</td>
<td>20 ± 0.5</td>
<td>307</td>
</tr>
<tr>
<td>Monocytes</td>
<td>20 ± 0.5</td>
<td>328</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>19 ± 1.0</td>
<td>75,000</td>
</tr>
<tr>
<td>Platelets</td>
<td>20 ± 2.0</td>
<td>397,000</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>---</td>
<td>2,532</td>
</tr>
</tbody>
</table>
cult to imagine how long-term alterations in CN can occur after a procedure such as plasmapheresis.

There is general agreement in the literature that hematologic cell lines other than the granulocyte series oscillate in CN with the same oscillation period as the granulocytes. Oscillations in the monocyte series are found consistently. Not all authors have observed periodic oscillations in the reticulocyte and platelet counts, which in view of the data in Fig. 1B, is not surprising, as oscillations in these cell lines may be relatively small. However, there exists some controversy regarding the phase relations between the oscillating cell lines. Using the method of auto- and crosscorrelation analysis (see Appendix), we determined the exact oscillation periods of the hematologic cell lines, which for our patient are very close to 20 days and thus in agreement with other reported cycle lengths in patients with CN. Cycling was found in the granulocyte, monocyte, reticulocyte, and platelet series, but no 20-day cycling was observed in the lymphocyte series. In addition, we determined the exact phase lags of the oscillations of the various cell lines with respect to the granulocyte oscillations. Knowledge of these phase lags should have important implications on the pathophysiologic understanding of CN: The phase lags together with the intramarrow maturation times of the various cell lines permit one, in principle, to determine whether the regulatory abnormality in CN primarily affects a granulocyte precursor leading to secondary oscillations in the other cell lines, or whether a noncommitted hematologic stem cell is primarily affected. In our case, assuming that the maximum granulocyte count occurs at day zero, we find that the maximum monocyte, reticulocyte, and platelet counts occur at -6.5 days, -8.0 days, and +2.0 days, corresponding to -0.33, (0.67), -0.43 (0.57), and 0.10 (-0.90) of a 20-day cycle. These results are consistent with the deductions from other literature data by one of us9 and are in qualitative agreement with the data of Wright et al.9

The observed phase lags can be interpreted in the following way. The monocytes appear to be produced simultaneously with the granulocytes, but because of the shorter maturation time (2-4 days versus 10 days), they appear much earlier in the periphery than the granulocytes, i.e., about 6-8 days earlier. The reticulocytes, on the other hand, appear in the periphery at about half cycle, i.e., about 10 days, out of phase with the granulocytes. As the reticulocyte maturation time is of the order of 7-8 days, it appears that the erythrocyte production occurs in competition with the granulocytes and monocytes. The platelets peak in the periphery shortly after the granulocytes, but correcting for their peripheral half-life of approximately 10 days, and allowing for their intra-bone marrow production time (4-7 days), suggests that their early precursors may be produced simultaneously with the reticulocyte precursors. Using this information, one may therefore predict that the abnormal feedback regulation primarily involves a stem cell which still has the capacity to differentiate into granulocytes and monocytes, but not into erythrocytes and platelets, these oscillations being the result of a "competition" coupling (alternating differentiation of pluripotent stem cells into either the granulocyte-monocyte series or the erythroid-platelet series). This view is consistent with the most recent information regarding the pedigree of the differentiation of hematologic cells, as well as with the notion that periodic production failure of CN occurs at an early level of differentiation.

NOTE ADDED IN PROOF

Twelve months after plasmapheresis, the patient experienced a cutaneous infection; serial blood counts 15 mo after plasmapheresis again showed marked cycling of the granulocytes with near-agranulocytotic episodes at the previously observed 20-day interval.

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APPENDIX

To determine the oscillation periods of the various hematologic
cell lines in our patient and to measure the phase lags of monocyte,
reticulocyte, and platelet oscillations with respect to the granulocyte
oscillations, we computed the auto- and cross-correlation functions.
The advantage of this approach over simple inspection of the cell
counts is that the full set of data available is used rather than just a
few data points. In this manner, it is possible to accurately determine
the oscillation periods and, in addition, the various respective phase
lags of the cell lines in CN.

The cross-correlation function $R_{xy}$ of two functions, $f(t)$ and $g(t)$,
may be defined as:

$$R_{xy}(r) = \frac{1}{T} \int_T^T f(t) g(t + r) dt$$

(A1)

where $r$ is a time long compared to the time scale of the oscillatory
phenomena to be analyzed. The autocorrelation function is simply equation A1, with $f(t)$ and $g(t)$ being identical.

In our case, the functions $f(t)$ and $g(t)$ (the various cell counts
minus the mean cell counts) are discrete functions with one value per
day. (In fact, missing daily data points in our cell counts were
obtained by linear interpolation.) For discrete functions $R_{xy}(r) = R_{xy}(mT)$, with $T = 1$ day and $n = 1, 2, 3, \ldots$, can be rewritten in
analogy to equation A1 as:

$$R_{xy}(mT) = \frac{1}{n-m} \sum_{k=m}^{n} f(nT + mT)$$

(A2)

$m = 0, 1, 2, 3, \ldots$

It is seen immediately that each value $R_{xy}(mT)$ is a sum of products of many function values of the functions $f$ and $g$, thus, that
a large part of the available data is used to compute $R_{xy}(mT)$. How
can the oscillation periods and phase lags be determined by using the
mathematical construct of equation A2?

Consider the following example. Assume that the cell count functions
$f$ and $g$ are sinusoidal, differing only by a phase shift of $2\pi T_{osc}/T_{max}$, thus that:

$$f(t) = \sin (2\pi t/T_{max} - 2\pi T_{osc}/T_{max})$$

and

$$g(t) = \sin (2\pi t/T_{max})$$

with $T$ defined only for $t = mT$, $T = 1$ day, $T_{osc}$ the oscillation period of the cycling blood counts, and $T_{max}$ the phase lag of any serial blood
count to the granulocyte serial count. Using these functions (as
continuous functions in equation A1) we obtain:

$$R_{xy}(r) = \frac{1}{T} \int_T^T \sin (2\pi t/T_{max} - 2\pi T_{osc}/T_{max})$$

$$\times \sin (2\pi (t + r)/T_{max} + 2\pi r/T_{max}) dt$$

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Fig. 4. Graphic representation of the monocyte-granulocyte (MONO-GC), the reticulocyte-granulocyte (RETI-GC), and the platelet-granulocyte (PLT-GC) crosscorrelation functions in arbitrary units. Without phase shift between cell counts, the first maximum of these functions would be found at day zero. The shift of the maxima (and minima) away from 0 yields the phase lags between the oscillation of the granulocyte and the other serial blood counts.

Thus, the correlation functions of two sinusoidally oscillating functions are oscillating functions themselves, and the same is true for near sinusoidal functions, as in fact, our cell counts are. The crosscorrelation function of two-phase shifted sine waves is another sine wave, phase shifted by $\pi/2 - 2\pi T_{av}/T_{osc}$ with the same oscillation period as the initial functions. For the autocorrelation functions $R_T(mT)$, $T_{av}$ is zero; thus, by plotting $R_T(mT)$ as a function of $m$, we obtain the oscillation periods for all the cell counts by determining the distances between the $mT$ axis intercepts, the maxima, or the minima of $R_T(mT)$. The results for the various cell counts are given in Table 1 and are close to 20 days. This justifies the assumption that the $T_{osc}$ are equal for all oscillating cell lines. Only under this assumption is the notion of a phase lag meaningful.

The crosscorrelation functions $R_T(mT)$ are plotted in Fig. 4 for the monocyte-granulocyte, reticulocyte-granulocyte, and platelet-granulocyte combinations. It is apparent that these curves are near sinusoidal. Also, we see that the distance between maxima, minima, and $T$-axis intercepts, respectively, is very close to 20 days for all curves. However, with respect to the reference day (day 0), the maxima of the curves occur at $-6.5$ days for the monocyte-granulocyte correlation function, at $-8$ days for the reticulocyte-granulocyte correlation function, and at $-18$ days for the platelet-granulocyte correlation function, as summarized in Table 1. These values represent oscillatory phase lags of the monocyte, reticulocyte, and platelet counts with respect to the granulocytes.
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