Neutrophil and Monocyte Kinetics in a Case of Cyclic Neutropenia

By G. Meuret and T.M. Fliedner

The kinetics of neutrophilic granulocytes and monocytes were examined simultaneously in a patient suffering from cyclic neutropenia. During the remission phase of the disease, the observed kinetic data for both cell types did not deviate significantly from the normal range. At a stage just before the minimal blood granulocyte count was reached, the total blood granulocyte pool was diminished and the granulocyte half disappearance time shortened to 2.3 hr. Moreover, the intravascular granulocyte distribution changed in favor of the marginal granulocyte pool. During the neutropenic phase, the intravascular monocyte pools were increased and monocyte half-disappearance time was prolonged to 14 hr; the monocyte turnover rate was 2.4-fold higher than during remission.

In patients with cyclic neutropenia, analysis of granulopoiesis revealed pronounced variations of its proliferative activity resulting in high-amplitude oscillations of the neutrophil blood count. Little information is available on the life span, turnover rates, and intravascular pool sizes of neutrophilic granulocytes and monocytes during the different parts of the cycle in this disease. It is, therefore, the purpose of this paper to report observations of these parameters in a patient with cyclic neutropenia.

CASE REPORT

The patient, a 53-yr-old woman, had been suffering for about 3 yr from symptoms which returned at regular intervals of about 26 days. These symptoms consisted mainly of weakness, abdominal pain, and psychic depression and occurred concomitantly with fever, neutropenia, and monocytosis as indicated in Figure 1. During the studies to be reported in this paper, several cycles like the ones shown in the figure were observed clinically and presented themselves always in a very typical and consistent pattern. For the sake of convenience, a cycle of about 26 days as derived from the neutrophil counts was divided into four phases: (1) Remission phase, lasting for about 12 days during which the neutrophil count was above 1000 cells/μl. (2) Phase of neutrophil descent, lasting for about 9 days during which the neutrophil count decreased slowly to levels below 100 cells/μl. (3) Phase of maximal neutrophil depression, at which time the number of neutrophils was below 100 cells/μl. (4) Recovery phase, of 5 days during which the number of neutrophils rose slowly to the level of the remission phase.

The clinical symptoms (fever, weakness, pain, psychic depression) developed during the second phase of the cycle, reached their peak during the third phase, and disappeared gradually during the fourth phase. The lymphocyte, reticulocyte, erythrocyte, and thrombocyte counts were normal throughout the cycle.
cycle. Despite physical examinations and appropriate laboratory studies (daily bacteria counting in urine, blood cultures for bacteria) the manifestation of infection could not be established.

MATERIALS AND METHODS

Blood-cell counts were carried out nearly every day at 8 a.m. Leukocytes and erythrocytes were counted with a Coulter Counter.

Neutrophil and Monocyte Kinetics

A modification of the autotransfusion technique described by Athens et al. was used. A sample of about 500 ml of blood was drawn into a plastic bag containing 75 ml ACD; 500 μCi 3H-diisopropylfluorophosphate (HDFP, dissolved in 0.2 ml propylene glycol; spec. act. 4.82 Ci/mM) were injected into the bag. The contents were then gently mixed and incubated at room temperature for 1 hr. At intervals of about 5 min, the bag was carefully inverted, in order to maintain a homogenous cell suspension. Subsequently autotransfusion was performed over a period of 10 min. During autotransfusion the plastic bag was agitated gently to avoid cell sedimentation. Samples withdrawn from the infusion set at the beginning, middle, and end of the transfusion were used to determine the neutrophil labeling index and the neutrophil concentration. The number of labeled neutrophils administered was calculated from the amount of blood-ACD transfused (determined by weighing), together with the average neutrophil count and neutrophil labeling index of the infusion fluid. At suitable intervals after transfusion, venous blood samples were taken for preparation of concentrated leukocyte smears. After radioautography (exposure time 80 days) and Giemsa staining, the labeling index of neutrophils and

Fig. 1. Signs and symptoms observed in the patient examined during hospitalization.
monocytes was determined microscopically by counting 3000 band and segmented neutrophils and 2000 monocytes in each sample.

The following cell kinetic parameters were determined:

- TBGP, TBMP: equals total blood granulocyte pool, total blood monocyte pool—calculated according to the principle of dilution using the number of transfused labeled neutrophils or monocytes and the labeling indices of these cells in the blood 5 min after completion of autotransfusion.
- CGP, CMP: circulating granulocyte pool, circulating monocyte pool equal to the blood cell count per $\mu l \times 10^8 \times ml$ blood volume (calculated according to reference 5).
- MGP, MMP: marginal granulocyte pool, marginal monocyte pool; equivalent to TBGP-CGP, TBMP-CMP.
- $T\frac{1}{2}$: intravascular half-disappearance time of labeled neutrophils and monocytes—estimated from the graph.
- GTR, MTR: granulocyte turnover rate, monocyte turnover rate—calculated by multiplication of TBGP or TBMP with $\ln 2$ and division by $T\frac{1}{2}$.

RESULTS

Kinetics of Neutrophilic Granulocytes and Monocytes

Studies of granulocyte and monocyte kinetics were carried out during a remission phase and during the last third of a neutrophil descent phase just before the minimal neutrophil blood count was reached (relapse). For the 30 hr of each kinetic study, neither the neutrophil nor monocyte blood counts changed significantly.

Radioautographic analysis of the concentrated leukocyte smears prepared from samples of the autotransfused blood indicated that 100% of the neutrophils and monocytes were labeled. The patterns of disappearance of the labeled neutrophils and monocytes from circulating blood after autotransfusion are shown in Fig. 2 for studies during phase 1 and 2 (remission resp. relapse).

During remission, the disappearance of neutrophils from the circulation followed a single exponential function. When the neutrophil disappearance was studied in the final third of the descent phase (relapse), a curve was obtained suggesting that the neutrophil disappearance is a two-component process.

The study of the monocyte behavior after autotransfusion indicated a two-component disappearance during phase 1 (remission) as well as phase 2 (relapse).
Table 1. Kinetic Data of Neutrophilic Granulocytes and Monocytes Determined Simultaneously During a Remission Phase ("Remission") and During the Last Third of a Phase of Neutrophil Descent ("Relapse")

<table>
<thead>
<tr>
<th></th>
<th>Remission</th>
<th>Relapse</th>
<th>Normal x (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils (per µl)</td>
<td>2400</td>
<td>240</td>
<td>3980* (3100-4550)</td>
</tr>
<tr>
<td>TBGP (x 10^7/kg)</td>
<td>59</td>
<td>10</td>
<td>55.3 (42.0-74.5)</td>
</tr>
<tr>
<td>CGP: MGP</td>
<td>0.4</td>
<td>0.2</td>
<td>1 (0.5-1.4)</td>
</tr>
<tr>
<td>T12 (hr)</td>
<td>5</td>
<td>2.3</td>
<td>7.6 (6.1-10.5)</td>
</tr>
<tr>
<td>GTR (x 10^7/kg/h)</td>
<td>8.2</td>
<td>3.0</td>
<td>5.2 (3.2-7.4)</td>
</tr>
<tr>
<td>Monocytes (per µl)</td>
<td>240</td>
<td>600</td>
<td>260† (135-370)</td>
</tr>
<tr>
<td>TBMP (x 10^7/kg)</td>
<td>4.7</td>
<td>16.2</td>
<td>8.1 (4.2-14.4)</td>
</tr>
<tr>
<td>CMP: MMP</td>
<td>0.6</td>
<td>0.4</td>
<td>0.3 (0.2-0.4)</td>
</tr>
<tr>
<td>T12 (hr)</td>
<td>9.5</td>
<td>14</td>
<td>8.4 (4.5-10.0)</td>
</tr>
<tr>
<td>MTR (x 10^7/kg/h)</td>
<td>0.34</td>
<td>0.80</td>
<td>0.7 (0.3-1.4)</td>
</tr>
</tbody>
</table>

* Nine subjects.
† Eight subjects.

The normal values were derived from studies in hematologically healthy subjects.6,7

The curves obtained showed a rapid loss of about 50% of the labeled cells during the first hour after autotransfusion and showed subsequent transition into a slowly declining exponential process.

The intravascular pool sizes and the turnover rates presented in Table 1 were calculated on the basis of the blood volume, the 5-min posttransfusion labeling indices, the number of labeled cells transfused, and the half-time of the slow component of the disappearance curves of the labeled cells. The kinetic data of neutrophilic granulocytes and monocytes obtained during the remission phase did not deviate significantly from the normal range.6,7 However, when the kinetics of neutrophilic granulocytes were studied during the last part of the neutrophilic descent phase (relapse), it was evident that the intravascular pools, the half-disappearance time, and the turnover rates were reduced. The intravascular granulocyte distribution changed slightly in favor of the marginal granulocyte pool. In contrast, monocytosis and increased intravascular monocyte pools were found during the neutrophil descent phase (Table 1). The monocyte half-disappearance time was prolonged to 14 hr. The monocyte turnover rate was found to be normal compared to the data obtained in normal subjects7 but about 2.4-fold higher than during the remission phase.

DISCUSSION

Methodological considerations. In the present study, the method to determine granulocyte kinetic data first described by Athens et al.4 was modified by using
tritiated DFP and single-cell radioautography. This modification allowed the simultaneous study of neutrophilic granulocytes and monocytes regardless of the concentration of the cells in the blood. The fact that human monocytes become labeled with tritiated DFP when incubated in vitro has been demonstrated previously. The monocyte labeling indices obtained after autotransfusion in this patient with cyclic neutropenia showed that the disappearance of labeled monocytes was not a single exponential process, but that an initial rapid disappearance of about 50% of the labeled cells was followed by a slowly declining component of the disappearance curve. The total blood monocyte pool was calculated according to the principle of dilution using the monocyte labeling index in the circulating blood 5 min after autotransfusion. This approach seemed justified by the results of skin window studies in hematologically healthy individuals in whom tritiated DFP-labeled monocytes were autotransfused 1 hr after establishment of skin windows. In these studies the macrophage labeling indices in skin windows 24 hr after autotransfusion equaled the 5-min posttransfusion labeling indices of blood monocytes. This observation suggested that the transfused monocytes had reached an equilibrium with the unlabeled intravascular monocytes.

The total blood granulocyte pool has also been calculated on the basis of the 5-min posttransfusion labeling index as in the method of Athens et al.

Neutrophilic granulocyte oscillations. The analysis of the patients' granulopoiesis reported that elsewhere, demonstrated that the neutrophil cycle was caused by an oscillation of the granulocyte production rate and release rate, the period being 26 days. Granulocyte kinetic data observed during the remission phase did not deviate significantly from the normal range. During the last third of the neutrophil descent phase (relapse) a reduction of the granulocyte pools, a change of the intravascular granulocyte distribution in favor of the marginal pool, and shortening of the half-disappearance time to 2.3 hr was observed.

An attempt was made to analyze the pattern of kinetic changes in the course of the neutrophil cycle. For this analysis, it was assumed that there is a linear positive correlation between the blood neutrophil count and the parameters expressing neutrophil kinetics such as granulocyte half-disappearance time, pool sizes, and turnover rate. These data were measured for the peak and the nadir of blood neutrophil concentrations (Table 1). Thus, if the above assumption is correct, the kinetic parameters could be calculated for each of 53 single neutrophil counts obtained during the two successive neutrophil cycles illustrated in Fig. 1. Each of the resulting curves of TBGP, MGP, $T_1^2$, and GTR showed three minima and two maxima. The mean maxima and minima of these parameters are given in Table 2.

<table>
<thead>
<tr>
<th>Oscillation</th>
<th>Neutrophils (per μl)</th>
<th>TBGP ($x 10^7$ /kg)</th>
<th>$GOD$ MGP</th>
<th>$T_1^2$ (hr)</th>
<th>GTR ($x 10^7$ /kg/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak</td>
<td>1733</td>
<td>43.8</td>
<td>0.36</td>
<td>3.8</td>
<td>7.3</td>
</tr>
<tr>
<td>Nadir</td>
<td>83</td>
<td>6.4</td>
<td>0.09</td>
<td>2.1</td>
<td>2.1</td>
</tr>
<tr>
<td>Mean</td>
<td>853</td>
<td>23.9</td>
<td>0.28</td>
<td>3.1</td>
<td>5.1</td>
</tr>
</tbody>
</table>

Peak to nadir amplitudes of oscillations in per cent of mean

|                  | 193 | 157 | 94.6 | 55 | 102 |
as “nadir” and “peak.” The “mean” of the oscillation is the average of all 53 data calculated for each parameter.

There is a difference in the values of the oscillation ranges of the neutrophil concentration and TBGP of 193% versus 157% of the mean. This is interpreted to indicate a damping effect brought about by margination of neutrophils during the neutropenic phases, as shown by the shift in the ratio of the circulating granulocyte pool (CGP) to the marginal granulocyte pool (MGP). The oscillation range of GTP was damped even further, reaching only 102% of the mean. Two mechanisms may be responsible for this effect: margination and shortening of granulocyte half-disappearance time during the neutropenic stages.

Margination and accelerated granulocyte disappearance were also observed in other forms of neutropenia as well as in acute infection. This supports the notion that similar changes may occur generally when neutrophil demands exceed neutrophil production rates, assuming a normal functional capacity of the granulocytes, such as emigration from the blood.

In three of 12 normal males Morley detected low amplitude oscillations of the neutrophil blood count; the cycle period amounted to 14, 22, and 23 days. Oscillations of that kind could be produced by computer simulation of a model of granulopoiesis which was characterized by two negative feedback loops. One linked the peripheral blood granulocyte concentration to the rate of differentiation of stem cells into granulocyte precursors (“production loop”). The other, acting upon the granulocyte storage pool, damped the oscillations by modulating the neutrophil release rate into the blood. When the stem cell differentiation rate was reduced in this model, the granulocyte storage pool became depleted, and the release-rate loop lost its damping ability, thus giving rise to high amplitude oscillations of the granulocyte blood count.

The profile of granulopoietic proliferation activity observed during the neutrophil cycle suggests that in this patient a further pathogenetic mechanism might have been operating. It is interesting to note that at the nadir of the blood granulocyte counts, with values below 100 per μl, a wave of granulopoietic hyperproliferation appeared. However, during the recovery phase, when the blood granulocyte concentration exceeded 300 per μl, a sudden fall of granulopoietic bone marrow cells occurred, and granulopoietic hyperplasia rapidly converted into hypoplasia. Similar results were reported by Dale et al., who studied granulopoiesis in grey collie dogs with cyclic neutropenia. These observations support the view that the operating point of the loop which adjusts the blood neutrophil count by regulation of the stem-cell differentiation rate may be set at a value much below the normal threshold of blood neutrophil concentration. Such a failure could be due to a decreased sensitivity of the stem cells to respond to the signal of the production loop or due to quantitative or qualitative defective production of a regulating factor.

**Monocyte kinetics.** The analysis of the kinetics of blood monocytes indicated a normal monocyte production rate during the remission phase. During the last part of the neutrophil descent phase, the monocyte blood count and the total blood monocyte pool were increased, the monocyte half-disappearance time was prolonged, and the monocyte turnover rate exceeded the remission value by a factor of 2.4. It can be deduced from these data that monocytosis occurring during
the neutropenic phases of the disease was caused by a rise in the monocyte production rate which approximately equals the monocyte turnover rate. Thus, the granulocyte production rate and monocyte production rate were inversely correlated.

REFERENCES

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