A mathematical model of erythropoiesis in mice and rats.
Part 4: Differences between bone marrow and spleen

K. Pantel*†, M. Loeffler*, B. Bungart* and H. E. Wichmann‡

*Medizinische Universitätsklinik I, LFI-EDV, Joseph-Stelzmann-Str. 9, D-5000 Köln 41; † Institut für Immunologie der LMU München, Goethestr. 31, D-8000 München 2 and ‡Universität Wuppertal, FB14 Arbeits sicherheit und Um weltmedizin, Gaußstr. 20, D-5600 Wuppertal 1, F.R.G.

(Received 8 May 1989; revision accepted 24 November 1989)

Abstract. In a preceding analysis we hypothesized that the most important parameter controlled by erythropoietic regulation in vivo is the degree of amplification (number of cell divisions) in the CFU-E and erythroblast cell stages. It was concluded that erythropoietic amplification in vivo is controlled according to a sigmoidal dose–response relationship with respect to the control parameter which is the haematocrit (or haemoglobin concentration). Here, this hypothesis is extended to include the differences in murine bone marrow and splenic erythropoiesis that are described and quantified by different dose–response relationships. Comparing several sets of experimental data with mathematical model simulations, this approach leads to the following conclusions: (i) in the unperturbed normal steady state at least one extra erythropoietic cell division takes place in the spleen compared with the bone marrow; (ii) a strong erythropoietic stimulus, such as severe bleeding or hypoxia, can induce five to six additional cell divisions in the spleen but only two to three additional divisions in the bone marrow; this results in a considerable increase in the spleen’s contribution to erythropoiesis from about 10% in normal animals to over 40% during strong stimulation; (iii) under erythropoietic suppression, such as red cell transfusion, a similar number of cell divisions is skipped in both organs and the splenic contribution to erythropoiesis remains unchanged. In conclusion, the concept that bone marrow and spleen microenvironments differ in the dose–response relationship for erythropoietic regulation provides an explanation for the changing contribution of splenic murine erythropoiesis following a variety of experimental treatments.

It is an interesting question concerning tissue regulation why a system of cellular proliferation and maturation like erythropoiesis can behave differently if located in different microenvironments. It is a well-known experimental observation in mice that the spleen can take over a large part of erythropoiesis under erythropoietic stimulation, while its contribution remains small under normal or suppressed conditions (reviewed by Loeffler & Wichmann, 1985a). The changes can be dramatic and can take place within a few days. Many experimental haematologists consider the splenic microenvironment as favourable for erythropoiesis in some

Correspondence: Dr M. Loeffler, Medizinische Universitätsklinik I, LFI-EDV, Joseph-Stelzmann-Str. 9, D-5000 Köln 41, F.R.G.
general qualitative sense, but this does not explain the differentiated response pattern observed. The objective of this paper is to demonstrate a new concept for describing and quantifying the microenvironmental differences in haemopoietic regulation to explain the varying relevance of splenic erythropoiesis in different experimental situations.

In a preceding model analysis we hypothesized that the most important parameter controlled by erythropoietic regulation \textit{in vivo} is the degree of amplification (number of cell divisions) in the erythroid colony-forming unit (CFU-E) and erythroblast stages (Loeffler \textit{et al.}, 1989; Wichmann \textit{et al.}, 1989; Wulff \textit{et al.}, 1989). It was concluded that erythropoietic amplification \textit{in vivo} is controlled according to a sigmoidal dose–response relationship with respect to the control parameter of peripheral red blood cell (haemoglobin) concentration. Here, this hypothesis is extended, advocating that murine erythropoiesis in the marrow and spleen differ in their quantitative parameters with their respective dose–response relationships. This implies that erythroid cells can adapt their programme of maturation and proliferation depending on their state of stimulation and location.

MODEL DESCRIPTION

Model compartments
The model is composed of nine compartments representing either different stages of the erythropoietic cell lineage or the regulating hormone erythropoietin (Fig. 1). In the marrow, we distinguish erythroid colony–forming units (CFU-E), proliferative erythropoietic precursors (PEP) and non-proliferative erythropoietic precursors (NPEP). The corresponding cell stages in the spleen are denoted with the prefix ‘S’ to be SCFU-E, SPEP and SNPEP. Bone marrow and spleen contribute to the production of blood reticulocytes and erythrocytes.

The bone marrow and blood compartments are described by differential equations, as discussed in detail by Loeffler \textit{et al.} (1989). For the splenic compartments, the same equations are used as in the corresponding compartments for the bone marrow.

Essential model parameters
We follow the concept of minimum assumptions and use the same parameters as described in previous publications as far as possible (Loeffler \textit{et al.}, 1989). Here, only for two-model parameters, new assumptions are introduced which we believe describe the essential difference between marrow and spleen erythropoiesis.

Cell influx to CFU-E and SCFU-E
The first assumption concerns the question of how many cells enter the stage of CFU-E from the more primitive progenitors in the spleen compared with the bone marrow. According to experimental data, the spleen contains about 5\% of all CFU-E and BFU-E in normal steady state in most mouse strains, while the bone marrow contains 95\% (Tables 1 and 2 in Loeffler & Wichmann, 1985a). This indicates that splenic stem cells produce only 5\% of all cells that are committed towards erythropoiesis. Therefore, it is assumed in all subsequent model calculations that the rate of cells entering the SCFU-E compartment is 1/20 that of CFU-E (Table 1).

Erythropoietic amplification factors
The second assumption is that the amplification factor \( f \) for erythropoietic precursors is not constant but regulated by erythropoietin feedback. The amplification factor \( f \) of a compartment is defined as the average number of cells leaving the compartment by differentiation per cell entering the compartment from more immature stages. In the steady state it is often convenient to relate \( f \) to the number of cell divisions, \( m \), taking place during a cell development within the compartment. In this case the relationship \( f = 2^m \) holds.
Fig. 1. The scheme of the proposed mathematical model of erythropoiesis. Compartments: (S)CFU-E = (spleen) colony-forming units of erythroblasts; (S)PEP = proliferative erythropoietic precursors; (S)NPEP = nonproliferative erythropoietic precursors; RETI = reticulocytes of the blood; ERYA = erythrocytes with an age-dependent destruction; ERYR = erythrocytes destroyed at random; EPO = erythropoietin; PV = plasma volume; Hct = haematocrit; Hb = haemoglobin; $p_{O_2}$ = arterial oxygen pressure; $p_{O_2}$ = tissue oxygen pressure; HbO$_2$ = haemoglobin oxygen. Shaded arrows represent transition between the compartments; full arrows represent regulatory influences of EPO; dotted arrows represent regulatory influences on the production of EPO.
Table 1. Numbers chosen as minimum, normal and maximum values of f for CFU-E and proliferative erythroblasts (PEP)

<table>
<thead>
<tr>
<th></th>
<th>Minimum</th>
<th>Normal</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>$f_{\text{Total CFU-E}}$</td>
<td>1</td>
<td>32</td>
<td>128</td>
</tr>
<tr>
<td>$f_{\text{Total PEP}}$</td>
<td>1</td>
<td>64</td>
<td>96</td>
</tr>
</tbody>
</table>

Table 1 shows the numbers chosen as minimum, normal and maximum values of f for CFU-E and proliferative erythroblasts for the previous model of total murine erythropoietic cell production. In the present model four such dose-response relationships are introduced, two for the spleen ($f_{\text{SCFU-E}, f_{\text{SCFU-E}}}$) and two for the marrow ($f_{\text{CFU-E}, f_{\text{PEP}}}$).

The following assumptions are made:

(i) The minimum amplification factor is 1 in all four cases.

(ii) The normal amplification factors are chosen differently for spleen and marrow. As mentioned above the spleen contains about 5% of an animal’s stem cells. However, in the normal steady state the spleen contains 10–15% of all erythropoietic cells (Loeffler & Wichmann, 1985a). Thus, the normal amplification factor appears to be about twofold larger in the spleen than in the marrow (one cell division more). The following assumptions take this into account: norm($f_{\text{CFU-E}}$) = 32, norm($f_{\text{SCFU-E}}$) = 64. For $f_{\text{PEP}}$ and $f_{\text{PEP}}$ the same values are chosen as in the previous model [norm($f_{\text{PEP}}$) = norm($f_{\text{PEP}}$) = 64].

(iii) The maximum amplification factors are also chosen differently for marrow and spleen. Following stimulation of erythropoiesis by bleeding or hypoxia, the spleen contains 5–10% of all CFU-S, while CFU-E can make up 30% and erythroblasts can exceed 40% of the respective cell numbers in the total animal (Loeffler & Wichmann, 1985a). The following assumption takes this difference into account: max($f_{\text{SCFU-E}}$) = 2048, max($f_{\text{CFU-E}}$) = 96. Thus, it is assumed that

Table 2. Model parameters for the spleen and bone marrow compartments during minimum, normal and maximum stimulation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Abbreviation</th>
<th>Minimum</th>
<th>Normal</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem cell ratio: spleen/marrow</td>
<td></td>
<td>1/20</td>
<td>1/20</td>
<td>1/20</td>
</tr>
<tr>
<td>Late progenitors (CFU-E, SCFU-E)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transit time (h)</td>
<td>$\tau_{\text{SCFU-E}} = \tau_{\text{CFU-E}}$</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Splenic amplification factor*</td>
<td>$f_{\text{SCFU-E}}$</td>
<td>16(1)†</td>
<td>64</td>
<td>1475(2096)†</td>
</tr>
<tr>
<td>Marrow amplification factor*</td>
<td>$f_{\text{CFU-E}}$</td>
<td>8(1)†</td>
<td>32</td>
<td>96</td>
</tr>
<tr>
<td>Proliferative erythropoietic precursors (SPEP, PEP)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transit time (h)</td>
<td>$\tau_{\text{SPEP}} = \tau_{\text{PEP}}$</td>
<td>65</td>
<td>54</td>
<td>27</td>
</tr>
<tr>
<td>Splenic amplification factor*</td>
<td>$f_{\text{SPEP}}$</td>
<td>20(1)†</td>
<td>64</td>
<td>96</td>
</tr>
<tr>
<td>Marrow amplification factor*</td>
<td>$f_{\text{PEP}}$</td>
<td>20(1)†</td>
<td>64</td>
<td>96</td>
</tr>
<tr>
<td>Non-proliferative erythroid precursors (SNEP, NPEP)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transit time (h)*</td>
<td>$\tau_{\text{SNEP}} = \tau_{\text{NPEP}}$</td>
<td>20</td>
<td>18</td>
<td>6</td>
</tr>
</tbody>
</table>

* For the regulated amplification factors and transit times the steepness factor BR = 0.7 has been used (for details see Loeffler et al., 1989).
† Theoretically, extreme values can be adopted for vanishing levels of EPO or indefinitely high values of EPO. These extreme values are given in brackets. Under practical circumstances the maximum is given by the minimum $pO_2$ of zero and the minimum by the maximum tolerable Hct of 0.75.
amplification can increase in the spleen much more than in the marrow. For \( f_{PEP} \) and \( f_{SPEP} \) the same values are chosen as in the previous model [\( \max(f_{PEP}) = \max(f_{SPEP}) = 96 \)].

Table 2 summarizes all these parameters and also gives the values for the transit times in each compartment. They are identical to the previous model. Using these parameters for minimum, normal and maximum amplification, one can calculate all intermediate values for any given haematocrit (Hct) or partial tissue oxygen pressure using the formulae (7)–(13), as given in Loeffler et al. (1989) for the total erythropoietic dose–response curve.

**Splenic contribution to total erythropoiesis**

Under steady state conditions, one can estimate the fraction of the total erythropoiesis present in the spleen \( (C_{spl/tot}) \) in terms of a ratio of amplification factors (Loeffler & Wichmann, 1985a):

\[
C_{spl/\text{tot}} = 0.05 \cdot f_{SP} / (0.95 \cdot f_{BM} + 0.05 \cdot f_{SP})
\]  

(1)

using the definitions

\[
f_{SP} = f_{\text{CFU}-E} \cdot f_{SPEP}
\]

(2a)

\[
f_{BM} = f_{\text{CFU}-E} \cdot f_{PEP}
\]

(2b)

Consequently, \( f_{SP} \) and \( f_{BM} \) are the total amplifications taking place in the spleen and bone marrow, respectively.

**Model technique for simulating experimental treatments**

The model techniques for simulating recovery behaviour after transfusion of erythrocytes (hypertransfusion), bleeding and hypoxia are described and discussed in detail in previous reports (Wichmann et al., 1989; Wulff et al., 1989). The most important assumptions involved are summarized in Table 3.

---

**Table 3. Model assumptions for the simulation of experimental treatments**

<table>
<thead>
<tr>
<th>Hypertension (Wulff et al., 1989)</th>
<th>Elevation of the initial values for reticulocytes and erythrocytes according to the number of transfused cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding anemia (Wichmann et al., 1989)</td>
<td>Reduction of the initial values for reticulocytes and erythrocytes according to the experimental blood loss</td>
</tr>
<tr>
<td>Hypoxia (Wichmann et al., 1989)</td>
<td>Reduction of the arterial oxygen pressure proportional to the atmosphere pressure (use of the barometric formula to simulate different altitudes)</td>
</tr>
<tr>
<td>Acute irradiation (Loeffler &amp; Wichmann, 1985b)</td>
<td>Reduction of the initial value of the late erythropoietic progenitors (CFU-E) and the proliferative erythropoietic precursors in bone marrow and spleen according to the data</td>
</tr>
</tbody>
</table>
Recovery from acute irradiation

For simulation of recovery from acute whole body irradiation, an initial cell loss of proliferating cells is assumed in the model. The cell numbers in the compartments CFU-E and PEP are reduced both in the bone marrow and spleen to the nadir values measured experimentally after irradiation. In addition, the influx from the stem cells into erythropoiesis is reduced temporarily. For radiation doses of about 3 Gy one can assume that the numbers of stem cells and progenitor cells are reduced to about 20% of normal, leading to a markedly reduced influx into marrow and splenic erythropoiesis (below 50% of the normal value) over 10 days (Loeffler & Wichmann, 1985b). After this period, the influx increases, overshoots and finally reaches its normal value at day 25. Furthermore, it is assumed that newly produced erythrocytes have a reduced life span (15 days) instead of the normal 42 days for the initial period of recovery (about 10 days). This extends a similar assumption about shortening of the erythrocytic life span during other stimulatory influences on erythropoiesis (Wichmann et al., 1989).

Splenectomy

Splenectomy is simulated by neglecting all spleen compartments and starting simulations at the appropriate new steady state values. Recovery from acute bleeding in splenectomized animals is simulated by using the same assumptions as before for bleeding (reduction of Hct, constant blood volume) and combining them with the assumption of a lack of a splenic contribution to blood reticulocyte (and erythrocyte) production.

RESULTS

Model dose–response relationship for erythropoietic amplification in the bone marrow and spleen

Based on the choice of parameters made in the previous paragraph, the mathematical formalism describes model dose–response relationships for the mitotic amplification of the in vivo regulation in the bone marrow ($f_{BM}$) and spleen ($f_{SP}$). The amplification factors are derived from those for the cell stages CFU-E and proliferating erythroblasts PEP by multiplication (e.g. $f_{BM} = f_{CFU-E} \cdot f_{PEP}$). Hct and partial tissue oxygen pressure ($pO_2$) are appropriate parameters of peripheral red cell demand to which $f_{BM}$ and $f_{SP}$ can be related. Fig. 2a shows the corresponding curves. They display the central model concept that erythropoietic amplification can be expanded in the spleen to a greater extent than in the marrow. Thus, a reduction of Hct to 0.35 (0.5 being assumed as normal), or of $pO_2$ to 32 mmHg (45 mmHg being normal), increases the amplification 2.8-fold in the marrow and fourfold in the spleen. For a Hct below 0.25 (or $pO_2$ of zero) this difference reaches its maximum (marrow: fourfold increase, spleen: about 30-fold increase). Consequently, splenic erythropoietic cell production ($C_{spl\,tot}$), normally contributing about 10% to the total production, becomes more prominent (Fig. 2b). At its maximum the splenic environment can support up to 45% of the total animal's red cell production due to the relatively larger increase in amplification. In situations of erythropoietic suppression (e.g. Hct > 0.5), no difference between marrow and spleen erythropoiesis builds up because the dose–response curves are parallel. Therefore, the relative contribution of the spleen remains about 10%.

It should be noted that the total amplification $f_{TO}$ can be calculated by the simple formula $f_{TO} = 0.95 \cdot f_{BM} + 0.05 \cdot f_{SP}$. The curve obtained (Fig. 2a) is consistent with the one used in the previous model of total erythropoiesis.

The dynamics of recovery (model simulations versus experimental data)

So far the concept of different dose–response relationships in bone marrow and spleen has been
introduced on the basis of steady state considerations using information from circumstances of minimum, normal and maximum stimulation (see erythropoietic amplification factors). This, however, gives only a static picture. We now demonstrate that this concept also allows us to understand the dynamic behaviour in a diversity of experimental situations. A comparison of model simulations with experimental recovery curves provides an important test of the dose–response curve concept, because large parts of the curves are involved during a recovery process. For example, during a recovery process from very low to normal Hct the entire left part of the curves is involved. We describe four such circumstances. The basic model assumptions involved in the corresponding simulations are summarized in Table 2.

Hypertransfusion

In the model, a transfusion of red cells is simulated by elevating the total mass of peripheral blood cells to four times normal. This increases the Hct up to 0·80. Due to the general
suppression of erythropoiesis, the Hct normalizes within about 20 days (Fig. 3f). The model curves for marrow and spleen erythropoiesis are similar because the dose–response curves for marrow and spleen are parallel for elevated values of Hct (Fig. 2a).

The model curves can be compared with the experimental data for erythroblasts, reticulocytes and Hct (Fig. 3 c–f). While the initial phase is described well by the model, discrepancies are apparent for the recovery phase. While CFU-E and erythropoietin numbers remain low in some experiments, reticulocytes recover in others, as expected by the model. Interestingly, CFU-E show a parallel behaviour in marrow and spleen in both data and model (Fig. 3a,b). However, there is a discrepancy between the minimum values of CFU-E in both experiment and model, suggesting that the real CFU-E dose–response curve for elevated Hct may be steeper than assumed.

**Fig. 3.** Changes in erythropoiesis after transfusion of red blood cells. Model curves are calculated for an elevation of the total mass of haemoglobin to 4 times normal (—). They are compared with data from Hara & Ogawa, 1977 (□); Seidel & Opetz, 1979 (■); Okunewick & Fulton, 1970 (X); Peschle et al., 1977 (Z); Kapa et al., 1984 (+); Brookhoff & Weiss, 1982 (★); Erleve et al., 1978 (Y); Hara, 1980 (♀); Gurney et al., 1965 (●).
Bleeding anaemia

In the model the effect of acute bleeding is taken into account by an initial reduction of the Hct to the experimental minimum. In Fig 4f we assume an Hct of 0.20 on day 0. According to the dose–response relationship shown in Fig. 2a, the decrease in Hct stimulates the amplification of the erythropoietic progenitors and precursors (Fig. 4a–e), leading to a fast restoration of the peripheral pool of red blood cells within 10 days (Fig. 4e,f). During this process the behaviour in the bone marrow and the spleen differs quantitatively. In the model, the production of splenic CFU-E increases to 12-fold normal values, compared with two-fold in the bone marrow (Fig. 4a,b). For erythroblasts the increase is 20-fold in the spleen and less than threefold in the marrow (Fig. 4c,d).

![Diagram of erythropoiesis model](image)

Fig. 4. Changes in erythropoiesis after acute bleeding. Model curves are calculated for a reduction of the total mass of haemoglobin to 0.40 (—). They are compared with data from Adamson et al., 1978 (□); Hara & Ogawa, 1977 (▲); Iscove, 1977 (*); Seidel & Kreja, 1985 (+); Metcalfe, 1969 (Z); Harrison & Russell, 1972 (●); Pannaciulli et al., 1977 (●); Lord, 1967 (●); Miller et al., 1976 (▲); Boggs & Patrene, 1985 (Y).
The model curves for marrow CFU-E are consistent with the experimental data (Fig. 4a). Although the maximum values are higher in some experiments and lower in others, the median characteristic of the data is reproduced. The model curve for the marrow erythroblasts (PEP) reproduces the data well (Fig. 4c). The time pattern for the splenic data is also reproduced. Although the amplitudes differ considerably between different data sets, the model curves for splenic CFU-E represent the median characteristic. The data for splenic PEP are reproduced well.

The relevance of the spleen’s contribution to red cell recovery during strong erythropoietic stimulation is further supported by the Hct recovery pattern in severely bled splenectomized mice. Model simulations have also been performed for these circumstances. Starting at a Hct of 0.20 the model for splenectomized animals predicts a Hct recovery to 0.35 within 6 days and to 0.45 within 11 days, compared with 4 and 8 days, respectively, in unsplenectomized animals (Fig. 4f). This compares well with the data of Boggs, Geist & Chervenick (1969), who indeed found a 2–3 day delayed recovery in splenectomized mice (data not shown).

**Hypoxia**

In the model, continuous hypoxia is simulated by reducing the arterial oxygen pressure to an atmospheric pressure of 360 mmHg (equivalent to an altitude of 6 km). Data on CFU-E could not be found, but during the initial phase a rapid increase of the erythropoietic precursors occurs (Fig. 5a,b). The erythropoietic precursors show a much more pronounced increase in the spleen compared with the marrow in both model and experiment (Fig. 5a,b). The initial time-course of the model simulations reproduces the experimental observations fairly well. However, after

![Graphs showing changes in erythropoiesis during hypoxia](image-url)

Fig. 5. Changes in erythropoiesis during hypoxia (corresponding to an altitude of 6 km). Model curves (—) are compared with data of Rickard et al., 1971 (16 days; ●); Turner et al., 1967 (6 days; Z); Harrison & Russell, 1972 (13 days; X); Bruce & McCulloch, 1964 (23 days; ▲); Mylea & Abbrecht, 1970 (33 days; >4); Kubanek et al., 1968 (24 days; △); Abbrecht & Littell, 1972 (11 days; eliac).
Erythropoiesis model

reaching their maximum values, erythroblasts as well as reticulocytes decrease more strongly in the data than in the model. Nevertheless, the model fits the observed changes in Hct well (Fig. 5d).

Acute irradiation

The model simulations start with a reduced number of CFU-E and erythroblast cells in bone marrow and spleen, but with normal values for peripheral blood cells. Due to the lack of erythropoietic cell production a mild anaemia develops, leading to a mild stimulation of the erythropoietic system. As the dose–response curves for bone marrow and splenic erythropoiesis are parallel in this area (Fig. 2a), the model curves for CFU–E and proliferative erythroblasts PEP are very similar in bone marrow and spleen (Fig. 6a–d). This model behaviour agrees with the experimental data which show similar changes in spleen and marrow (Fig. 6a–d).

Fig. 6. Changes in erythropoiesis after an acute irradiation (doses between 1.5 and 3.5 Gy). Model curves (--) are compared with experimental data from Monette, Ziegelstein & Hunter, 1984 (□); Seidel & Kreja, 1985 (+); Smith et al., 1980 (*); Chervenick & Boggs, 1971 (∆); Russell & Keighley, 1972 (†); Krupienicz & Wiktor-Jedrzejczak, 1984 (Y).
The objective of this investigation was to examine whether differences in bone marrow and spleen erythropoiesis can be attributed to different dose–response relationships in the regulation of mitotic amplification. In particular, we wanted to explain why splenic erythropoiesis becomes relevant under situations of considerable erythropoietic stimulation but not during moderate stimulation or suppression. For this, it was necessary to show in a general sense that the unifying concept of variable amplification factors regulated in a dose-dependent way is comprehensive and is able to generate the correct patterns of recovery consistently for a variety of circumstances.

During suppression of erythropoiesis (e.g., following transfusion of erythrocytes) the number of CFU-E and erythroblasts decreases quite similarly in the bone marrow and in the spleen. The contribution of splenic erythropoiesis remains small. Acute irradiation with doses of 1.5–3.5 Gy leads to a moderate erythropoietic stimulation due to a mild anaemia. Again, the recovery in bone marrow and spleen is quite similar and the contribution of splenic erythropoiesis remains small. The model suggests a simple explanation for these observations. For Hct values larger than 0.40 the dose–response relationships for the bone marrow \( f_{BM} \) and the spleen \( f_{Sp} \) behave in parallel (Fig. 2a). Therefore, the splenic contribution to erythropoiesis remains at 10% (Fig. 2b).

Severe bleeding (Hct below 0.40), or chronic hypoxia (tissue or venous oxygen pressure below 35 mmHg), lead to a strong stimulation of erythropoiesis in general and to a more pronounced splenic response. According to the model, this can be explained by considerable differences between the amplification factors of \( f_{BM} \) and \( f_{Sp} \) in the marrow and spleen (Fig. 2a). This increases the splenic contribution to erythropoiesis up to 45% (Fig. 2b), resulting in a more efficient compensation of the severe demand for red blood cells.

The model presented here is the first to our knowledge in which a distinction between erythropoietic marrow and spleen properties is introduced and justified. Regarding the differences between data and qualitative model simulations, it should be emphasized that the present model was not designed to generate quantitative fits to each particular set of data but to match the mean characteristic of a large variety of data. Therefore, only one set of parameters was used in all model calculations. This 'average mouse model' was compared with data obtained from several mouse strains and different laboratory conditions.

Although the model gives a consistent and simple explanation for many observations, one should acknowledge its limitations. First, the simplifying assumption is made that amplification factors of the proliferative compartments affect the 'cells' only at the 'entrance' to a model compartment, i.e., it influences only the new CFU-E or new erythroblasts, but does not instantaneously influence all the cells in this compartment. This simplification causes problems if one intends to simulate short-term changes on a time scale of hours.

Second, it is that the stem cells are considered in a largely simplified way, as already discussed in the previous papers of this series (Loeffler et al., 1989; Wichmann, et al., 1989; Wulff et al., 1989). Adding a stem cell model to the model of erythropoiesis is the next logical step of development, and preliminary studies using such an advanced model indicate no major changes to the above conclusions.

Third, and probably most unrealistically, the effect of migration of stem and progenitor cells from the bone marrow to the spleen is neglected. Previous experimental data have suggested that migration of stem cells (CFU-S) and early erythropoietic progenitors (BFU-E) from the bone marrow to the spleen may occur during the initial period after severe bleeding (Adamson, Tavok-Storb & Lin, 1978; Marsh et al., 1968; Pannaciulli et al., 1977; Quesenberry et al., 1973;
Seidel & Kreja, 1985; Iscove, 1977; Hara & Ogawa, 1977). In particular, for phenylhydrazine-induced haemolytic anaemia migration of CFU-S and BFU-E seems to be a very important mechanism (Hara & Ogawa, 1977). Under these circumstances, the splenic erythropoietic contribution can reach 80% of the total red cell production reviewed by Loeffler & Wichmann, 1985a). Therefore, phenylhydrazine treatments have been excluded from the present analysis. A more extended modelling is presently being performed to investigate whether the concept of different marrow and spleen dose–response curves still remains valid if migration is specifically taken into account. Such a model has to include a description of the haemopoietic stem cells, which are primarily involved in the migration process.

Having pointed out these limitations, it is clear that the in vivo dose–response curves of CFU-E and PEP predicted for marrow and spleen may be subject to some modifications as more information becomes available. At the present stage of limited available information it was important to restrict the choice of parameters for the four dose–response curves (f_{CFU-E}, f_{SCFU-E}, f_{PEP}, f_{SPEP}) to a minimum. Except for the normal and maximum values of f_{CFU-E} and f_{SCFU-E}, all other parameters were identical to those of the previous erythropoiesis model. In particular, the functional form (including the steepness parameter BR) is the same for all curves [see equation (7) in Loeffler et al., 1989]. The somewhat unsatisfactory decline of the model CFU-E during hypertransfusion (Fig. 3a,b) suggests that the dose–response curve should be steeper for CFU-E and flatter for PEP, respectively. However, a correction does not seem to be justified because there is no more accurate data.

The model presented here is an extension of a previous one which required specification of 23 relevant parameters. Formally the distinction between the marrow and spleen compartments required specification of another 21 parameters. However, 19 of them were identical for marrow and spleen and cannot be considered as new. Only two relevant parameters [norm (f_{SCFU-E}), max (f_{SCFU-E})] are new introductions. As already discussed in the first paper of this series (Loeffler et al., 1989) not all parameters are sensitive for the behaviour of the model. The essential ones are those related to the shape and slope of the dose–response curves. We believe that they are fairly precisely defined for each mouse strain and that it would be important to design experimental protocols to directly assess these dose–response curves in vivo. The model analysis suggests that they are more crucial parameters for the temporal dynamics of the system than, e.g., maturation times. An increase in the slopes of the dose–response curves in Fig. 2a by 30%, for example, would shorten the hematocrit recovery after bleeding to a Hct of 0.20 considerably (reading 0.35 1–2 days earlier and 0.45 3–4 days earlier).

The concept proposed above has biological implications. Cells entering erythropoiesis would not have a predetermined programme with a specified number of cell divisions they have to undergo. They would only have a 'knowledge' that they can undergo a limited maximum number of cell divisions during differentiation. Whether this limited division capacity is in fact achieved depends on two major factors, the degree of erythropoietin stimulation and the microenvironmental localization. Under normal circumstances the maximum capacity is not exploited, neither in the marrow nor in the spleen. At present it is not known which factors determine the different maximum division capacities in the marrow and spleen. It would be of particular interest to see how rapidly marrow cells transferred to the spleen or vice versa can adapt their behaviour. This might stimulate experiments involving highly enriched and specifically labelled erythropoietic progenitor cells derived from marrow and spleen and reciprocal transplantation.

In conclusion, this analysis suggests that during murine erythropoiesis different numbers of cell divisions occur in the splenic and marrow environment, respectively. Under normal steady state conditions, they differ by one cell division. Under severe erythropoietic stimulation, two to three mitoses may be added in the marrow but up to five to six in the spleen. It is suggested that
these differences can be understood in terms of the different dose–response relationships for the erythropoietic control mechanisms.

ACKNOWLEDGEMENTS

We would like to thank Henderikus Goris, Willem Nijhof (Groningen, Holland) and Horst Franke for a very fruitful discussion and S. Gontard for the technical assistance in completing the manuscript. K. Pantel was fellow of the ‘Deutsche Krebshilfe Mildred-Scheel-Stiftung für Krebsforschung’.

This work was supported by grant LO 342/1–2 of the Deutsche Forschungsgemeinschaft (FRG), granted to M. Loeffler.

REFERENCES


Erythropoiesis model


