Hemopoiesis during Thiamphenicol Treatment. II. A Theoretical Analysis Shows Consistency of New Data with a Previously Hypothesized Model of Stem Cell Regulation

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Abstract. In a recent theoretical model of stem cell regulation, specific quantitative assumptions were made about an in vivo feedback process from erythroid and granuloid precursor cell stages to the spleen colony-forming units (CFU-S), erythroid burst-forming units (BFU-E), and granulocyte-macrophage colony-forming units (CFU-GM). Utilizing specific effects of the antibiotic thiamphenicol (TAP), new experiments have been performed to challenge this model. Here these data are treated in an analysis that implies three steps. First, model assumptions about TAP toxicity are justified. The toxic TAP effects on erythroid and granuloid precursors are quantified as a continuous reduction of the normal amplification coefficient for CFU-E (down to \(\frac{1}{2}\)), proerythroblasts, basophilic erythroblasts, and proliferating granuloid precursors (down to \(\frac{1}{3}\)). Second, the original model predictions for the behavior of CFU-S, CFU-GM, and BFU-E are compared with the corresponding data. Third, discrepancies are discussed and it is demonstrated that adjustment of one single parameter resolves most of them. Thus one can quantitatively explain the experimental results for CFU-S, BFU-E, and CFU-GM by an activation of the regulatory process postulated: the decline in erythroid (and granuloid) cell numbers enhances the cycling of CFU-S while their self-renewal probability is reduced; consequently CFU-S numbers decline; as more cells differentiate towards BFU-E and CFU-GM per unit time the cell numbers of these cell stages increase. Thus the new data on stem cell behavior during TAP treatment support the hypothesis of a feedback from erythroid and granuloid precursors to the stem cells.

Key words: Hemopoietic stem cells — Regulation — Thiamphenicol — Mathematical model

Quantitative modeling of theoretical biological concepts can contribute to the understanding of complex biological systems for several reasons. First, the assumptions implied in the concept have to be stated very clearly in order to allow a translation into mathematical terms (and vice versa). Second, the contribution of individual parameters in a complex system becomes traceable. Third, one can examine whether the concept can give a consistent interpretation of different types of experiments that are otherwise not directly comparable (different doses or manipulations). Fourth, specific predictions can be formulated that can be tested by new experiments. The logic of model testing implies that a fit of data to predictions does not prove the truth of the model but only supports further application. If a fit is not convincing one has to examine whether minor or major assumptions of the model have to be corrected. The latter implies rejection of the original model.

We have recently suggested a theoretical model of stem cell regulation in which specific quantitative assumptions were made about the feedback controls involved [1–5] (see Wichmann et al. [6] for review). The model scheme is summarized in Figure 1. The basic concept is that three interrelated feedback loops govern hemopoietic proliferation and differentiation (feedback I, II, and III).

Recent data suggested that thiamphenicol (TAP) is a suitable drug to challenge the feedback II hypothesis (feedback from erythroid and granuloid precursor stages to the stem cells) due to its effects on specific cell stages of the erythroid and granuloid lineages [7–9]. The essential assumptions on feedback II can be summarized as follows: two distinct parameters of the primitive hemopoietic cells are regulated—the cyclic activity (of spleen colony-forming units [CFU-S], erythroid burst-forming units [BFU-E], and granulocyte-macrophage colony-forming units [CFU-GM]) and the self-renewal (of CFU-S). The specific values of both parameters depend in a complicated (nonlinear) way on the actual cell numbers of CFU-S, granuloid (G), and erythroid (E) precursors. This concept of mutual interactions of CFU-S, E, and G numbers on cyclic activity (of CFU-S, BFU-E, and CFU-GM) and self-renewal (of CFU-S) leads to a consistent explanation of hemopoietic behavior under such different circumstances as acute and continuous irradiation (CFU-S, E, and G are all reduced), red cell transfusion and posthypoxia (E is reduced, but CFU-S and G slightly increase), and different kinds of anemia (E is increased, G slightly decreases, and CFU-S remains unchanged) (see Discussion) [2, 6].

On the basis of this concept it was predicted that TAP should lead to an increase in cyclic activity (of CFU-S, BFU-E, and CFU-GM), to a decrease in CFU-S number, and to an increased cell differentiation. To obtain an independent test for this model new and more comprehensive experiments were undertaken and reported by Goris et al. [10]. A part of these data serves the quantification of the toxic effects of TAP and is used as input to the model. Based on this, model

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predictions are generated. They are compared with the remaining part of the data and the necessity of modifications of the model is discussed.

Methods

Standard model and definitions

The mathematical model used is a combination of a previous stem cell model [2] and a recent model of mature erythropoiesis including erythroid colony-forming units (CFU-E), erythroblasts, reticulocytes, and erythrocytes [3]. All technical details are given in these references. Therefore, only a brief summary and some definitions are given.

The mathematical description is based on the compartment concept. Each compartment refers to one biologically identifiable cell stage. It is characterized by a transit time “T,” a cyclic activity “a,” and either an amplification coefficient “Z” (in non-self-sustaining compartments) or a self-renewal probability “p.” The compartments are linked by cell flows. Changes in a compartment size Y with time t are described by ordinary differential equations of the type:

\[ \frac{dC}{dt} Y = C' Z - a Y / T \]

with C representing the cell input rate. The amplification coefficient Z appears in the input term and the output is proportional to the compartment content (first order kinetics). This allows for simple numerical calculations but implies a biologically unrealistic random age structure within such a compartment. The model is designed to represent the “total” hemopoietic cell production of the marrow and spleen. The corresponding experimental values can be obtained by pooling the splenic and femoral cell numbers, assuming that one femur represents 6% of the whole bone marrow [2, vol II, Appendix 2].

Control processes. The essential model assumptions about the three interrelated feedback loops are: a) The CFU-S exert self control on their self-renewal and cyclic activity (feedback I). b) The E and G progenitors and precursors also have a regulatory impact on the self-renewal of CFU-S and on the cyclic activity of CFU-S, BFU-E, and CFU-GM (feedback II). c) Amplification in the highly proliferating erythroid cell stages (CFU-S, pro–, baso–, and polychromatric erythroblasts) is controlled by the demand for red blood cells that is mediated by erythropoetin (EPO) (feedback III).

Cyclic activity (proliferative fraction). In the model cyclic activity of a cell stage is defined as the fraction of cells in cell cycle. It is denoted by “a.” Experimentally this parameter correlates (e.g., to the measurements) by the triated thymidine ([3H]Tdr) suicide technique. An experimental [3H]Tdr suicide of 60% is the maximum value frequently found and therefore corresponds to a = 1.0 [2, vol I, chapter 4, 10]. In the model variable cyclic activities are only assumed for the three cell stages CFU-S (denoted as \(a_s\)), BFU-E (\(a_{be}\)), and CFU-GM (\(a_{gm}\)). Their normal steady state values are assumed to be 0.15, 0.33, and 0.33, respectively. This implies that the cyclic activity can be increased 6.0-fold for CFU-S and three-fold for BFU-E and CFU-GM. \(a_s, a_{be}, a_{gm}\), and \(a_e\) are monotonously decreasing functions of CFU-S (feedback I), and of E and G numbers (feedback II) [2, chapter 4].

Self-renewal probability. Self-renewal is defined as the property of CFU-S to maintain, after cell division, the same characteristics as the mother cells. It is quantified by the self-renewal probability “p.” If all daughter cells keep the properties of the mother stem cells, p = 1. If all daughter cells change to a different cell type, p = 0. A steady state is characterized by \(p = 0.5\). In constantly growing cell populations \(p\) can be quantified experimentally by reseeding assays [11, 12]. For dynamically changing growth situations no experimental assay is available. In the model \(p\) can vary between 0.6 and 0.4. It is assumed that \(p\) is a monotonously decreasing function of CFU-S (feedback I) and a monotonously increasing function of E and G numbers (feedback II) [2, chapter 4].

Amplification. Amplification is defined as the increase in cell numbers by cell division in transient (not self-sustaining) cell stages.
only be obtained if the amplification coefficient is reduced to about 0.250 of the normal value for the entire 4-day period (Fig. 2a) (implying an effective amplification coefficient Z of 0.128 for a definition of Z see formula (1) in the Methods section). Assuming a selective effect on the CFU-E alone implies that the subsequent compartments exhibit a sequential decline due to reduced cell influx (upper curves, scenario A, Fig. 2b–f). However, this scenario can be rejected because the more mature cell stages decline later in the model than actually observed (Fig. 2d–f). Likewise scenario D can be excluded, in which all erythroblast cell stages are assumed to be affected. The decline in mature cell stages of the model is much faster than actually observed (lowest curve, Fig. 2d and e). In scenario B (crossed lines) a toxic effect of TAP is assumed on the CFU-E and proerythroblasts with a reduction of Z to 0.250 of normal at the CFU-E and to 0.250 of normal at the proerythroblast stage. In scenario C (full lines) an additional effect on basophilic erythroblasts is assumed. Both scenarios B and C are sufficient to explain the data of most cell stages except for the hematocrit, which in all model scenarios decreases too slowly.

A similar analysis can be undertaken to quantify and localize the toxicity of TAP on granulopoiesis. Three scenarios are shown in Figure 3. One can conclude that TAP reduces the amplification coefficient to about 0.250 of normal at the level of morphologically recognizable granuloid precursors.

Based on this analysis, it is justified to assume in the subsequent model simulations that TAP is toxic to CFU-E, pro- and basophilic erythroblasts, and morphologically recognizable granuloid precursors, reducing their amplification coefficient to 0.250 of 0.250 and 0.250 of normal, respectively. Taken together the total erythropoietic amplification is reduced by a factor of 1000. No (negligible) toxic TAP effects are assumed for CFU-S, BFU-E, CFU-GM, and polychromatic erythroblasts.

**Somatic cell behavior under TAP treatment (standard model)**

Having justified these assumptions about the TAP effect on specific erythroid and granuloid cell stages, the standard model predicts the behavior for CFU-S, BFU-E, and CFU-GM on the basis of feedback II. Figure 4 shows the corresponding model curves for the CFU-S, BFU-E, and CFU-GM. These curves originate in the following way: the TAP-induced depletion of the erythroid and granuloid precursors leads via feedback II to two effects. First, the feedback induces an activation of the cyclic activity, which increases in the model from 0.15 to 1.0 for CFU-S (Fig. 4c) and from 0.33 to 1.0 for BFU-E and CFU-GM (Fig. 4f) (full curves, Fig. 4f). Second, the feedback causes a decrease of the self-renewal probability of the CFU-S (Fig. 4b). Both effects lead to an increased cell flux out of the CFU-S compartments towards differentiation. The CFU-S cell numbers decline (Fig. 4a) while an increased number of cells enters into the progenitor compartments per unit time, enlarging the cell flux through the BFU-E and CFU-GM about twofold. Due to the high turnover (high a_{s} and a_{c}) in these model compartments, this increased cell flux, however, is largely masked and only appears as a slight increase in BFU-E and CFU-GM numbers (full lines, Fig. 4d and e).

The comparison of the standard model curves (full lines)
with the experimental data shows a qualitatively correct pattern (decline in CFU-S, increase in BFU-E and CFU-GM, and increase in CFU-S cycling). But quantitatively, the increase of the BFU-E and the CFU-GM numbers is not sufficiently large compared with the experimental data (Fig. 4d and e). On the other hand Figure 4f shows a clear discrepancy between the cyclic activity of BFU-E and CFU-GM in the standard model (full line, increasing) and the actual [H]Tdr kill in the data (almost constant in the marrow and the spleen). In particular, the control values of untreated C57bl/6 animals already exhibit a higher cyclic activity than assumed in the standard average mouse model (day 0, Fig. 4f).

**Stem cell behavior under TAP treatment (modified model)**

Based on the experimental evidence provided by Gors et al. [10], it is justified to introduce a modification into the standard model with respect to the variability of the cyclic activity of BFU-E and CFU-GM. It has to be assumed that BFU-E and CFU-GM exhibit a constant and high cyclic activity (at least in this particular case). Therefore the values are chosen as \( \alpha_{BFU-E} = \alpha_{CFU-GM} = 0.66 \approx \text{const.} \). This corresponds to the median 40% [H]Tdr kill found in marrow and spleen [10] (Fig. 4f). Using this as the only modification, the corresponding model curves (dashed lines) now show much clearer increases of the BFU-E and CFU-GM numbers (Fig. 4d and e) to about 200% of normal. This compares with the magnitude of change actually measured.

Thus, adjusting the standard model only for the one parameter of normal cyclic activity of BFU-E and CFU-GM (which was measured) generates a satisfactory explanation of CFU-S, BFU-E, and CFU-GM cell numbers and of CFU-S cycling under the application of TAP.

**Discussion**

It was the major objective of this experimental-theoretical cooperation to subject the model hypothesis of a feedback II to a test with a new experiment and to compare the data to the predictions.

The first part in the analysis concerns the quantification and description of TAP toxicity in model terms. It is concluded that TAP reduces the normal amplification process at the stage of CFU-E, proerythroblasts, basophilic erythroblasts, and morphologically recognizable granuloid precursors. Erythroid amplification in total appears to be reduced by a factor of at least 1000 continuously during the 4-day period. This implies that on average 1 cell entering a CFU-E produces only 1 erythrocyte instead of 1000 as normal. In granulopoiesis the amplification is reduced by a factor of about two to four.

In previous papers [7-9] it was suggested that a specific block at the BFU-E-to-CFU-E boundary is an important TAP effect. The present analysis confirms that the CFU-E is indeed the most immature erythroid cell stage affected. The reduction of the normal amplification coefficient to an effective \( Z \) below 1 (\( Z = 0.128 \)) implies a strong effect not only on proliferation but also on differentiation that can be interpreted as a block (i.e., out of ten cells entering the CFU-E stage on average only one cell manages its way to the next stage). However, the analysis also shows that additional effects on erythroblast cell stages are necessary to explain the data satisfactorily.

Biologically, several mechanisms could be responsible for reduction of amplification: 1) there could be ineffective hemopoiesis with ongoing cell division but subsequent cell death; 2) there could be a block of differentiation and proliferation; and 3) there could be a deletion of some or all of the cell divisions with ongoing differentiation. At the present stage a decision between these possibilities is not possible. Therefore it is preferable to use the term "reduction of the amplification coefficient," which is neutral with respect to the biological mechanisms implied.

The second part in the analysis concerns the behavior of CFU-S, BFU-E, and CFU-GM. It is concluded that the data on CFU-S, BFU-E, and CFU-GM can be explained on the basis of a compensatory regulatory process acting from E and G cell stages to the more primitive cell stages (feedback II). The modification introduced to the standard model (\( \alpha_{BFU-E} = \alpha_{CFU-GM} = \text{const.} \) ) can be considered as a minor model modification because the general concept of a feedback II is not changed, in particular with respect to CFU-S regulation. It is very likely that the difference has to do with strain differences.

Comparing model predictions quantitatively with the data, one should bear in mind that at least three important simplifications have been made in the model, because appropriate data are not available. First, the model is designed for the total body hemopoiesis in mice. Therefore the model is
compared with the data pooled from bone marrow and spleen. This appears justified because marrow and spleen exhibit a similar pattern of reactions during TAP treatment with some minor quantitative differences. Model refinements considering the conditions in marrow and spleen separately are in preparation.

Second, the description of granulopoiesis is not very realistic, because no CSF effects are assumed to act on CFU-GM. It is possible that during the 4-day application of the antibiotic TAP a specific stimulation of CFU-GM growth by CSFs or other granulopoietic growth factors takes place. This could explain why the CFU-GM data are higher than predicted by the model curve. This could also explain why CFU-GM increase more than BFU-E in the data. At present it is difficult to make quantitative assumptions about the in vivo feedback system governing granulopoiesis. Further experimental research seems necessary to provide data for an appropriate modeling.

Third, one may question the description of CFU-S as a homogeneous self-renewing stem cell in the light of reports on stem cell hierarchies [13–15]. However, it should be kept in mind that the present model is designed to describe the behavior of erythromyeloid stem cells on a time scale of a few days. It is not very likely that the behavior of more primitive stem cells will play a considerable role in such short-term circumstances. In addition, it was pointed out that the present description can often be interpreted as an averaged description of a heterogeneous stem cell population [2, 6]. Nevertheless the future availability of data on mixed lineage colony-forming units (CFU-GEMM) and pre-CFU-S might make it possible to abandon the simplistic description of a homogeneous stem cell population.

It is certainly possible to imagine other ways to explain the data on CFU-S, BFU-E, and CFU-GM during TAP application than by feedback II activation. TAP could specifically stimulate amplification of BFU-E and CFU-GM, although this would be surprising with respect to the effects on more mature cells. Alternatively, maturation could be slowed down, leading to an accumulation of BFU-E and CFU-GM, but the constant cyclic activities argue against this. Changes in microenvironmental conditions have not been investigated and cannot be ruled out. Due to these possibilities it would certainly be unjustified to take the TAP data alone as sufficient evidence for the feedback II process. However, they supplement a series of other experiments that all fit into this concept, thus suggesting consistency and comprehensiveness. This is illustrated by four examples.

First, this feedback II concept explains that during continuous whole body irradiation peripheral hemopoiesis can be maintained at almost normal levels although stem cell numbers stay at low values. This is due to a massive stimulation of stem cells to produce more differentiating progeny [2, vol I, chapter 9].

Second, in the case of erythropoietic suppression by red cell transfusion as well as posthypoxic conditions, significant increases in CFU-S numbers can be attributed to a feedback II activation. At the beginning the reduction in erythroid precursors E has two effects: an increase in CFU-S cycling and a decrease of self-renewal. A wave of differentiation results, enlarging BFU-E and CFU-GM numbers (note that up to here the explanation for the TAP effects is identical). As a consequence the number of G increases. Due to the strong role of G in feedback II this leads to a change of the self-renewal of CFU-S, which then start to increase. Thus, after several days CFU-S, BFU-E, and CFU-GM numbers are all elevated [2, vol II, chapters 2 and 4]. There is a specific difference between these circumstances and those found during TAP treatment. In the TAP experiments the number of G does not increase but remains below normal. This causes CFU-S to continue their decline.

Third, in the case of erythropoietic stimulation by hypoxia, the increase in E numbers leads to a suppression of CFU-S cyclic activity, resulting in a lower differentiation rate visible in a decline of BFU-E, CFU-GM, and G that is in agreement with the data [2, vol I, chapter 15].

As a final example, experiments with iron 55 need to be mentioned. The isotope is incorporated into heme-synthesizing cells and kills them. A decline in CFU-S numbers to 50% within 1 day was observed. This was interpreted by Reinke et al. [16] and Reincke and Cronkite [17] as evidence for an intramedullary feedback. A recent model analysis of their data qualitatively supported the conclusions, but also demonstrated that the CFU-S decline seen is more pronounced and more rapid than should be expected from the model. Reinke et al. [16] and Reincke and Cronkite [17] found about a 50% reduction in ortho- and polychromatic erythroblasts and no changes in granuloid precursors. The effects during TAP treatment are much more pronounced on erythroblasts and also affect granulopoiesis. Nevertheless, with regard to the different mode of action, the data on iron 55 and TAP application can be considered as supplementary.

The feedback II concept has gradually developed in the past and it is difficult to find its root. Lajtha and Schofield [18] and Lajtha [19] suggested a feedback from erythroid cells to the CFU-S, Reinke et al. [16] and Reincke and Cronkite [17] argued along similar lines based on the iron 55 experiments mentioned. Blackett and Botnick [20] argued for a regulatory impact of granulopoiesis on CFU-S based on data obtained after cyclophosphamide treatment and irradiation. Their hypothesis, however, lacked some precision [21]. Some authors described such feedback concepts in terms of mathematical models [22–24], but usually considered only one cell lineage, either erythropoiesis or granulopoiesis. Consequently we consider the proposed model concept as more comprehensive because it allows the description of the behavior for various combinations of CFU-S, E, and G numbers under different situations. The model assumptions on how CFU-S, E, and G numbers quantitatively influence cyclic activity and self-renewal in vivo have up to now resisted a series of tests and can therefore be recommended for further application.

Clearly a rigorous proof of feedback II requires a molecular basis of this process. Little is known at the moment about possible control factors and the role of the microenvironment under such circumstances, but it may be important to focus interest on this topic. A better understanding of feedback II may also be interesting because of its potential clinical implications. Because, at the moment, growth factors of feedback III (EPO, GM-CSF, and G-CSF) enter clinical practice, it is interesting to know whether manipulations of the precursor cell numbers by growth factors can indirectly influence stem cells.
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