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Bone marrow regeneration under cytotoxic drug regimens: behaviour ranging from homeostasis to unpredictability in a model for hemopoietic differentiation

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In the process of hemopoiesis, bone marrow stem cells differentiate into the various types of mature blood cells. We present a model for bone marrow dynamics, which retrieves its ability to continuously modulate the balance between self-renewal and differentiation, even under periodic cytodestructive perturbations. Yet, a temporally stochastic perturbation results in chaotic-like behaviour which has no deterministic source.

Keywords: Cellular automata; Nonlinear dynamics; Chaos; Stem cells; Colony-forming units.

In the process of hemopoiesis, a small population of pluripotent stem cells continuously produces the whole variety of mature blood cells, even under harsh cytodestructive treatment. The hemopoietic system is a good example of a homeostatic, self-organizing, biological system. Bone marrow stem cells give rise to cells that are irreversibly committed to differentiate into one of the various hemopoietic lineages. Committed cells further differentiate into immature, lineage-restricted cells that mature into eight main types of specialized cells. Proliferation and differentiation are controlled by protein signals (cytokines) that are secreted by hemopoietic cells themselves, by bone marrow stromal cells and by other organs (Metcalf, 1989; Moore et al., 1990). The spatial structure of the bone marrow plays a role in the control process: a differentiated neighbourhood seems to favour differentiation, while a stem-cell neighbourhood favours self-renewal (Zipori, 1988). This poorly understood feedback (Lord and Schofield; 1979, Zipori, 1990a,b) modulates the balance between the number of stem cells that differentiate and those that self-renew, ensuring the existence of the required numbers of any cell type (Wichmann et al., 1988). Considerable research efforts have recently been directed towards the identification of the most primitive hemopoietic stem cell, which is possibly the one bearing the CD34 surface antigen (Visser and Van Bekkum, 1990; Bertoncello et al., 1991; Kobayashi et al., 1991), and assessing its self-renewal capacity (Sutherland et al., 1990; Okada et al., 1991). The most studied, though probably not the most primitive, are the spleen colony-forming units (CFU-S). In a steady state, 90% of these cells are proliferatively quiescent (Schofield, 1978; Lord and Testa, 1988), but after a severe depletion, which may be caused by chemotherapy or irradiation, as few as one or two dozens of CFU-S suffice for complete regeneration (Zipori, 1990a; Spangrude et al., 1988).

How the bone marrow homeostasis is maintained and under which conditions it is upset are

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questions of major importance, especially now when drug therapy is considered in conjunction with bone marrow reconstitution (Atkinson, 1990). The aim of the present work is to extract the resilient properties of the bone marrow from its basic dynamics and to analyze the effect of different drug regimens on its regeneration ability. The rationale of our mathematical modelling is to find the minimal assumptions necessary to retrieve the observed phenomena. Accordingly, we assume that each cell may be in one of three possible states: one is the stem cell and the two other states are cells already committed to one of two branches of differentiation. A stem cell in our model represents the most primitive stem cell, which has a high capacity (infinite, in the model) of self-renewal (the exact identity of the stem cell is irrelevant). A stem cell in the model switches from self-renewal into differentiation according to the states of its neighbouring cells. Thus, the balance between self-renewal and differentiation is controlled only through communication between neighbouring cells, while proliferation is controlled only by available space. We assume that additional factors (like cytokine signals) which are involved in the global control of the system and not in the local cell-cell interactions, are of second-order importance relative to local interactions (Zipori, 1990b). For this reason, in our model we do not include such factors, again striving to construct a minimal description. Inter-cell interactions in real bone marrow may be stimulatory (Broxmeyer et al., 1991; Quesenberry et al., 1991; McNiece et al., 1991; Bernstein et al., 1991; Okada et al., 1991) or inhibitory (Graham et al., 1990; Zipori, 1990a,b; Bungart et al., 1990; Johnson et al., 1991). However, the existence of growth inhibitors in the bone marrow is as yet to be fully understood (Zipori, 1991). No specific inhibition on stem-cell proliferation or differentiation is assumed in our model: the stem cell tends to decide its next move according to the principle that an environment composed mainly of stem cells encourages self-renewal, and a differentiating environment encourages differentiation. We will not argue that there is no inhibition, but, rather, we will examine the possibility that the basic properties of hemopoiesis can be retrieved without assuming inhibition. Below we describe in detail our model and its dynamics.

Our model for hemopoiesis is a cellular automaton model, in which each cell may be in one of three possible states: 0, +1 and -1, denoting a stem-cell state and two possible differentiated states. The cell processes external input according to its present state, the result being its new state. The input the cell uses is a stochastic variable, whose probability distribution is determined only by the cell's nearest neighbours: a stem cell neighbour induces self-renewal of a stem cell with a high probability p, or differentiation of the stem cell with probability $(1 - p)$; a differentiated neighbour induces self-renewal of a stem cell with a probability $\tilde{p}$, $(\tilde{p} < p)$, or differentiation of the stem cell with probability $(1 - \tilde{p})$. The self-renewal probability of the stem cell is the average of the self-renewal probabilities induced by all its neighbours (Fig. 1). Differentiated cells self-renew and die after τ divisions. Each of the four nearest neighbours has an equal chance to split and proliferate into the empty place created by such cell death.

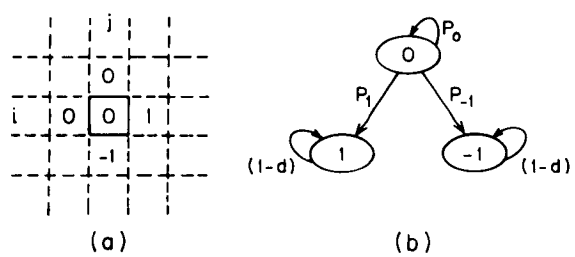

Fig. 1. Our model for the bone marrow is a two-dimensional, three-state cellular automaton with nearest-neighbour connectivity. One possible configuration of cells is shown in (a). The operation of a cell in state 0 depends on its nearest neighbours: denote by l_0, l_1, l_{-1} and l, the stem-cell, state 1, state -1 numbers, and total number of neighbours of the cell (i,j), respectively $(l_0 + l_1 + l_{-1} = l$ and $0 \leq l \leq 4)$. A cell in state 0 remains in the 0 state, i.e. it self-renews, with probability $P_0 = p(l_0/l) + \tilde{p}[(l_1 + l_{-1})/l]$, with p, $\tilde{p}$ as defined in the text; it differentiates to state 1 with probability $P_1 = [(1 - p)/2](l_0/l) + (1 - \tilde{p})(l_1/l)$, or to state -1 with probability $P_{-1} = [(1 - p)/2](l_0/l) + (1 - \tilde{p})(l_{-1}/l)$ (obviously $P_0 + P_1 + P_{-1} = 1$). Cells in state 1 or -1 are removed after τ iterations; cells in state 0 are never removed. The diagram of allowed transitions, with the corresponding probabilities, is given in (b).

At the initiation of the process the system is composed of 10^4 stem cells. At every time-step, all cells are processed in parallel, using periodic boundary conditions. As long as the system is unperturbed and its differentiation rate is not larger than the stem cells' self-renewal rate, it evolves to a steady state where the proportion of the various cell types in the population stays roughly the same. If the differentiation rate exceeds the stem cells' self-renewal rate, the pool of stem cells is exhausted and all cells eventually die.

Homeostasis is exhibited in our model by a full recovery from severe (up to 97%) stem-cell loss. Recovery is basically exponential with superimposed damped oscillations of period τ, generated by synchronization of cell death and proliferation in a large fraction of the population after one sharp depletion. Similar oscillations were observed in bone marrow stem cells of mice and explained as the result of a hypothetical autoregulation of stem cells through a proliferation-inhibiting factor (Nečas and Znojil 1988; Hauser and Nečas 1988). Our work suggests that there need be no such factor, since the synchronized cell death following each perturbation can result in proliferation oscillations due to the sudden enlargement of available space (which represents here all other limiting resources).

Simulating various regimens of toxic-drug administration by repeated depletion perturbations, we have shown that homeostasis can be maintained when drug administration is fully periodic (Fig. 2a). In contrast, when time intervals between administrations become stochastic, chaotic-like behaviour emerges (Fig. 2b) and the stem-cell pool is rapidly depleted. The temporal behaviour of the stochastically perturbed system is highly sensitive to initial conditions: a change in the state of as little as 0.001 of the cells in the initial configuration results in totally different time series. The system also exhibits almost complete memory loss, measured by the Gade–Amritkar method (Gade and Amritkar, 1990): $\nu_{(t>0)}/\nu_{(t=0)} = 2.0 \pm 0.1$, where doubling of the generalized exponent ν_t indicates complete memory loss, characteristic of chaotic time series. The periodically perturbed series exhibit only partial memory loss: $\nu_{(t>0)}/\nu_{(t=0)} = 1.5 \pm 0.1$. Below we focus on the analysis of this behaviour, via the time-evolution equations for the stem-cell pool.

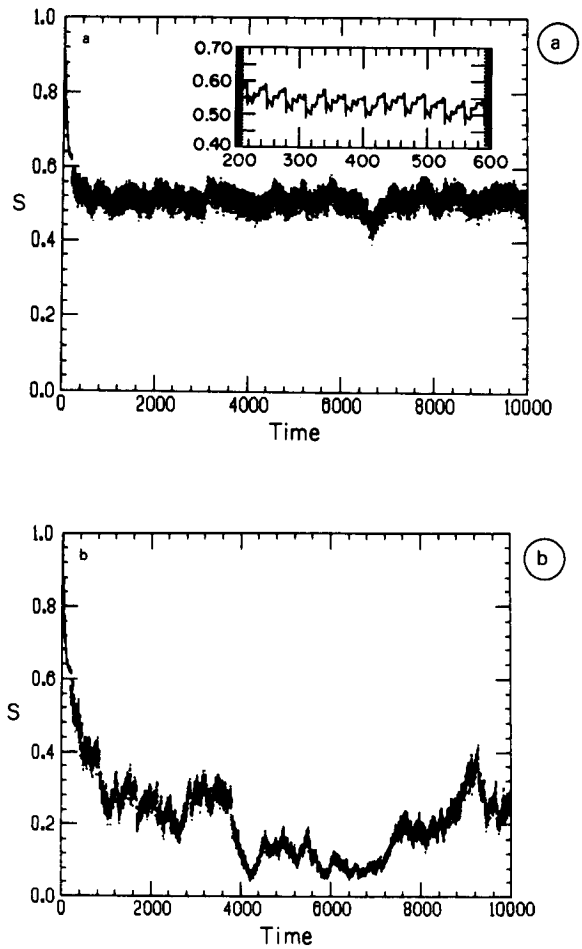

Fig. 2. The cellular automaton simulations, performed on a square grid of 10^4 cells. Initially all cells are in the 0 state (stem cells), but there is a finite probability $(1 - p)/2$ for a stem cell to spontaneously differentiate to one of the two branches. Drug treatment is initiated after the system has reached a steady state. Time series of the fraction of stem cells are shown here for two cases: periodic and stochastic drug treatment. (a) A time series of a periodic drug treatment ($T = 30$). Inset: part of the series enlarged to show the periodicity. (b) Stochastic drug treatment: chaotic-like ($\overline{T} = 30$, $\sigma = 1$) time series. The other parameters (for both (a) and (b)) are: $p = 0.96$; $\tilde{p} = 0.92$; $\tau = 9$, $D = 1/3$.

In order to construct such equations for the fraction of stem cells in the system, we look only at averaged quantities (averaged over the whole grid of cells), which is equivalent to making the simplifying assumption of homogeneity. The average stem cell self-renewal probability (averaged over all stem cells) per cell-cycle is: $ps_n + \tilde{p}(1 - s_n)$, where s_n denotes the fraction of stem cells in the population in generation n. The death probability per cell cycle of non-stem cells is d, so that the fraction of sites vacated at each cycle is $d(1 - s_n)$ and the fraction of such sites reoccupied by stem cells is $ds_n(1 - s_n)$. Thus we reach the time-evolution equation for s_n:

$$\begin{aligned} s_{n+1} &= [ps_n + \tilde{p}(1 - s_n)]s_n + ds_n(1 - s_n) \\ &= (\tilde{p} + d)s_n + (p - \tilde{p} - d)s_n^2 \end{aligned} \quad (1)$$

which resembles the logistic mapping (May, 1974) and has the same bifurcations, and the same accumulation point of bifurcation values, as the logistic. The only non-zero steady-state fraction of stem cells is $\hat{s} = (1 - \tilde{p} - d)/(p - \tilde{p} - d)$ which is stable and obeys $0 < \hat{s} < 1$, when $p < 1 < (\tilde{p} + d) < 3$. Since p, $\tilde{p}$, d are probabilities, our calculations are always performed in the one fixed-point regime. Starting from any $0 < s_{n=0} < 1$ and iterating (1), we arrive at $\hat{s}$ in less than 200 generations. The trivial steady state, $\hat{s} = 0$, is stable when $(\tilde{p} + d) < 1$, i.e. when the differentiation rate is larger than the self-renewal rate.

To simulate the administration of drug, we introduce perturbations in Eqn. (1) by instantaneously eliminating a fraction D of the stem cells. We observe a periodically repeated recovery when the perturbation is fully periodic and an erratic behaviour that looks chaotic when the intervals between successive perturbations are normally distributed random variables (Fig. 3). Dimension measurements performed on these chaotic-like series in the Grassberger–Procaccia method (Grassberger and Procaccia, 1983a,b) yield a dimension of 0.6 ± 0.2. This value lies between the dimension of the chaotic attractor of the logistic equation (0.5) (May, 1974), and that of the time series of our equation (0.7 ± 0.2) when using chaotic-regime

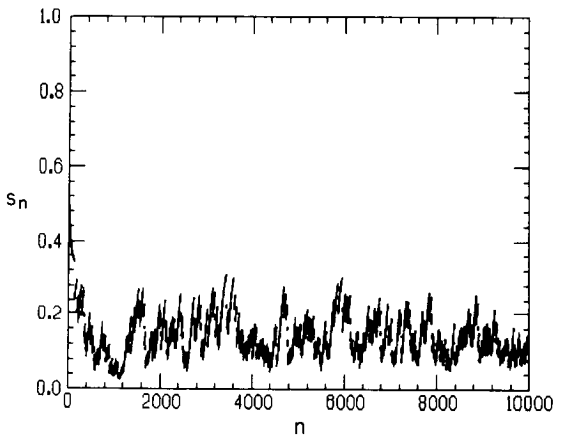

Fig. 3. Chaotic-like ($\overline{T}$ = 30, σ = 1) time series, generated from Eqn. (1) with perturbations. p = 0.96, $\tilde{p}$ = 0.92, d = 0.1, D = 0.3.

parameters ($\tilde{p} + d = 4.0$). The dimension of the periodic series is smaller, 0.2 ± 0.1.

To examine the source of the chaotic-like behaviour, we will focus attention on the stem-cell fraction on the instant just prior to the perturbation. Define S_j as the value of s_n at the instant prior to the jth perturbation. Between perturbations s_n recovers through (1) and after each perturbation, which eliminates a fraction D of the stem cells, s_n equals $(1 - D)S_j$. Hence S_j (which is a Poincaré mapping (Eckmann and Ruelle, 1985) of our time series) evolves in time through

$$S_{j+1} = [(1 - D)S_j - \hat{s}] \exp(-\beta t_j) + \hat{s} \quad (2)$$

where t_j is the time between the jth perturbation and the $(j + 1)$th perturbation, and β is the rate of the exponential recovery of s_n to equilibrium after each perturbation: $\beta \equiv -\ln[\tilde{p} + d + 2(p - \tilde{p} - d)\hat{s}] > 0$.

In the periodically perturbed case, $t_j = T$ = constant. The mapping is linear and as such has only one (stable) fixed point,

$$\hat{S} = [1 - \exp(-\beta T)/1 - (1 - D)\exp(-\beta T)]\hat{s} \quad (3)$$

This resembles the mode locking phenomenon

characteristic of periodically driven systems, appearing also in neural network models (Sompolinski et al., 1988; Renals and Rohwer, 1990).

In the chaotic-like case, t_j in Eqn. (2) is a random variable, normally distributed with an average $\overline{T}$ and a standard deviation σ. S_j, which depends on t_j, may fluctuate faster than the system can adjust, i.e. the perturbation hits at a different point of the recovery to equilibrium every time, so that the system is unable to settle on a periodic motion (unless $\overline{T}$ is so large – of the order of 200 – that the system returns to the same point before each perturbation). This noise in t_j can never drive s_n to the chaotic regime of (1), because the calculations are performed only in the one fixed-point regime. The source of the chaotic-like behaviour we see must then lie in the stochastic driving itself; it destabilizes the system in a way comparable to the case described by Newhouse (1974, 1979, 1980), where there are many complex attracting periodic orbits of arbitrarily high periods, with very narrow and convoluted domains of attraction, so that any amount of noise leads to chaotic behaviour.

Low-dimensional erratic behaviour has recently been observed in several biological systems (Babloyantz and Destexhe, 1986; Skarda and Freeman, 1987; Pool, 1989) and described as deterministic chaos. It was furthermore conjectured that the chaotic mechanism plays the role of a maximal-information basic state of the systems considered. We suggest, based on our model for hemopoietic stem-cell dynamics, that a behaviour that looks chaotic may sometimes result from stochasticity in the intervals between successive perturbations and is not necessarily deterministic. Moreover, results presented here support our previous conclusions (Agur, 1985; Agur and Deneubourg, 1985) that the distribution of inter-disturbance intervals is the major factor in determining population persistence. The possibility that random intervals between high-dose drug applications may impede bone marrow regeneration should be further investigated. In this context it should be noted that mathematical work and laboratory experiments also suggest that a well-calculated fully periodic drug application may minimize the cytotoxicity to the bone marrow (Agur et al., 1988).

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