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ORIGINAL ARTICLE

A Model of Oscillatory Blood Cell Counts in Chronic Myelogenous Leukaemia

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Abstract In certain blood diseases, oscillations are found in blood cell counts. Particularly, such oscillations are sometimes found in chronic myelogenous leukaemia, and then occur in all the derived blood cell types: red blood cells, white blood cells, and platelets. It has been suggested that such oscillations arise because of an instability in the pluri-potential stem cell population, associated with its regulatory control system. In this paper, we consider how such oscillations can arise in a model of competition between normal (S) and genetically altered abnormal (A) stem cells, as the latter population grows at the expense of the former. We use an analytic model of long period oscillations to describe regions of oscillatory behaviour in the S–A phase plane, and give parametric criteria to describe when such oscillations will occur. We also describe a mechanism which can explain dynamically how the transformation from chronic phase to acute phase and blast crisis can occur.

Keywords Chronic myelogenous leukaemia · CML · Chronic phase · Oscillations · Delay equations · Blast crisis

1 Introduction

Chronic myelogenous leukaemia (CML) is a progressive, malignant disease characterized by a large number of abnormal blood cells in the bone marrow and peripheral blood (Hoffbrand and Pettit 1993; Whittaker 1987; Druker et al. 2001). Abnormal blood cells contain the Philadelphia (Ph) chromosome, the result of a reciprocal translocation between the chromosomes 9 and 22 (De Klein et al. 1982;

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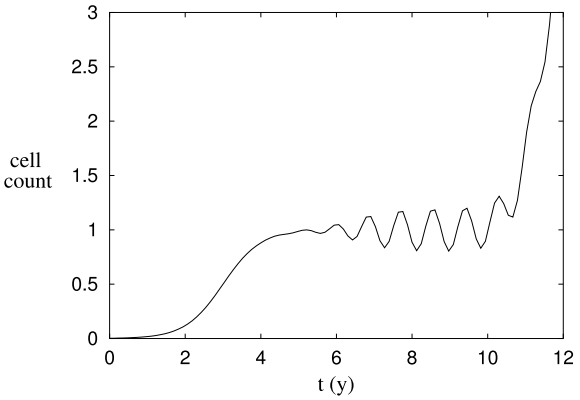

Fig. 1 An entirely schematic representation of the progression of CML through three distinct phases. The units of cell count are arbitrary (as the graph is schematic) and the time is indicated in years for a typical progression of the disease. After an initial period of 5 to 10 years during which the abnormal cell count rises to a relatively steady (and possibly oscillatory) level, the system reaches a chronic phase during which the disease is diagnosed. After several years in the chronic phase, an instability arises which proves fatal after a period of the order of months

Nowell and Hungerford 1960). Cells with this new form of chromosome have a proliferative advantage over the normal ones because by the time the disease has become sufficiently advanced to permit a diagnosis to be made, which is approximately 8 years after the initial cell alteration (Kamada and Uchino 1978), the Ph chromosome is present in a majority of blood cells (Goldman 1997; Strife and Clarkson 1988). This leads to the conclusion that the initial transformation starts in a single pluripotent hematopoietic stem cell in the bone marrow (Buckle et al. 2000). However, in the majority of patients at diagnosis, it is found that the bone marrow still contains a significant number of normal stem cells, and it is only later that these normal Ph negative cells decline with time (Strife and Clarkson 1988; Frassoni et al. 1999).

Somewhat like AIDS, the clinical course of chronic myelogenous leukaemia when diagnosed can be divided into an apparently stable chronic phase, a transitional accelerated phase, and a subsequent acute phase (Faderl et al. 1999; Hill and Meehan 1999; Cortes et al. 1996). The chronic phase, lasting for 3–6 years, is characterized by a larger than normal but relatively constant number of abnormal cells which nevertheless function almost normally, so the disease can be controlled (Eaves et al. 1998). Eventually, the disease transforms into a more advanced phase characterized by an accelerated increase of the abnormal cells, which is manifested by a sudden expansion in the number of abnormally proliferated immature blood cell types (Goldman 1997). This acute phase leads to fatality in a period of 3–9 months (Cortes et al. 1996). Figure 1 shows a schematic progression in time through these phases.

The progression from the chronic, seemingly stable, phase to the advanced phase has intrigued experimental haematologists for several decades. One possible explanation for this progression is that during the chronic phase, leukaemic cells develop additional mutational events, which cause partial or complete loss of

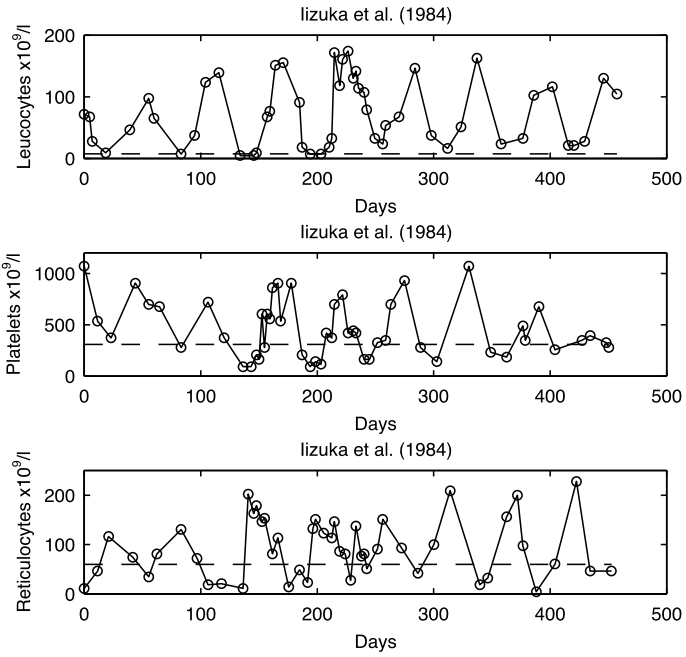

Fig. 2 Oscillations in leukocytes, platelets, and reticulocytes in a patient with CML. Data from Iizuka et al. (1984). The period is about 60 days. Figure supplied courtesy of M.C. Mackey

the cells' ability to undergo differentiation and maturation (Frassoni et al. 1999; Strife and Clarkson 1988).

A way in which such mutational events might be mathematically modelled is through the slow evolution of parameters with time, and a consequent interpretation of the onset of the acute phase is that it occurs through the loss of stability of a slowly evolving quasi-steady state, in which there is a balance in the interaction between normal and abnormal stem cells (the most primitive cells in the bone marrow) and their progeny, and possibly also the immune system response. It is this possibility which motivates our study of the dynamics of the blood cell lineages.

A related issue, and one which also concerns us here, is the presence of regular oscillations in blood cell counts in patients with CML. Such oscillations have been found in many different CML patients, and many cases have been documented by Fortin and Mackey (1999) and Bennett and Grunwald (2001), including that shown in Fig. 2, while these and other such oscillatory blood cell diseases are reviewed by Haurie et al. (1998) and Guerry et al. (1973). When oscillations occur in blood cell counts, these are seen in all blood cell types, consistently suggesting that the oscillations originate in the stem cell population. This was suggested by Mackey (1978, 1979, 1997), who also provides a model for the control of the stem cell population which can be subject to oscillatory instability. It is not at all clear whether cell oscillations are a side effect of the disease, or are instrumental in controlling the course of its progression. In order to further our understanding of this question, we are here concerned with models which can describe such oscillations, and we aim to do so in the

context of a presumed competition between normally and abnormally proliferating stem cells.

Stem cells are a small group of cells in the bone marrow which possess an extensive capacity to maintain the blood cell production in the body (Lodish et al. 1995; Alberts et al. 1989; Fox 1996; Potten 1997; Schwarzenberger et al. 2002). The most primitive stem cell is the pluripotent haematopoietic stem cell. This cell gives rise to myeloid and lymphoid stem cells. The myeloid line differentiates into various types of myeloid progenitor cell which eventually generate erythrocytes, neutrophils, eosinophils, basophils and mast cells, monocytes, and platelets. The lymphoid line gives rise to lymphocyte progenitor cells that eventually mature into T lymphocytes, B lymphocytes, and natural killer (NK) cells. The process of blood cell formation is called *haematopoiesis*; it takes place in the bone marrow, and it involves the progressive development of structural and functional characteristics specific for a given cell type (maturation), and cell proliferation (Hughes-Jones and Wickramasinghe 1997; Hoffbrand and Pettit 1993).

As maturity increases, the ability of cells to proliferate and self-renew decreases (Hoffbrand and Pettit 1993). When the fully matured and functional cells are formed, they leave the bone marrow and enter the blood. In healthy individuals, some stem cells enter the blood as well, but they are present in such small numbers that they cannot be counted or identified in the usual type of blood count.

Although some of the process of hematopoiesis is known, there are a lot of open questions concerning the role of the stem cells in this process. One of the questions of interest for our model is whether the stem cells self-renew (i.e., replicate themselves in order to maintain their number). Gordon and Blackett (1998) offer two different hypotheses for this: either sufficient stem cells are produced during embryogenesis to supply the needs of the adult animal throughout life, which would mean that stem cells are quiescent until they are needed to supply mature blood cells; or adult animals contain only a small number of stem cells that can self-replicate to produce more stem cells, in which case when the stem cells divide, not all of the daughter cells differentiate.

In the second case, it is believed that the molecular abnormality that arises in stem cells in CML causes an abnormality in the delayed negative-feedback mechanisms of the cell cycle (Gordon et al. 1999) which then results in the overproduction of cells of the granulocytic series. However, the link between the molecular and cellular phenotypes remains poorly understood. It is believed that some or all of the normal stem cell proliferation rate, differentiation rate, apoptosis rate, and the time the cell spends in the proliferative cycle, are perturbed in abnormal cells (Frassoni et al. 1999; Eaves et al. 1998; Gordon et al. 1999; Jorgensen and Holyoake 2001; Kummermehr and Trott 1997).

An alternative view is that of the discordant maturation hypothesis of Strife and Clarkson (1988) and Strife et al. (1988), which proposes that the more mature proliferating cells in chronic phase CML are responsible for the expansion of the leukemic population. A mathematical model of this has been proposed by Rubinow and Lebowitz (1976). This is in contrast to the Dowding hypothesis, which suggests that leukemic stem cell proliferation alone can explain the expansion (Gordon et al. 1987).

The two models give rise to very similar model structures, as discussed below. In this paper, we will largely for simplicity follow the idea of the Dowding hypothesis

and will look into the regulation of the proliferation of the leukemic stem cell population. Jorgensen and Holyoake (2001) investigated the influence of the Ph chromosome on the behaviour of the leukemic cells, and they concluded that the altered gene may induce neoplastic transformation, enhance cellular proliferation and differentiation, increase stem cell turnover, inhibit apoptosis, and produce defects in adhesion to bone marrow. Eaves et al. (1998) indicate three biological changes affecting the development of leukemic cells: an increased probability of differentiation at the level of the most primitive leukemic stem cells, an increased turnover rate of the leukemic progenitors at all stages of differentiation, and their increased ability to survive under conditions of growth factor deprivation. Gordon et al. (1999) offer several explanations for the expansion of the leukemic progenitor: reduced apoptosis in leukemic stem cells, reduction in cell cycle time, an increase in stem cell proliferation rate, and an increase in stem cell self-renewal rate. Bedi et al. (1994) suggest that CML cells have a decreased rate of apoptosis.

This summary of evidence reported by these investigators leads us to make several assumptions concerning the effect of CML on the model parameters:

1. The rate of apoptosis in normal stem cells is higher than in leukemic stem cells.
2. The time spent in the cell cycle is higher in normal stem cells than in the leukemic stem cells.
3. The rate of differentiation is smaller in normal stem cells than in the leukemic stem cells.
4. The recruitment rate is smaller in the normal stem cell than in the leukemic stem cells.

2 The Mackey Model

Stem cells go through a cycle indicated in Fig. 3 comprising four activities: they may be in an inactive resting phase (which is the most common phase (Gordon et

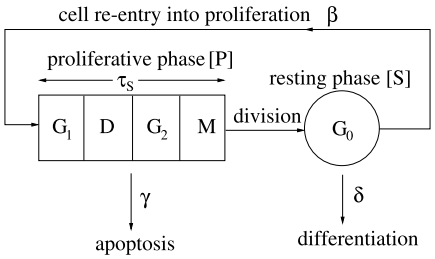

Fig. 3 A schematic representation of the control of pluripotent stem cell regeneration. The resting state is designated G_0 and its population size is denoted S, while the proliferative phase (of population size P) is composed of various sub-phases, including those labelled D, DNA synthesis, and M, mitosis, as well as intermediary phases G_1 and G_2; see Mackey (1981) for further details. The proliferative phase takes a time τ_S, γ is the specific rate of cellular death (apoptosis) during proliferation, δ is the specific rate of differentiation into all of the committed stem cell populations, and the specific recruitment rate $\beta(S)$ is usually taken to be in the form of the Hill function given in (2). Reasons for choosing a Hill function are given in the Appendix. Apoptosis during the resting phase is not generally considered (Mackey 1981), but would in any case only have the effect of altering the value of δ

al. 1999)), they can proliferate (by dividing to produce two daughter cells), they can differentiate (becoming more mature and beginning the progression to fully differentiated blood cells), and they can undergo apoptosis; in other words die. There is a delay in the model, because it takes a time τ_S for cells to go through the proliferative phase.

Mackey's model of the replication control system shown in the figure consists of two delayed differential equations that simulate the evolution of a stem cell population. The model is based on earlier formative work by Lebowitz and Rubinow (1969), and Rubinow and Lebowitz (1975); the latter paper also discusses the maturation of the cells. Mackey's equations are given by

$$\begin{aligned} \frac{dP}{dt} &= -\gamma P + \beta\big\{S(t)\big\}S(t) - e^{-\gamma\tau_S}\beta\big\{S(t-\tau_S)\big\}S(t-\tau_S), \\ \frac{dS}{dt} &= -\big[\beta\big\{S(t)\big\} + \delta\big]S(t) + 2e^{-\gamma\tau_S}\beta\big\{S(t-\tau_S)\big\}S(t-\tau_S), \end{aligned} \tag{1}$$

where $\beta(S)$ is given by the Hill function

$$\beta(S) = \frac{\beta_0\theta^n}{\theta^n + S^n}. \tag{2}$$

2.1 Oscillatory Behaviour

Under certain circumstances, the steady state of $(1)_2$ is oscillatorily unstable, and the cell population undergoes spontaneous oscillations. These oscillations can have long periods (by comparison with τ_S), as shown in Fig. 2, which can be understood in terms of $(1)_2$ when the differentiation rate δ is relatively small.

Fowler and Mackey (2002) analysed the stem cell regulation model $(1)_2$ when δ is small, and we begin by recapitulating the main points of their findings here. The equations can be written in dimensionless form by scaling S and t as $S \sim \theta$, $t \sim \tau_S$; the resulting equation for the dimensionless resting population S is

$$\frac{\mathrm{d}S}{\mathrm{d}t} = g(S_1) - g(S) + \varepsilon\big[\mu_S g(S_1) - S\big], \tag{3}$$

where $S_1 = S(t-1)$, and

$$g(S) = b_S S h(S), \quad h(S) = \frac{1}{1+S^n}, \tag{4}$$

and the parameters are defined by

$$b_S = \beta_S\tau_S, \qquad \varepsilon = \delta_S\tau_S, \qquad \mu_S = \frac{2e^{-\gamma_S\tau_S} - 1}{\delta_S\tau_S}. \tag{5}$$

Fowler and Mackey (2002) analysed this class of delay differential equations using singular perturbation analysis, based on the assumption that b_S and μ_S are $O(1)$, but $\varepsilon \ll 1$. (Typical estimates based on clinical data were $b_S = 3.9$, $\mu_S = 2.6$, $\varepsilon = 0.11$.)

Oscillations occur when $\varepsilon \ll 1$ if $b_S > b_c$, where $b_c = 3$ for $n = 3$, and are relaxational; the period is of dimensional order $1/\delta_S$, and is controlled by the differentiation rate. Long period oscillations in the model thus result when the differentiation time is long.

A good deal of work has been done on this G_0 stem cell model, and variations thereof. Models which consider only the stem cell population, and with a constant time delay include those by Mackey (1978, 1979, 1997), Fowler and Mackey (2002), Pujo-Menjouet and Mackey (2004), and Pujo-Menjouet et al. (2005). Colijn and Mackey (2005) and Colijn et al. (2006) synthesized several previous mathematical models of hematopoietic stem cell dynamics (Mackey 1978, 1979, 1997), and models for the regulation of neutrophils (Haurie et al. 2000), platelets (Bélair and Mackey 1987), and erythrocytes (Mahaffy et al. 1998) into a single model for the regulation of the hematopoietic system.

Adimy et al. (2005a) use the G_0 model but assume that the time delay (or proliferating phase duration) is uniformly distributed on an interval. The main objective is to investigate the effect of time delay on the dynamical solutions. It is shown that there exist some critical values of the time delay such that a local Hopf bifurcation occurs at the non-trivial equilibrium. Adimy et al. (2005b) apply a similar approach on a maturation model. More recently, sufficient conditions for the stability of delay differential equations with distributed delay have been obtained by Bernard et al. (2001). They used some properties of the distribution to prove these results. However, the authors focused on sufficient conditions for stability, there is no necessary condition in these studies, and these results are not applicable directly to the maturation model used by Adimy et al. (2005b).

Other approaches which do not use the G_0 model have also been reviewed. Michor et al. (2005) used a four-compartment model consisting of stem cells, progenitor cells, differentiated cells, and terminally differentiated cells and involved both normal and leukaemic cells to explain the kinetics of the molecular response to imatinib in a 169-patient data set. Their model was a simple competition model comprising a system of ordinary differential equations. Bessonov et al. (2006) use a population dynamics approach and consider a population of individual cells instead of a density which is usually considered in the continuous models. This allows them to describe the behaviour of cells and their interactions in a more explicit way. In their work, they introduce a new software created to study hematopoiesis at the cell population level with this individually based approach. Their main focus is to use it as an interface between theoretical works on population dynamics and experimental observations. Moore and Li (2004) model the interaction between the immune system (naïve T cells and effector T cells) and CML cancer cells in the body, using a system of ordinary differential equations which gives rates of change of the three cell populations.

Here, we use the G_0 model, and focus on the approach described by Fowler and Mackey (2002), where they analysed a single population stem cell model (1). We extend their method by introducing an additional population of stem cells, and look at the competition of the normal and the abnormal, leukaemic cells. In Sect. 3, we describe the competition model. We then analyse this model and its fixed points and stability in Sect. 4. We investigate the oscillatory behaviour of the model in Sect. 5. Finally, we discuss and interpret the results in Sect. 6.

3 A Competitive G_0 Model

We now suppose that there are two types of stem cell, the normal population (denoted S) and the abnormal, genetically altered population, denoted A. We suppose that A is controlled by a similar equation to that of S, but with different parameters, as described below. A model of essentially this type was introduced by Rubinow and Lebowitz (1976) in modelling acute myeloblastic leukaemia. However, they considered the precursor cells to be myeloblasts rather than stem cells, so that although the model was quite similar to that given here, the appropriate parameter choice was quite different. In order to write an appropriate model, we need to generalise how the Hill function $h(S)$ (cf. (4)) should be written when there are two cell populations present. It is by no means obvious how to do this and, therefore, in the Appendix, we examine a conceptual model of stem cell recruitment which is able to motivate the choice in (4), and also to provide a plausible controller for the present purpose. The result of this discussion is to suggest that the effects of crowding should lead to a modification of the definition of β in (2) to the form

$$\beta(S) = \beta_S \frac{\theta^n}{\theta^n + cS^n}, \tag{6}$$

where c is a crowding factor which depends on total cell density Θ. In the discussion leading to the derivation of (67), we derive the form

$$c = \frac{1}{\left(1 - \frac{\Theta}{\Theta_c}\right)^n}, \tag{7}$$

but the main point is that c should be an increasing function of Θ.

The competition of the two populations of cells in the bone marrow is modelled by the equations

$$\begin{aligned} \frac{dS}{dt} &= 2e^{-\gamma_S \tau_S} \beta_S S_{\tau_S} h(S_{\tau_S}/\theta, \Theta_{\tau_S}) - \left[\delta_S + \beta_S h(S/\theta, \Theta)\right] S, \\ \frac{dA}{dt} &= 2e^{-\gamma_A \tau_A} \beta_A A_{\tau_A} h(A_{\tau_A}/\theta, \Theta_{\tau_A}) - \left[\delta_A + \beta_A h(A/\theta, \Theta)\right] A, \end{aligned} \tag{8}$$

where

$$\Theta = S + A, \tag{9}$$

$S_{\tau_S} = S(t - \tau_S)$, $A_{\tau_A} = A(t - \tau_A)$, β_S and β_A represent maximum recruitment rate, and the feedback function h is the Hill function

$$h(\xi, \Theta) = \frac{1}{1 + c(\Theta)\xi^n}. \tag{10}$$

We non-dimensionalisz the variables using θ as the scale for A and S, and τ_S as the scale for t. The scaled version of (8) can then be written in the form

$$\begin{aligned} \frac{dS}{dt} &= (b_S + \varepsilon\lambda_S)S_1 h(S_1, \Theta_1) - \big[\varepsilon + b_S h(S, \Theta)\big]S, \\ \alpha\frac{dA}{dt} &= (b_A + \varepsilon\alpha d_A\lambda_A)A_\alpha h(A_\alpha, \Theta_\alpha) - \big[\varepsilon\alpha d_A + b_A h(A, \Theta)\big]A. \end{aligned} \tag{11}$$

The parameters are defined by

$$\begin{aligned} &b_S = \beta_S\tau_S, \qquad \varepsilon = \delta_S\tau_S, \qquad \lambda_S = \frac{(2e^{-\gamma_S\tau_S} - 1)\beta_S}{\delta_S}, \\ &b_A = \beta_A\tau_A, \qquad \alpha = \frac{\tau_A}{\tau_S}, \qquad d_A = \frac{\delta_A}{\delta_S}, \qquad \lambda_A = \frac{(2e^{-\gamma_A\tau_A} - 1)\beta_A}{\delta_A}. \end{aligned} \tag{12}$$

It should be noted that we have used the same value of θ in each Hill function. This is largely for convenience. Differing values of θ can effectively be included by allowing different definitions of the crowding coefficient in the Hill functions for each controller. We omit such delicacies.

Previously (Fowler and Mackey 2002), we estimated values of $b_S = 3.9$, $\mu_S = 2.6$, $\varepsilon = 0.11$; noting that $\lambda_S = \mu_S b_S$, this implies $\lambda_S \approx 10.14$.[1] On the basis that abnormal CML stem cells differentiate more rapidly, have a shorter cell cycle time, proliferate at a greater rate, and die less often, we would surmise that

$$\delta_A > \delta_S, \qquad \tau_A < \tau_S, \qquad \beta_A > \beta_S, \qquad \gamma_A < \gamma_S. \tag{13}$$

If this is the case, then the corresponding values of the dimensionless parameters would be

$$d_A > 1, \qquad \alpha < 1, \qquad b_A \sim b_S, \qquad \lambda_A \sim \lambda_S. \tag{14}$$

The equivalence of b and λ is due to the effect of competing inequalities in their definitions. We have a mild preference for $b_A > b_S$, on the basis that the alteration in recruitment rate β may be larger than that in cell cycle time τ. We also suppose that it is likely that $\lambda_A > \lambda_S$, on the basis that both quantities in the numerator increase, whereas only the single quantity in the denominator does so.

[1] The astute reader will note that the high value of λ_S appears to nullify the whole basis of the slowly varying approximation, since in fact $\varepsilon\lambda_S \sim 1$. Although one could simply choose to proceed in any case, formally assuming that $\lambda_S = O(1)$, it may be pointed out that what is necessary is that $\lambda_S h = O(1)$, and this is suggested by the steady state values in (23) below, which indicate precisely this balance.

4 The Slowly Varying Phase Plane

Our strategy in solving the competitive model (11) is to treat it in the same way as we dealt with (3). First, we rewrite (11) in a similar form:

$$\begin{aligned} \frac{dS}{dt} &= b_S\big[S_1 h(S_1,\Theta_1) - Sh(S,\Theta)\big] + \varepsilon\big[\lambda_S S_1 h(S_1,\Theta_1) - S\big], \\ \alpha\frac{dA}{dt} &= b_A\big[A_\alpha h(A_\alpha,\Theta_\alpha) - Ah(A,\Theta)\big] + \varepsilon\alpha d_A\big[\lambda_A A_\alpha h(A_\alpha,\Theta_\alpha) - A\big]. \end{aligned} \tag{15}$$

Next, we assume that S and A are slowly varying on a time scale of $O(1/\varepsilon) \gg 1$; expanding the delayed terms, we have

$$\begin{aligned} S_1 &\approx S - \dot{S} + \cdots, \qquad & A_\alpha &\approx A - \alpha\dot{A} + \cdots, \\ \Theta_1 &\approx \Theta - \dot{\Theta} + \cdots, \qquad & \Theta_\alpha &\approx \Theta - \alpha\dot{\Theta} + \cdots, \end{aligned} \tag{16}$$

and thus, defining the slow time

$$\tau = \varepsilon t, \tag{17}$$

we derive the slowly varying approximation for S and A,

$$M\begin{pmatrix} S' \\ A' \end{pmatrix} = \begin{pmatrix} \{\lambda_S h(S,\Theta) - 1\}S \\ d_A\{\lambda_A h(A,\Theta) - 1\}A \end{pmatrix} \tag{18}$$

where $S' = dS/d\tau$, $A' = dA/d\tau$, and the matrix M is given by

$$M = \begin{pmatrix} 1 + b_S H(S,\Theta) & b_S J(S,\Theta) \\ b_A J(A,\Theta) & 1 + b_A H(A,\Theta) \end{pmatrix}, \tag{19}$$

where

$$H(\xi,\Theta) = h + \xi(h_\xi + h_\Theta), \qquad J(\xi,\Theta) = \xi h_\Theta. \tag{20}$$

Inverting this, we have

$$\begin{pmatrix} S' \\ A' \end{pmatrix} = \Delta^{-1}\begin{pmatrix} 1 + b_A H(A,\Theta) & -b_S J(S,\Theta) \\ -b_A J(A,\Theta) & 1 + b_S H(S,\Theta) \end{pmatrix}\begin{pmatrix} \{\lambda_S h(S,\Theta) - 1\}S \\ d_A\{\lambda_A h(A,\Theta) - 1\}A \end{pmatrix}, \tag{21}$$

where the determinant of M is given by

$$\begin{aligned} \Delta = \det M &= 1 + b_S H(S,\Theta) + b_A H(A,\Theta) \\ &\quad + b_S b_A\big[H(S,\Theta)H(A,\Theta) - J(S,\Theta)J(A,\Theta)\big]. \end{aligned} \tag{22}$$

4.1 Fixed Points and Stability

We will assume that $\lambda_A, \lambda_S > 1$, so that both cell populations are viable. It is easy to see from (18) that there are three fixed points (apart from the origin). Two of these

correspond to steady states of one or other cell population. These two steady states are given by $S = S^*$, $A = 0$, and $S = 0$, $A = A^*$, where S^* and A^* are the roots of

$$h(S^*, S^*) = \frac{1}{\lambda_S}, \qquad h(A^*, A^*) = \frac{1}{\lambda_A}. \tag{23}$$

The third steady state corresponds to steady co-existing cell populations $\bar{S}$, $\bar{A}$ satisfying

$$h(\bar{S}, \bar{\Theta}) = \frac{1}{\lambda_S}, \qquad h(\bar{A}, \bar{\Theta}) = \frac{1}{\lambda_A}. \tag{24}$$

With h defined by (10), we have

$$\bar{S} = \bar{A}\left(\frac{\lambda_S - 1}{\lambda_A - 1}\right)^{1/n}, \tag{25}$$

where $\bar{A}$ is the unique positive root of

$$A^n c\left[\left\{1 + \left(\frac{\lambda_S - 1}{\lambda_A - 1}\right)^{1/n}\right\}A\right] = \lambda_A - 1. \tag{26}$$

The solution is evidently unique for any monotonically increasing function $c(\Theta)$.

To examine the stability of these fixed points, we note first that the S and A axes are invariant. The stability of the fixed points to perturbations of the non-zero population only (i.e., perturbations of S only for $(S^*, 0)$ or A^* for $(0, A^*)$) is described by the sign of $1 + g'$ (Fowler and Mackey 2002), where here $g(S) = b_S S h(S, S)$. Now, evaluation of $\Delta = \det M$ on, for example, the S axis, shows that

$$\Delta = [1 + b_A][1 + g'] \quad \text{on} \quad A = 0, \tag{27}$$

and thus $\operatorname{sgn}\Delta = \operatorname{sgn}(1 + g')$ on the S axis; a similar result holds on the A axis. Therefore, the stability of either fixed point to along axis perturbations is determined by the sign of Δ at the fixed point. Since in fact we know that the consequence of $\Delta < 0$ at either fixed point is to promote relaxation oscillations, we assume for the moment that $\Delta > 0$.

Let us consider the behaviour of trajectories near $(S^*, 0)$. We write

$$S = S^* + s, \tag{28}$$

and linearise the equations, taking $A, s \ll 1$. This yields, using the definitions of H and J in (20),

$$M^* \begin{pmatrix} s' \\ A' \end{pmatrix} \approx \begin{pmatrix} \lambda_S(Hs + JA) - s \\ d_A(\lambda_A - 1)A \end{pmatrix}, \tag{29}$$

where H and J are evaluated at $(S^*, 0)$, and we have used the facts from the assumed controller in (10) that

$$H(0, \Theta) = 1, \qquad J(0, \Theta) = 0. \tag{30}$$

The matrix $M^* = M|_{(S^*,0)}$ is given by

$$M^* = \begin{pmatrix} 1 + g'(S^*) & b_S J(S^*, 0) \\ 0 & 1 + b_A \end{pmatrix}; \tag{31}$$

from this we see that

$$(1 + b_A)\dot{A} = d_A(\lambda_A - 1)A, \tag{32}$$

and thus that, with this choice of controller, the steady state $(S^*, 0)$ is always unstable (a saddle) when $\lambda_A > 1$. Similarly, the steady state $(0, A^*)$ is an unstable saddle, and in the absence of the delay, we would surmise that the coexistence state $(\bar{S}, \bar{A})$ would be stable.

4.2 Chronic Phase Stability

We identify the steady state $(\bar{S}, \bar{A})$ with a chronic phase of the disease. Putting

$$S = \bar{S} + s, \qquad A = \bar{A} + a, \tag{33}$$

the linearisation of (18) yields, after a little algebra,

$$M \begin{pmatrix} s' \\ a' \end{pmatrix} = N \begin{pmatrix} s \\ a \end{pmatrix}, \tag{34}$$

where

$$M = \begin{pmatrix} 1 + b_S H_S & b_S J_S \\ b_A J_A & 1 + b_A H_A \end{pmatrix}, \qquad N = \begin{pmatrix} \lambda_S H_S - 1 & J_S \\ J_A & \lambda_A H_A - 1 \end{pmatrix}, \tag{35}$$

and

$$\begin{aligned} H_S &= H(\bar{S}, \bar{\Theta}), \qquad & J_S &= J(\bar{S}, \bar{\Theta}), \\ H_A &= H(\bar{A}, \bar{\Theta}), \qquad & J_A &= J(\bar{A}, \bar{\Theta}). \end{aligned} \tag{36}$$

The solutions s, a are proportional to $e^{\sigma t}$, where the two possible values of σ are the eigenvalues of $M^{-1}N$. The stability of the fixed point depends on the sign of $\operatorname{Re}\sigma$, which is determined by $\operatorname{tr} M^{-1}N$ and $\det M^{-1}N = \frac{\det N}{\Delta}$, where $\Delta = \det M$. We have, in particular,

$$\Delta = 1 + b_S H_S + b_A H_A + b_S b_A (H_S H_A - J_S J_A). \tag{37}$$

Let us suppose firstly that $\Delta > 0$. It is straightforward to show, assuming $h_\xi < 0$, $h_\Theta < 0$, that $\det N > 0$, and thus that $\det M^{-1}N > 0$. Therefore, the stability of the fixed point is determined by $\operatorname{tr} M^{-1}N$, and the point is a node or a spiral. We define

$$\operatorname{tr} M^{-1}N = \frac{T}{\Delta}, \tag{38}$$

where we have

$$\begin{aligned} T = {} & (1 + b_A H_A)(\lambda_S H_S - 1) \\ & - b_A \lambda_S J_A J_S + d_A \big\{ (1 + b_S H_S)(\lambda_A H_A - 1) - b_S \lambda_A J_A J_S \big\}. \end{aligned} \tag{39}$$

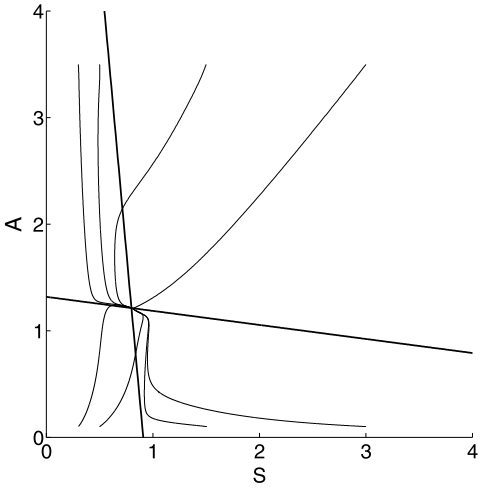

Fig. 4 Phase portrait of (15) when $b_A = b_S = 1.5$, $\lambda_A = 4.5$, $\lambda_S = 2$, $n = 3$, $\Theta_c = 10$, $d_A = 1.1$, $\varepsilon = 0.05$, $\alpha = 0.1$. The chronic steady state is stable, since both $\Delta > 0$ and $T < 0$. The two nullclines of the slowly varying system (18) are *the thick straight lines*

It is easy to see that $\lambda H - 1 < 0$ for both A and S and, therefore, $T < 0$ for sufficiently small b_A and b_S. Hence, also $\operatorname{tr} M^{-1}N < 0$, and the fixed point is a stable node or spiral.

This is illustrated in the phase portrait (of the full system) of Fig. 4. Suppose that $b_S, b_A \ll 1$, thus the stem cell recruitment rates into the renewal cycle are small; then $M \approx I$, the identity, $\Delta \approx 1$, and thus

$$\begin{aligned} S' &\approx (\lambda_S h - 1)S, \\ A' &\approx d_A(\lambda_A h - 1)A. \end{aligned} \tag{40}$$

The phase plane is easily studied in this case, and one can show that the fixed point is a stable node. Obviously, this is perturbed for larger values of b_S and b_A. However, consideration of the limit $\Theta \to 0$ shows that trajectories always move away from $(0, 0)$ as an unstable node. So long as $\lambda_S, \lambda_A > 1$, the S and A nullclines must leave the S and A axes as in Fig. 4, and as we have seen, the intersection of the nullclines at the fixed point $(\bar{S}, \bar{A})$ is unique. Therefore, the topology of the phase portrait of Fig. 4 in the trapezoidal region bounded by the axes and the nullclines is the only possible one, at least if $\Delta > 0$. The example in Fig. 4 illustrates this when b_A and b_S are not small.

5 The Onset of Oscillations

There are two ways in which the chronic phase fixed point can lose stability. If the nullclines are not both negatively sloped at the fixed point, then trajectories can cycle round, and a Hopf bifurcation can occur if $T > 0$. We have seen that this is not the case if b_A and b_S are small. Conversely, if they are both large, then

$$\Delta \approx b_A b_S (H_A H_S - J_A J_S), \tag{41}$$

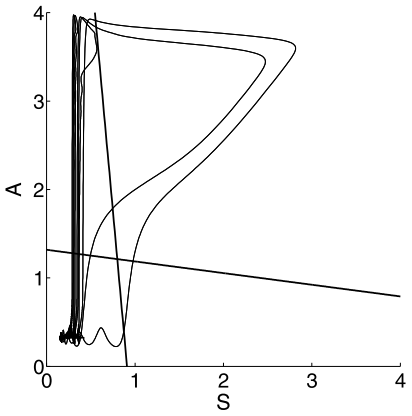

Fig. 5 Approximately periodic solutions of (15) when $b_A = 7$, $b_S = 7$; other values as in Fig. 4. For these values, we have $\Delta > 0$ and $T > 0$, and the slowly varying system has a periodic solution

while

$$T \approx b_A\{\lambda_S(H_A H_S - J_A J_S) - H_A\} + d_A b_S\{\lambda_A(H_A H_S - J_A J_S) - H_S\}. \tag{42}$$

For the particular choice (10), we have at the chronic fixed point,

$$-H = \frac{(\lambda-1)(B+n-1)-1}{\lambda^2}, \qquad -J = \frac{(\lambda-1)B}{\lambda^2}, \tag{43}$$

where

$$B = \frac{\xi c'}{c} > 0, \tag{44}$$

and thus $\Delta > 0$ providing $\lambda_A, \lambda_S \gtrsim 1 + \frac{1}{n-1}$; and if the inequalities are only just satisfied, we will also have $T > 0$. In this case, (slow) oscillations will occur, and an example is shown in Fig. 5.

The preceding discussion is appropriate as long as $\Delta > 0$. Indeed, it still applies if $\Delta < 0$, except that the trajectory directions are reversed. However, we know from the one-dimensional system (3) that regions where $\Delta < 0$ are 'no-go' areas, and when these are encountered, the variables undergo a rapid transition to another 'safe' area: this is described further below. Therefore, we need to know where $\Delta < 0$.

For small b_A and b_S, $\Delta \approx 1$ and is positive. As $\Theta \to \Theta_c$, we find

$$\Delta \approx 1 - \frac{b_S}{\Theta_c S^{n-1}} - \frac{b_A}{\Theta_c A^{n-1}}, \tag{45}$$

so that in fact there are small regions near $S = 0$, $A = \Theta_c$ and $A = 0$, $S = \Theta_c$ where $\Delta < 0$. That near $S = 0$, for example, is the approximate wedge

$$0 < \Theta_c - \Theta < \left\{\frac{b_S \Theta_c}{(1+b_S)\left(1 - \frac{b_A}{\Theta_c^n}\right)}\right\}^{1/2} S^{(n+1)/2}, \tag{46}$$

assuming $b_A < \Theta_c^n$. Furthermore, (27) (or its equivalent for $S = 0$) shows that $\Delta < 0$ on $A = 0$ if $1 + g'_S < 0$ there, where

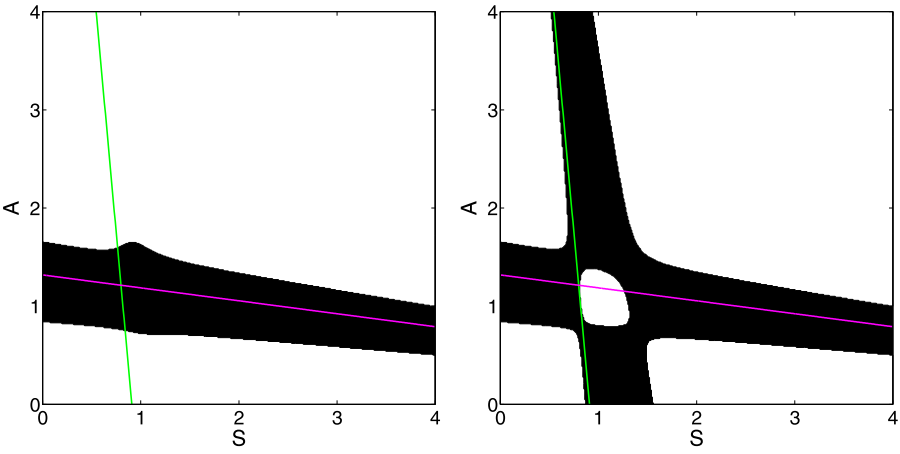

Fig. 6 *Left*: region (*shaded*) where $\det M < 0$, when $(S^*, 0)$ is stable but $(0, A^*)$ is unstable; parameter values $b_A = 4$, $b_S = 2$; other values as in Fig. 4. *Right*: region (*shaded*) where $\det M < 0$, when $(S^*, 0)$ and $(0, A^*)$ are both unstable; parameter values $b_A = 4$, $b_S = 3.5$; other values as in Fig. 4

$$g_S = b_S S h(S, S), \tag{47}$$

and on $S = 0$ if $1 + g'_A < 0$ (with g_A defined analogously), and intervals on the S and A axes where this is the case exist if $b_S > b_c$ and $b_A > b_c$, respectively (cf. the discussion after (5)). (The precise definition of b_c will be slightly different if $c(S) \neq 1$, but not very much if, as we suppose, Θ_c is reasonably large.)

What this suggests is that closed regions where $\Delta < 0$ will grow as b_S and b_A are increased from their initial locations near $(0, \Theta_c)$ and $(\Theta_c, 0)$, and from $(0, A^*)$ when $b_A > b_c$ (and from $(S^*, 0)$ when $b_S > b_c$). We recall from (12) that the parameters b_S and b_A are dimensionless measures of the proliferative recruitment rate of normal and abnormal cells, respectively; b_c is the critical value such that oscillations in a normal S population occur when $b_S > b_c$. We might suppose in a healthy individual that $b_S < b_c$, and additionally that as the chronic phase progresses, increasing mutation causes the slow evolution of abnormal parameters such as b_A. As b_A increases, the negative Δ regions may join up, and if the chronic phase stationary state (or its associated periodic orbit) migrates into this region, there is a prospect of rapid migration through this forbidden zone toward $\Theta = \Theta_c$. This latter evolution would correspond to the onset of blast crisis and the acute phase. This model therefore contains the potential for a dynamic understanding of the progression to chronic and then acute phase; it remains to be seen whether such a sequence of events does actually occur in this model. Figure 6 shows two examples where $\Delta < 0$; in both there is an A-interval where $\Delta < 0$; in the left figure there is no such corresponding S-interval, whereas in the right figure there is.

5.1 Oscillations

Oscillations will occur when the slow trajectories encounter a region where $\Delta < 0$. These regions are determined by b_S and b_A, whereas the shape of the slow trajectories

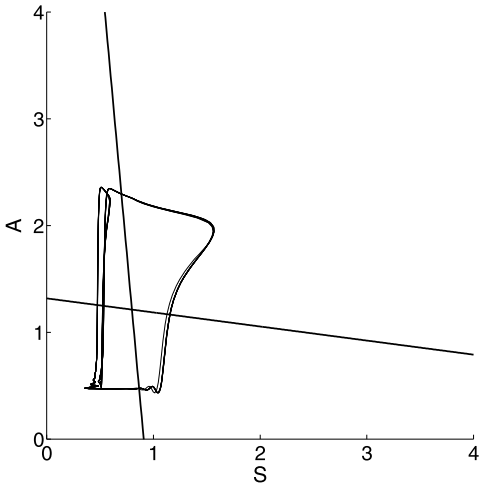

Fig. 7 Slow approach to an oscillatory chronic phase; parameter values as in Fig. 6 (*left*)

is largely controlled by λ_A and λ_S. Various possibilities can occur depending on the values of these parameters, and we focus on two of particular interest.

The first is shown in Fig. 7. There is a negative Δ field which separates the (slowly) unstable S steady state from the stable A state. The connecting trajectory in the slow phase space reaches the curve $\Delta = 0$, and there is a fast transition, in which

$$\begin{aligned} \frac{\mathrm{d}S}{\mathrm{d}t} &\approx b_S\big[S_1 h(S_1, \Theta_1) - Sh(S, \Theta)\big], \\ \alpha\frac{\mathrm{d}A}{\mathrm{d}t} &\approx b_A\big[A_\alpha h(A_\alpha \Theta_\alpha) - Ah(A, \Theta)\big]. \end{aligned} \tag{48}$$

It is easy to show that the values of S and A across such a transition are related by

$$\begin{aligned} \big[S + b_S Sh(\Theta)\big]_-^+ &= 0, \\ \big[A + b_A Ah(\Theta)\big]_-^+ &= 0, \end{aligned} \tag{49}$$

and this provides a map to take points (S_-, A_-) to (S_+, A_+) outside the negative Δ field, where they resume their slow evolution toward the steady A state. Thus, in this case, there is an initial slow evolution of the abnormal cell population, followed by a sharp rise. This is somewhat reminiscent of the transition from chronic to acute phase. Figure 8 shows the corresponding time series for A.

We can get some geometric understanding of the fast transition by defining the new variables

$$\begin{aligned} \tilde{S} &= S + b_S Sh(S, \Theta), \\ \tilde{A} &= A + b_A Ah(A, \Theta); \end{aligned} \tag{50}$$

note that M is the Jacobian of the transformation, i.e.,

$$M = \frac{\partial(\tilde{S}, \tilde{A})}{\partial(S, A)}. \tag{51}$$

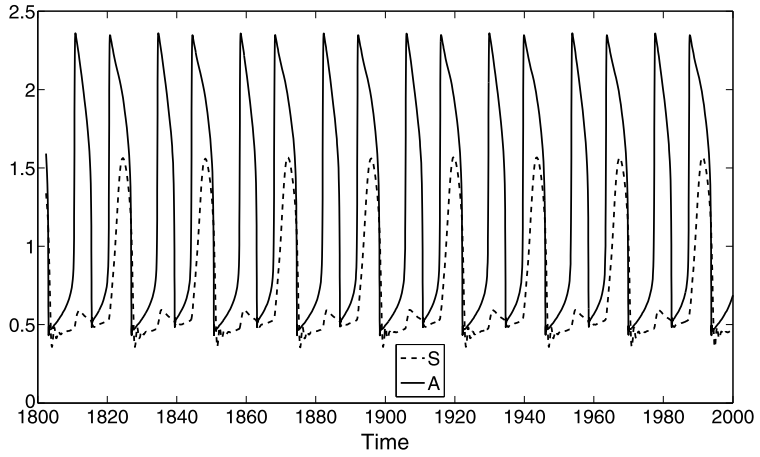

Fig. 8 Time series of A (*full line*) and S (*dashed*) corresponding to the phase diagram of Fig. 7. The parameters are also those used in Fig. 7, i.e., the default parameters of Fig. 4, except that $b_A = 4$, $b_S = 3.5$. The units of S and A are dimensionless, as are those of time t. The segment shown starts at $t = 1\,800$ so as to eliminate transient effects

Inverting (50), we have

$$\Theta = \frac{\tilde{S}}{1 + b_S h(\Theta)} + \frac{\tilde{A}}{1 + b_A h(\Theta)}. \tag{52}$$

The right-hand side of (52) is sigmoidal, and there are generally either three values of Θ or one value for each $\tilde{S}$ and $\tilde{A}$, and exceptionally two. The curve in $(\tilde{S}, \tilde{A})$ space on which there are two is the image of the $\Delta = 0$ curve in (S, A) space, because of (51). Thus, $\Theta(\tilde{S}, \tilde{A})$ is represented as a folded sheet in $(\tilde{S}, \tilde{A})$ space, with the folded portion inside the image of $\Delta = 0$, where Θ has three possible values. During a rapid transition, Θ jumps rapidly in $(\tilde{S}, \tilde{A})$ space while $\tilde{S}$ and $\tilde{A}$ remain the same. In (S, A) space, this is manifested as a jump across the negative Δ field to the other side, as shown in Fig. 7.

The jumps which occur when $\Delta < 0$ are described by (49). For increasing values of b_A, the large A values begin to approach the overcrowding limit, which we identify with the onset of blast crisis. We associate this with increasing values of b_A due to further cell abnormalities. Figure 9 shows a case where the parameter b_A is quite large. The initial slow evolution of A is accompanied by oscillations in S as the trajectory repeatedly encounters $\Delta = 0$, but as A increases, the oscillations reach a threshold where A increases rapidly, and Θ reaches Θ_c: blast crisis is initiated. Figure 10 shows the corresponding time series.

6 Discussion

There are two principal dynamic features of CML which one would hope that a model could explain. These are the long period oscillations which are sometimes found, and

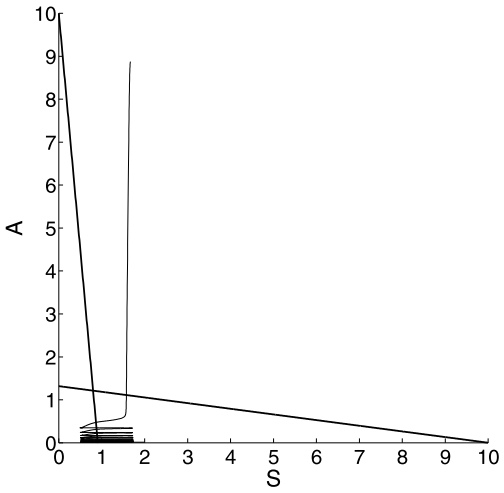

Fig. 9 Slow oscillatory 'chronic' phase followed by an acute phase; parameter values are $b_A = 20$, $b_S = 3.5$; other values as in Fig. 4

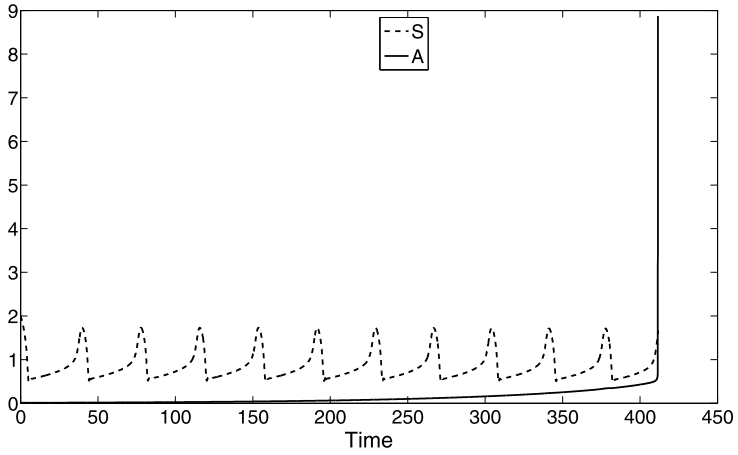

Fig. 10 Time series of A and S corresponding to the parameters of Fig. 9

the long duration of the chronic phase before the onset of the acute phase. It is not clear whether these two features are related, but we would hope that a model might shed light on both features, and in that way suggest possible therapeutic strategies for the management of the disease. In this paper, we have formulated what is perhaps the simplest model of the diseased state, in which normal and abnormal stem cells compete with each other. Nevertheless, the model is complicated by the fact that it consists of two coupled delay differential equations, and this fact makes useful analysis hazardous.

We have adapted a novel technique developed by Fowler and Mackey (2002) to analyse systems of this type, and in this way we are able to describe the evolution of the system, and the onset of oscillations, in a fairly thorough analytical way, despite the functional character of the equations. In this particular model, we find that normal

and abnormal states can exist together in a steady state, but that this state can be oscillatorily unstable, and long period oscillations can occur. Although there are a number of parameters in the model, we find that the principal controlling parameter is the abnormal stem cell recruitment rate, as represented in the coefficient b_A. Essentially, as this is increased, we find a transition to periodic solutions, but for sufficiently large b_A, the trajectories lead to unlimited growth of the abnormal cell population, which we identify with the onset of blast crisis. This sequence of events is very similar to that which is observed clinically.

It should be emphasised that our study is essentially a proof of concept. There are so many parameters, both dimensional and dimensionless, in the model, that our strategy of illustrating the transition from stable coexistence to oscillations to an acute phase has focussed on altering one particular parameter, b_A, while keeping the others fixed within reasonable limits. That said, our parameter choice is motivated by realistic values, and we find, for example, in Fig. 10 that if we take the stem cycle delay $\tau_S \approx 2.2$ days (Fowler and Mackey 2002), then the oscillations have periods of about 80 days, while the chronic phase shown lasts some 2.5 years. These values are not unreasonable.

The change in b_A from its 'normal' value $b_A \approx 4$ to the value $b_A = 20$ used in the simulation in Fig. 10 represents a fivefold increase. Since we took $\alpha = 0.1$, this corresponds to a fiftyfold increase in recruitment rate. Such a low value of α is probably extreme, and a tenfold increase of abnormal recruitment is in line with our model results. Increased abnormal proliferation rate is line with our expectation (see (13)), but it is a hazard of our procedure that changing a single dimensional parameter (β_A) has the effect of altering both b_A and λ_A (see (12)). It is a matter for future work to explore parameter space more fully with a view to mapping out where oscillatory and acute behaviour can be found.

Figure 10 shows oscillations only in the normal stem cells. Data such as that shown in Fig. 2 does not typically distinguish between normal and abnormal cells, but we might expect oscillations in the abnormal population as well or instead. The simulation in Fig. 8 shows that the model is well able to produce such oscillations, but we have not extended our parameter search to combine abnormal oscillations with blast crisis.

Although it is reasonable that any model of CML will involve competing normal and abnormal stem cell populations, the particular model we have studied falls short of reality in several respects. In particular, we have omitted discussion of the developing maturation of the nucleated cells (Mackey and Rudnicki 1994), nor have we included the effects of the immune system reaction (Neiman 2000; Moore and Li 2004); other shortcomings could be mentioned. However, it is in the nature of disease modelling of this type that it is as yet unclear what the important constituents are, and in fact the purpose of exploring models such as that presented here is to attempt to elucidate what such constituents might be.

One particular and potentially predictive feature of the model is the identification of a critical parametric inequality $b_A > b_S$ in order that the disease progress. In turn, this may suggest possible clinical strategies. It remains to be seen what the effect of treatment in the model is on the oscillations and rapid transitions we have identified here. Clearly, the aim of a clinical strategy would be to keep Δ positive.

Within the confines of the present competitive model, we have delineated one mechanism which may be important in determining the onset of oscillatory cell counts during the chronic phase, and that is the effect of the delay in the proliferative cycle. Although this is short (two days) compared with the much longer time scales of oscillations or of secular increase of abnormal cell populations, it can have a dramatic effect on the course of the disease, as shown in Figs. 8 and 10. In particular, we have found that the onset of oscillatory behaviour as a consequence of the transition to the chronic diseased state can be explained relatively simply, as can an abrupt transition to the acute phase, in terms of the slow dynamics of the variables, which are largely determined by the regions of phase space where the determinant Δ is negative. So far as we are aware, no other model has been able to provide a rational basis for the transition from chronic to acute phase.

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Appendix A

The use of Hill functions in cell control models, or similar Monod growth functions in cell population models, can be motivated by reaction schemes similar to those describing cooperative enzyme kinetics. To this end, we consider a cartoon description of the resting stem cell population, in which the stem cell population S releases signalling molecules M which bind to the stem cells. In this description, we then suppose that a series of complexes C_i, $i = 1, \ldots, n$ are formed, in which C_i has i molecules of M bound to the cell. Thus, we suppose multiple binding sites for M, and we suppose that only C_0 cells can be recruited to the proliferative phase, at a constant specific rate r. The signalling molecules are released by the whole stem cell population, but the restriction of recruitment to C_0 cells provides for an inhibitory effect.

A reaction scheme to describe this cartoon, if there are n binding sites per cell, is

$$\begin{aligned} C_0 &\overset{r}{\rightarrow} R, \\ C_0 + M &\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C_1, \\ C_1 + M &\underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} C_2, \\ &\cdots \\ C_{i-1} + M &\underset{k_{-i}}{\overset{k_i}{\rightleftharpoons}} C_i, \\ &\cdots \\ C_{n-1} + M &\underset{k_{-n}}{\overset{k_n}{\rightleftharpoons}} C_n. \end{aligned} \tag{53}$$

The recruited cell population is R, and we define the total stem cell population

$$S = \sum_0^n C_i. \tag{54}$$

Then the law of mass action applied to (53) gives the system of equations

$$\begin{aligned}
\dot{R} &= rC_0,\\
\dot{C}_0 &= -(k_1 MC_0 - k_{-1}C_1) - rC_0,\\
\dot{C}_i &= k_i MC_{i-1} - k_{-i}C_i - (k_{i+1}MC_i - k_{-(i+1)}C_{i+1}), \quad 1 \le i \le n-1,\\
\dot{C}_n &= k_n MC_{n-1} - k_{-n}C_n,\\
\dot{M} &= \sum_1^n (k_{-j}C_j - k_j MC_{j-1}) + k_+ S - k_- M,
\end{aligned} \tag{55}$$

where we have added to the kinetics of (53) a source of M proportional to total stem cell density S, and a degradation rate proportional to M.

These equations can be written more simply by defining

$$R_i = k_i MC_{i-1} - k_{-i}C_i, \quad 1 \le i \le n, \tag{56}$$

whence

$$\begin{aligned}
\dot{C}_0 &= -R_1 - rC_0,\\
\dot{C}_i &= R_i - R_{i+1}, \quad 1 \le i \le n-1,\\
\dot{C}_n &= R_n,\\
\dot{M} &= -\sum_1^n R_j + k_+ S - k_- M,
\end{aligned} \tag{57}$$

and $\beta = rC_0/S$ is the specific recruitment rate, which is to be found in terms of S. Note that using (56) and (57), $\dot{S} = -\beta S$.

It is conventional in considering such systems of equations to suppose that the binding reactions are fast, so that all the bound complex equations for C_i, $i \ge 1$ are in equilibrium. With this assumption, we put $R_i = 0$, and if in addition M rapidly equilibrates, then

$$M = LS, \tag{58}$$

where

$$L = \frac{k_+}{k_-}. \tag{59}$$

The equations $R_i = 0$ define a sequence of difference equations for C_i, and we solve these to find

$$C_m = \left(\prod_1^m K_j\right) M^m C_0, \tag{60}$$

whence we determine C_0 in terms of S as

$$C_0 = \frac{S}{\sum_{k=0}^{n}\{\prod_{j=1}^{k} K_j\}M^k}, \tag{61}$$

and the consequent definition of the specific recruitment rate is

$$\beta = \frac{r}{\sum_{k=0}^{n}\{\prod_{j=1}^{k} K_j\}L^k S^k}. \tag{62}$$

The product within the summation takes the value 1 when $k = 0$.

We can write this in the form

$$\beta = \frac{r}{\sum_{k=0}^{n}\left(\frac{S}{\theta_k}\right)^k}, \tag{63}$$

where we define

$$\theta_k = \frac{1}{L\{\prod_{j=1}^{k} K_j\}^{1/k}} \tag{64}$$

(and $\theta_0 = 1$). Evidently, the Hill function as in (4) follows from the identification of $\beta_S = r$ and the assumption that $\theta_k \gg \theta_n \ \forall\ 1 < k < n$; this follows if K_n is large enough.

A.1 Overcrowding

The question now arises, how should we specify control when there are two stem cell populations S and A, and in particular, how should we generalise (63) to allow for the effects of spatial crowding. The 'derivation' of (63) using the law of mass action implicitly involves the idea that cells and molecules have access to the entire volume, i.e., the populations are 'dilute'. When using the law of mass action, we need to distinguish between the effects of signalling molecule density and that of cell density. For the population S, we still suppose that $M \propto S$ (i.e., only normal cells produce a signalling control for S). We also suppose that the dissociation rate factors k_{-i} remain constant and independent of crowding, since they should depend only on cell density. However, the rate of binding depends on total cell density, for the following reason. The law of mass action assumes dilute 'solutions', so that the rate of meeting of cell and molecule is proportional to the density of each per unit volume of the medium. However, when the cells occupy significant volume, then the rate of binding of M will be proportional to its actual density in the intercellular space, which is inversely proportional to $\left(1 - \frac{\Theta}{\Theta_c}\right)$, where

$$\Theta = S + A \tag{65}$$

is the total cell density, and Θ_c is the total cell capacity in the bone marrow. Consequently, this suggests that we put k_j, and thus K_j, dependent on Θ as

$$K_j = \frac{K_j^0 \Theta_c}{\Theta_c - \Theta}. \tag{66}$$

In this case, we could modify (63) to be

$$\beta = \frac{r}{\sum_{k=0}^{n} \left\{ \frac{\kappa(\Theta) S}{\theta_k} \right\}^k}, \tag{67}$$

where

$$\kappa(\Theta) = \frac{\Theta_c}{\Theta_c - \Theta} \tag{68}$$

is an increasing function of Θ. A similar expression for the A controller then follows, with the same crowding factor. This then motivates our choice of controller in (10).

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