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SPECIAL ISSUE ARTICLE

Understanding, Treating and Avoiding Hematological Disease: Better Medicine Through Mathematics?

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Abstract This paper traces the experimental, clinical and mathematical modeling efforts to understand a periodic hematological disease—cyclical neutropenia. It is primarily a highly personal account by two scientists from quite different backgrounds of their interactions over almost 40 years and their attempts to understand this intriguing disease. It's also a story of their efforts to offer effective treatments for the patients who suffer from cyclic neutropenia and other conditions causing neutropenia and infections.

Keywords Cyclical neutropenia · Granulocyte colony-stimulating factor · G-CSF · Chemotherapy

1 Introduction

Human hematopoiesis produces the equivalent of our body weight of red blood cells (RBCs), white blood cells and platelets every 10 years of life (Mackey 2000). Throughout life, hematopoiesis usually proceeds flawlessly probably implying the existence of finely tuned control mechanisms. Thus this cellular renewal system is ideal for a study of the normal regulation of tissue proliferation and maturation from the single cell to whole organ level, as well as the study of derangements of these processes.

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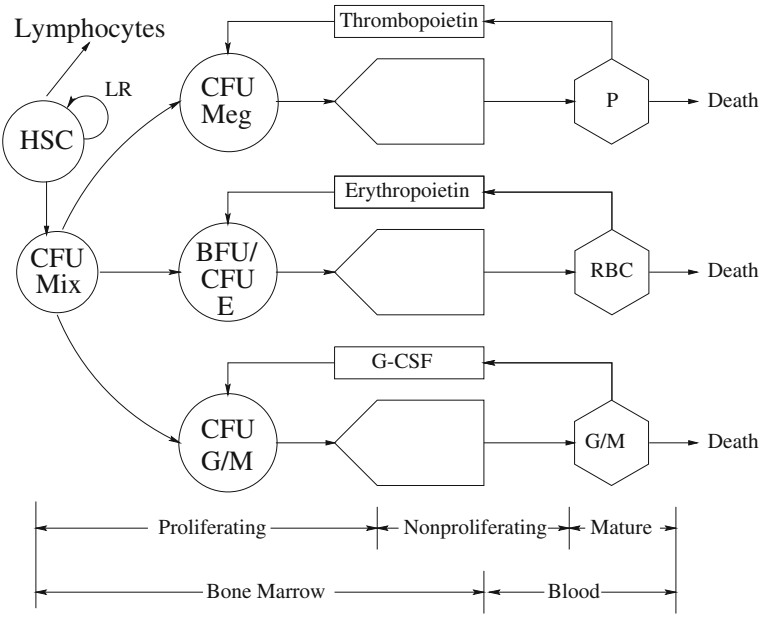

Fig. 1 A schematic representation of the architecture and control of mammalian hematopoiesis. All blood cells are formed from a hematopoietic stem cell (*HSC*), and this figure summarizes mammalian platelet (*P*), red blood cell (*RBC*), monocyte (*M*) and granulocyte (*G*, including neutrophil, basophil, and eosinophil) production. Control over these processes is mediated by a variety of cytokines [e.g., thrombopoietin (*TPO*), erythropoietin (*EPO*), and granulocyte colony-stimulating factor (*G-CSF*) are the main ones] and there are also local regulatory (*LR*) effects within the HSC population. *CFU/BFU* refers to the various in vitro analogs of the in vivo committed stem cells. This figure is taken from Haurie et al. (1998) with permission

Blood cells are formed from a hematopoietic stem cell (HSC). Though this process, known as hematopoiesis, is incredibly complicated the broad outlines can be summarized as in Fig. 1 which schematically shows the major aspects of mammalian platelet (P), red blood cell (RBC), monocyte (M), and granulocyte (G, including neutrophil, basophil, and eosinophil) production. Control over this process is mediated by a family of hematopoietic growth factors and regulatory cytokines. Three of the major players are thrombopoietin (TPO), erythropoietin (EPO), and granulocyte colony-stimulating factor (G-CSF) which also have local regulatory (LR) effects within the HSC population. CFU/BFU refers to the various in vitro analogs of the in vivo committed stem cells.

The study of dynamic behavior of cellular replication systems in the whole animal is hampered by the lack of good quality temporal data on cell numbers and cytokine levels in response to perturbation. Ironically, the best source of data currently available comes from clinical studies of patients with hematological disease. Of the vast array of documented hematological disease, the periodic hematological diseases (periods from weeks to months) have been some of the most instructive in terms of elucidating the control mechanisms regulating hematopoiesis (Foley and Mackey 2009a).

These periodic hematological diseases fall into two types. The first, with oscillations in numbers of a single circulating cell type, is probably due to a destabilization of a

peripheral control mechanism, e.g., cyclical thrombocytopenia with periods of 13–65 days (Swinburne and Mackey 2000; Apostu and Mackey 2008). The second type has oscillations in several circulating cell types and may be due to a destabilization at the stem cell level. An example is cyclical neutropenia with periods of 14–45 days (Haurie et al. 1999a). Both offer exciting tests for the range of dynamics that cellular control systems can exhibit, and illuminate aspects of the regulation of proliferation and maturation that probably would never have been discovered in a laboratory setting because of the time scales involved.

In this expository paper, we use the example of cyclical neutropenia to illustrate how the interplay between clinical and laboratory studies and mathematical modeling has increased our understanding of the disease and contributed to its treatment.

2 Cyclical Neutropenia

Cyclic neutropenia (CN) is a rare hematological disease characterized by regular oscillations in blood neutrophil counts (the absolute blood neutrophil count or ANC) (Dale and Hammond 1988a). Severe neutropenia (ANC less that 0.5×10^9/L) fever and mouth ulcers recur usually at 21 day intervals (Wright et al. 1981). The severity of symptoms and clinical manifestation can usually be related to the depth and duration of neutropenia. The ANC may go to zero and extremely low counts can last for 3–5 days. Although CN is usually discovered in very young children, the disease lasts for the lifetime of the individual. In 1949 Reiman reported that CN is an inherited disorder in his classic description of periodic diseases (Reimann and DeBerardinis 1949). Several years later, Morley showed that CN is dominantly inherited, through careful studies of five Australian families (Morley et al. 1967). Important physiological studies in 1950s by Page and Good (1957) pointed to interrupted cell production by the bone marrow as a physiological cause of CN. Their clinical observations showed that the cycling of blood cells is independent of the 28 day menstrual cycle and thus the pituitary-hypothalamic axis does not control the oscillations. These and subsequent studies showed that there are cyclic variations in all blood cells, suggesting that the intrinsic defect is in the HSCs (Guerry et al. 1973).

In the 1960s, veterinary researchers discovered that a championship line of collie dogs with a distinctive gray fur had CN and severe bacterial infections (Lund et al. 1967). They named the condition the "lethal gray collie syndrome." Blood and bone marrow studies showed that all blood cell counts in these dogs oscillated with a periodicity of about 13 days attributable to cyclic production of these cells by the bone marrow (Dale et al. 1972). The urine of the dogs had high levels of a colony-stimulating activity, substances which stimulate in vitro growth of colonies of myeloid cells, when the dogs were severely neutropenic (Dale et al. 1971). Bone marrow transplantation studies showed that the disease could be transferred or corrected with infusions of bone marrow cells (Dale and Graw 1974). Because it was feasible to collect blood samples from these dogs on a daily basis, they provided very valuable data for modeling hematopoiesis, of particular value because canine and human hematopoiesis are so similar.

A "typical" patient with cyclical neutropenia has a circulating neutrophil level that oscillates from normal to virtually zero with a period of 19–21 days (Guerry et al. 1973;

Dale and Hammond 1988b). However, patients with periods of up to 40 days have also been reported (Haurie et al. 1999a). The curious and suggestive aspect of this dynamic disease is that usually there are concomitant oscillations in the reticulocytes (red cell precursors), lymphocytes, and platelets with the same period as the neutrophils but out of phase with them.

The classic description of CN, however, does not fit perfectly to all the biological data. For example, there are reports of acquired CN and patients in whom the disease onset was not in early childhood (Wright et al. 1981). There are patients that have been described with cycle lengths longer and shorter than 19–21 days (Haurie et al. 1999a). Although there are readily observed cycles of all blood cells in some patients, other patients do not have distinct cycles of reticulocytes, erythrocytes, platelets or lymphocytes. However, monocyte oscillation, in opposite phase to the neutrophil oscillation, is an almost uniform finding. Finally there are patients who appear to have CN at times, and at other times the counts are low without apparent oscillations (Dale et al. (2000)). There are also a variety of other human diseases in which oscillations of neutrophils have been reported, e.g., auto-immune neutropenia (Barrachina Barbera et al. 2005), mutations genes for myeloperoxidase (Erbagci 2003), glucose 6 phosphatase component 3 [G6PC3 mutations, Alangari et al. (2013)] and Barth syndrome (TAZ mutations, Marziliano et al. (2007) and others), raising the question as to whether CN is a single disease or reflects a perturbation of the dynamics of blood cell production which may have a variety of causes. Understanding these reports and observations requires both examination of the number and consistency of the blood count observations and a systematic method for determining if there is good evidence of regular oscillations or variations without enough measurement to determine the presence of regular oscillations. Fortunately, techniques developed for the analysis of astronomical data (Lomb 1976; Scargle 1982) have proved to be of immense help in the characterization of the periodic hematological diseases (Haurie et al. 1999a; Fortin and Mackey 1999; Swinburne and Mackey 2000).

2.1 Insight into CN from Molecular Biology

Advances in molecular biology and our understanding the genetic, molecular, and cellular mechanisms governing hematopoiesis have greatly increased our understanding of diseases of the hematopoietic system. We know now that specific regulators, e.g., EPO and TPO, govern the production of red cells and platelets, respectively. In general, in a steady state the level of the regulator is inversely proportional to the level of the target cell type. Thus with severe anemia the level of EPO is high, unless there is a serious abnormality of the producing organ, the kidneys (Bunn 2013). Similarly, TPO levels are high with thrombocytopenia, unless there is severe disease of the producing organ, the liver (Hitchcock and Kaushansky 2014). Severely anemic patients also provided the biological materials for purification of EPO and determining its amino acid and DNA sequence, leading to the availability of recombinant EPO to treat anemia (Erslev 1991).

The perturbations of neutrophil production in canine and human CN were important for the discovery and understanding of the substances regulating neutrophil and

monocytes production, substances called colony-stimulating factors. As noted above, it was observed that in dogs and in humans with cyclic neutropenia the neutrophil colony-stimulating factor now called "granulocyte colony-stimulating factor", or "G-CSF", increases in the urine at the nadir in blood neutrophil counts and the peak level antecedes the recovery of blood neutrophils (Dale et al. 1971). Subsequent studies showed that G-CSF is the principal regulator for the production and deployment of blood neutrophils (Hammond et al. 1991; Christopher and Link 2007). Tumor tissue producing large amounts of G-CSF were used to purify this glycoprotein, determine its amino acid and DNA sequence and ultimately produce recombinant G-CSF (rG-CSF) for treatment of patients with neutropenia (Welte et al. 1985).

Recent developments in molecular biology have permitted discovery of the genetic basis for many inherited diseases. Beginning in the late 1980s investigators began collecting blood samples from families with CN for DNA sequencing. As methods improved, particularly the development of automated sequencing of DNA, it was possible to exclude portions of the DNA and focus investigations on areas of higher and higher probability of harboring the mutant gene (Palmer et al. 1994). Subsequently, a mutation at locus 19q13, the location for the gene for three proteases found in the granules of neutrophils, was identified (Horwitz et al. 1999). Sanger sequencing showed mutations in the gene for neutrophil elastase, now called ELANE, were consistently found in all affected individuals but not in the unaffected family members.

Because of similarities in the diseases CN and severe chronic neutropenia (SCN) and because some SCN patients showed cycling of their blood neutrophils on rG-CSF, researchers sequenced ELANE in SCN patients (Dale et al. 2000). They found that most of the patients with a diagnosis of SCN had ELANE mutations, indicating at the genetic level there was an overlap in these diseases. Some authorities now refer to this condition as *ELANE* associated neutropenia. Subsequent studies have shown that the pattern of mutations differs between the two clinical entities with mutations causing CN clustering in exon 4 and 5 and intron 4 (mutations affecting the reading of DNA in exon 4 or 5) whereas mutations causing SCN are more widely distributed across the gene. Genotype-phenotype correlations show that some specific mutations cause SCN with more severe disease, less likely to respond to rG-CSF and more likely to evolve to acute leukemia, whereas other mutations are associated with a more benign clinical outcome. There are also a set of overlapping mutations for CN and SCN; that is, mutations seen in both clinical groups. These overlapping mutations are associated with a benign clinical outcome (Makaryan et al. 2012).

Understanding cyclic and congenital neutropenia at the cellular and molecular level was aided greatly by the mathematical modeling studies performed earlier by Mackey (1978a). This work had suggested that CN might be due to a defect within the HSC compartment that caused abnormal apoptotic loss of cells from the proliferative phase of the cell cycle. Mackeys work also suggested that there might be transitions between very severely impaired production with very low counts at all times and the cyclic production state if the severity of apoptosis or apoptotic rate for the early progenitor cells could be modified. These concepts were important for linking congenital and CN as potentially very closely related diseases, and were the fundamental reason patients with severe congenital neutropenia were hypothesized to also have *ELANE* mutations. Based on these concepts it was also hypothesized that decreasing the severity of

apoptosis might shift a patient from the congenital category to the cyclic category. It was then observed that patients with congenital neutropenia treated with G-CSF, which decreases apoptosis, caused the patient's blood neutrophils to begin to oscillate. On G-CSF many of the congenital patients showed cyclical oscillations (Haurie et al. 1999a).

Laboratory investigations using HL 60 cells transfected with mutant or normal *ELANE* subsequently provided more support for these concepts. These studies showed that HL 60 cells expressing mutant *ELANE* undergo accelerated apoptotic death (Aprikyan et al. 2001, 2003). Current understanding at the cellular level indicates that production of the mutant protein in the endoplasm reticulum is followed by failure to transient normally to the neutrophils primary granules for storage. The presence of the mutant protein in the cytoplasm then triggers the death of the developing neutrophil by apoptosis, i.e., programmed cell death (Kollner et al. 2006; Xia and Link 2008; Nanua et al. 2011). The mutant protein initiates cell death through the unfolded protein response, a cellular process for handling abnormal proteins in cells. It is thought, but not yet proven, that the magnitude of this reaction depends upon the specific properties of the mutant protein. Under this hypothesis, the mutations which caused the most severe disease would cause an accentuated unfolded protein response, whereas if the mutant protein has minor abnormalities the disease is less severe, and if the mutant protein is removed the protein response is normal. Additionally, it is hypothesized that the disease CN is associated with more moderate abnormalities of the protein than is severe congenital neutropenia.

2.2 Early Mathematical Modeling Efforts

The interesting thing about the cytokine control pathways diagrammed in Fig. 1 is that they all are examples of what mathematicians would call negative feedback regulation. This is because, for example with the G/M control mediated by G-CSF, a fall in peripheral cell numbers leads to an elevation in G-CSF and this is, in turn, followed by an increase in the number of immature precursor cells entering into the maturation process. The other aspect of this that turns out to be of crucial importance in understanding the possible dynamics of these control loops is that there is a significant time delay τ between when the control signal is delivered to its target (the immature cell population) and when the ultimate effect (an increase in the number of mature circulating cells) is actually experienced. (The dynamics in the stem cell compartment are slightly different and will be discussed later.)

To aid in an understanding of the explanations in this and subsequent sections of mathematical modeling efforts directed at an understanding of cyclical neutropenia, refer to Fig. 2 taken from Bernard et al. (2003). In that figure, the rectangle at the top of the figure represents the proliferative stem cells while the circle represents the resting phase cells in the G_0 phase of the cell cycle. Both are components of what is known as the G_0 phase cell cycle model of Burns and Tannock (1970). The resting phase stem cells may differentiate into the erythroid, platelet or neutrophil lines and only the latter is depicted there.

Bernard et al. (2003) presented a two variable delay differential equation model that couples the stem cell population model with the neutrophil compartment:

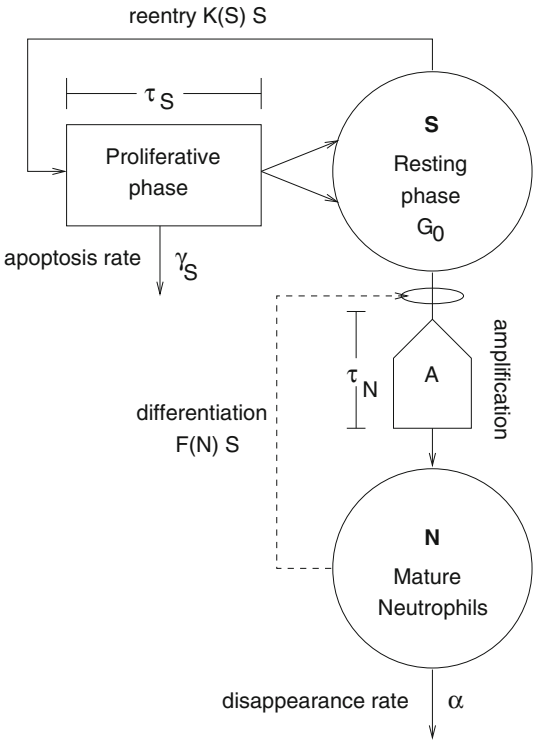

Fig. 2 A diagrammatic representation of the relationships between stem cells and neutrophils. Resting G_0 phase stem cells (S) may either stay in that phase indefinitely, re-enter the proliferative phase at a rate $K(S)$ dependent on S or differentiate into the neutrophil line at a rate $F(N)$ where N is the circulating number of mature neutrophils (differentiation into the erythrocyte and platelet lines is neglected in this treatment, but it is unimportant for the considerations here). Following differentiation into the neutrophil line, a stem cell undergoes an amplification (box with a peak) A during a maturation period lasting τ_N days before being released into the circulation (*bottom circle*) as a mature neutrophil and dying at a random rate α. Taken from Bernard et al. (2003) with permission

$$\frac{\mathrm{d}S}{\mathrm{d}t} = -(K(S) + F(N))S + 2\mathrm{e}^{-\tau_S \gamma_S} K(S_{\tau_S}) S_{\tau_S}, \tag{1}$$

and

$$\frac{\mathrm{d}N}{\mathrm{d}t} = -\alpha N + AF(N_{\tau_N}) S_{\tau_N}, \tag{2}$$

where $x_\tau \equiv x(t-\tau)$,

$$F(N) = f_0 \frac{\theta_1^{s_1}}{\theta_1^{s_1} + N^{s_1}}, \quad K)(S) = k_0 \frac{\theta_2^{s_2}}{\theta_2^{s_2} + S^{s_2}}.$$

This model neglects the compartments erythrocyte and platelet lineages but this fact is unimportant for the discussion here (Colijn and Mackey 2005a, b).

From a modeling perspective, the time delayed cytokine negative feedback described above is exactly captured in Eq. 2. If the stem cells were in a steady state S_* such that $\dot{S} = 0$ then the equation governing N would reduce to

$$\frac{\mathrm{d}N}{\mathrm{d}t} = -\alpha N + \tilde{A} F(N_{\tau_N}) \quad \text{where} \quad \tilde{A} = AS_*. \tag{3}$$

Cyclical neutropenia has attracted the attention of mathematical modelers for some time. The earliest work that we are aware of is to be found in a number of papers

by Morley (Morley et al. 1969; Morley and Stohlman 1970; Morley 1970; King-Smith and Morley 1970) which were followed by (Morley and Stohlman 1970; Morley 1970; King-Smith and Morley 1970). Later work by Reeve (1973), MacDonald (1978), Kazarinoff and van den Driessche (1979), von Schulthess and Mazer (1982), G.K. von Schulthess and Dahinde (1983), Shvitra et al. (1983), Wichmann et al. (1988),Schmitz et al. (1990), and Schmitz et al. (1995) followed in much the same vein as the earliest treatments of Morley and collaborators.

In all of these papers, the explicit assumption was that cyclical neutropenia is due to a destabilization of the peripheral neutrophil control loop, and almost all of the models were more or less like Eq. 3. This was somewhat curious in that all of these authors ignored the parallel oscillations in cell lines other than the neutrophils. Though the origin of the fundamental hypothesis of a destabilization of the neutrophil control loop is of course not clear, and may have varied from author to author, we speculate that the assumption had its origin at least partially in a fundamental property of equations like (3). Namely that if the steady state N_* of (3), defined implicitly by $\frac{dN}{dt} = 0$ so $\alpha N_* = \tilde{A}F(N_*)$, becomes unstable (which occurs via a supercritical Hopf bifurcation) then there will be a limit cycle oscillation with a period T satisfying $2\tau_N \leq T \leq 4\tau_N$. This simple fact (which is easy to prove (Mackey 1996)), combined with the clinical estimate that $\tau_N \simeq 9.7$ days for the neutrophil lineage would imply that an instability in this control loop would manifest itself in an oscillation with a period between 20 and 40 days–a figure almost exactly bracketing the clinically observed values. However, the work of Hearn et al. (1998) cast doubt on this explanation, by showing that any alternations of parameters in the peripheral control system consistent with the extant laboratory and clinical data on cyclical neutropenia are unable to reproduce either the characteristics of clinical cyclical neutropenia or its laboratory counterpart in the gray collie (Hearn et al. 1998; Haurie et al. 2000).

In a different vein, Mackey (1978a) took note of the frequent concomitant oscillations in all of the mature circulating blood cells and hypothesized that the origin of the defect in cyclical neutropenia had to reside in the stem cell compartment that gives rise to all of the differentiated cell lines. His starting point for the physiology of the stem cells was the G_0 model for the cell cycle illustrated schematically in Fig. 2. The dynamics of this stem cell population is governed (Mackey 1978b, 1979) by the differential delay Eq. 1, and was sufficiently simple that it was possible to perform a complete bifurcation analysis (Mackey 1978a). This model has two possible steady states. There is a steady state corresponding to no cells ($S_* = 0$), which is stable if it is the only steady state. The second positive steady state S_* exists for small HSC apoptosis rate γ_S. The stability of the positive steady state depends on the value of γ_S. When $\gamma_S = 0$, this steady state cannot be destabilized to produce oscillatory dynamics of cyclical neutropenia. For $\gamma_S > 0$, increases in γ_S lead to a decrease in the HSC numbers, and destabilize the steady state when a critical value of γ_S is reached, $\gamma_S = \gamma_{\text{crit},1}$, at which a supercritical Hopf bifurcation occurs. When γ_S is further increased, a reverse bifurcation occurs at a critical value $\gamma_S = \gamma_{\text{crit},2}$, where the positive steady state becomes stable, and approaches the zero steady state as γ_S increases. For all values of γ_S satisfying $\gamma_{\text{crit},1} < \gamma_S < \gamma_{\text{crit},2}$, there is a periodic solution of (1) whose period is in good agreement with that seen in cyclical neutropenia (Mackey 1978a).

These results suggested that cyclical neutropenia might be due to defects, possibly genetic, within the HSC population that lead to an abnormal apoptotic loss of cells from the proliferative phase of the cell cycle. It was also noted that the same type of bifurcation could arise if the differentiation rate out of the HSC was increased, and this turned out to be crucial.

G-CSF appears to act as an anti-apoptotic, and the stem cell model predictions were that decreasing γ_S in the range $\gamma_{\text{crit},1} < \gamma_S < \gamma_{\text{crit},2}$ would lead to a decreased period of the oscillation as well as an increased amplitude and early experiments with G-CSF (see below) in gray collies were consistent with this. However, the model also predicted that further decreases in γ_S toward $\gamma_{\text{crit},1}$ would lead to a *decrease* in the oscillation amplitude (which was never observed) before the oscillation was finally obliterated through a reverse Hopf bifurcation (which does occur). Thus the modeling predictions of Mackey (1978a) were partially consistent and partially inconsistent with the observed behavior in the gray collie. However, "partially inconsistent" in mathematical modeling is the death knell for any model and led to other attempts to understand the genesis of cyclical neutropenia.

2.3 More Complicated Models and Our Current Understanding of CN

After a period of data collection and analysis in the gray collie and humans, the discrepancies noted at the end of the previous section made it clear that the original hypothesis of a primary defect at the stem cell level (Mackey 1978a) causing cyclical neutropenia could not be the whole story, and led to a renewed effort to understand this curious disorder. This led to a consideration (Bernard et al. 2003) of the model depicted in Fig. 2.

Using a combination of mathematical analysis and computational tools, the results of Bernard et al. (2003) indicated that the probable origin of cyclical neutropenia is to be traced to an increased apoptosis rate in the neutrophil precursors (which leads to a decrease in A). This leads to a decrease in the steady state level N_* of circulating neutrophils which, in turn, increases the rate of differentiation F out of the stem cell compartment and this destabilizes the stem cell dynamics through a supercritical Hopf bifurcation. This has the effect of generating oscillations in the HSC population. This hypothesis was shown to be consistent with the existing clinical data on humans as well as the experimental data from grey collies.

A more sophisticated model that includes not only the neutrophils and HSC, but also the platelets and red blood cells was developed by Colijn and Mackey (2005a, b). This model included not only the stem cell and neutrophil dynamics but also erythrocyte and platelet compartment models (Mahaffy et al. 1998; Santillan et al. 2000).

Colijn and Mackey (2005b) used a simulated annealing approach and data from dogs and humans to estimate the model parameters for CN. The model supported the hypothesis for the origin of cyclical neutropenia put forward in Bernard et al. (2003) that the oscillations observed in all of the differentiated hematopoietic cells results from an increased apoptosis rate in the neutrophil precursors.

The concept of a graded effect of the mutant protein, depending on specifically how the protein is mutated, fits nicely with mathematical modeling proposals for the mechanisms of cyclic neutropenia. Because neutrophil elastase is synthesized

very early in development of neutrophils at the stage for the formation of the first or primary granules in the cells, cell death at this stage could lead to an extremely low production of neutrophils, i.e., SCN, or to ineffective production because of cell loss in the developmental process with oscillations in the peripheral blood cell counts depending upon the strength of the feedback stimulus, presumably the stimulus of endogenous G-CSF. The latter circumstance is consistent with the clinical disease CN. It is also consistent with the concept that treatment of SCN with rG-CSF could transform production from a pattern of consistently low blood neutrophil counts to an oscillatory state by stimulating production, suppressing apoptosis and more effectively delivering neutrophils through the marrow into the blood.

3 Treating Hematological Disease

3.1 Cyclical Neutropenia and G-CSF

rG-CSF has been used to treat CN for more 25 years (Bonilla et al. 1994; Dale et al. 1993). The long-term results have been very gratifying to patients and families (Dale et al. 2006). Daily or alternate day rG-CSF administered subcutaneously in doses of about 3 mcg/kg/day will make the ANC peaks higher, and the nadirs shorter and overall the neutropenia less severe (Hammond et al. 1989) Interestingly, rG-CSF also shortens the cycle length from about 21–14 days. The effects of this treatment last indefinitely; the longest treated patient has self-administered rG-CSF daily for 25 years. For SCN patients somewhat higher doses of rG-CSF are generally required, but more than 90% respond and the effects are also long term. The effectiveness of G-CSF is attributed a combination of effects: stimulation of production of cells, acceleration of release of neutrophils from the marrow into the blood and promotion of the survival of neutrophils and their progenitors (Price et al. 1996; Kaushansky 2006). rG-CSF is also used quite effectively for treatment of another disease, severe congenital neutropenia (SCN) (Dale et al. 1993). One other surprising observation was that these patients whose neutrophil counts were always extremely low and who had very serious problems with infections had oscillations in their blood neutrophil levels when started on rG-CSF (Haurie et al. 1999a). All of these effects are also seen in the gray collie animal model with canine G-CSF therapy (Haurie et al. 1999b).

rG-CSF is widely used in the treatment of neutropenia in other hematological diseases including the neutropenia associated with other congenital disorders and patients with the myelodysplastic syndrome. Before the development of highly effective antiretroviral therapies, rG-CSF was also widely used to treat neutropenia in patients with HIV infection. There are some adverse effects but these are usually not severe. The overriding concern, however, is that chronic rG-CSF treatment might lead to leukemia. Patients with CN have not developed leukemia, but there are leukemia cases in patients with SCN (Rosenberg et al. 2006, 2010). Because leukemia has been observed in SCN patients before the availability of rG-CSF therapy, it is difficult to establish whether G-CSF is a causal, contributing or non-contributory factor (Dale et al. 2006).

Within a modeling context, the qualitative effects of G-CSF on the amplitude and period are simply mimicked by the assumption that it acts to decrease the rate of cell

death within the neutrophil precursors (increase in A) (Colijn and Mackey 2005b), but recent modeling work has achieved more quantitative agreement by also including the effects of G-CSF on the maturation time on the neutrophil precursors (Colijn et al. 2007; Foley and Mackey 2009b; Lei and Mackey 2011; Brooks et al. 2012).

3.2 The Frequency of G-CSF Administration

rG-CSF is usually administered subcutaneously on a daily basis, i.e., once every 24 h. The non-glycosylated rG-CSF most widely available has a half-life in the body of about 3–4 h and a duration of the biological effect lasting for 1–2 days (Wang et al. 2001). A longer effect of its administration can be achieved by increasing the molecular weight of the drug through pegylation (Roskos et al. 2006). The increase in molecular size retards renal clearance, leaving the drug in the body longer to completely saturate receptors for G-CSF as they are generated by developing bone marrow precursors of neutrophils.

Because of the time delay between a stimulus to neutrophil production and the delivery of cells with blood and their availability to enter the tissues to fight infection, rG-CSF is most effective as a preventive strategy in the management of neutropenia. Patients with CN are usually treated every day or every other day to try to maintain a stable production of neutrophils under the influence of rG-CSF. It would be attractive to patients to avoid daily injections and receive rG-CSF only in one phase of the cycle. It is however difficult to predict exactly when this will be in patients treated with rG-CSF because of the effects of the treatment on the patients cycle length. There are no clinical trials establishing whether or not intermittent treatment is as effective as daily therapy and usually intermittent treatment is associated with more bone pain and other acute side effects.

Using a mathematical model for cyclical neutropenia, Foley et al. (2006) also examined the effect of different delivery schedules on simulated cyclical neutropenia and were able to show that often an every second day delivery could achieve almost the same results as same day delivery but noted that the choice of delivery method depends on a fairly detailed knowledge of the time course of the neutrophil numbers.

3.3 Timing of G-CSF: Is There a 'Black Hole'?

One of the most intriguing results of the model analysis of Bernard et al. (2003) was the observation that in their model for canine cyclical neutropenia there is a locally stable steady state coexisting with the limit cycle corresponding to the cyclical neutropenia. Foley et al. (2006) went on to explore the implications of this bistability and were able to show that for the grey collie it is theoretically possible to stop the cycling by the administration of G-CSF during any point in a specific portion of the cycle. Though this experiment has not been carried out there is circumstantial evidence (Foley et al. 2006, Fig. 9) that this is in fact possible. That it has not been done can be traced to the paucity of good clinical data and the current difficulty of collecting sufficiently detailed data, see Sect. 5. Other studies of the bifurcation properties of these hematopoietic control models have revealed the same bistable behavior when the models are biased (in the

sense that parameters are chosen to mimic those determined by fitting patient data to the model) to reproduce cyclical neutropenia in humans (Lei and Mackey 2011). The implication of these theoretical results is that it might be possible to actually *stop* the neutrophil cycle in cyclical neutropenia by a single properly timed dose of G-CSF if one knew the nature of the neutrophil time course in sufficient detail. This is an example of what Winfree (1980) called a 'black hole' in the dynamics since being in the basin of attraction of a locally steady state makes it difficult to escape.

4 Avoiding Hematological Damage from Chemotherapy

4.1 Hematological Side Effects of Chemotherapy

Cancer chemotherapy usually involves administration of cytotoxic drugs which kill cancer cells but also damage the hematopoietic cells of the bone marrow (Dale 2013). Chemotherapy causes hematological damage because of the high proliferative activity of the precursors of blood cells. The progenitors for neutrophils are the most proliferative cells in this compartment, and therefore decreased neutrophil production and neutropenia are the critical, dose-limiting toxicity for most cancer chemotherapy regimens. This is a serious problem because to be effective cancer chemotherapy generally must be administered at its dose-limiting toxicity, but knowing the individuals tolerance for cancer drugs is not easy to predict.

Since the 1960s, cancer chemotherapy has been administered in cycles predicated on the concept that a series of treatments will steadily decrease the number of residual cancer cells. Because the hematopoietic tissues are exposed to this repetitive toxicity, a consequence of serial treatment is gradual reduction in the population of hematopoietic progenitor cells to repopulate the marrow and restore normal hematopoiesis. Although all hematopoietic lineages are suppressed by chemotherapy, it is neutrophil production, far more than erythrocyte or platelet production, which is impaired. Furthermore, because erythrocytes and platelets can be readily collected, stored and transfused, whereas this is not feasible for neutrophils, the risk of neutropenia limits chemotherapy doses and dose intensity.

Cancer therapy changed dramatically with the availability of the recombinant hematopoietic growth factors, particularly rG-CSF. Clinical trials have established that administration of G-CSF beginning approximately 24 h after cytotoxic drugs will limit the hematopoietic injury and lessen the severity of chemotherapy-induced neutropenia (Crawford et al. 1991). Because of the time delay between administration of rG-CSF and its effect on blood neutrophil counts, it is most effective as a preventive treatment. Clinical trials have shown the largest effect if the drug is administered 1–2 days after the completion of a cycle of chemotherapy. If one delays until the ANC is extremely low, i.e., less than 0.5×10^9/L G-CSF has little effect on clinical outcomes, presumably because by this time there is already a surge in production of endogenous G-CSF. If one delays longer, i.e., until a patient has neutropenia with fever and evidence of infection, there is no effect, again because of the time delay for the hematological response and the likelihood that endogenous G-CSF levels are already high. Administration of rG-CSF can only increase blood neutrophils if there are enough normal neutrophil

progenitor cells to respond. In patients with neutropenia due to aplastic anemia, neutropenia after very high dose chemotherapy, neutropenia due to hematopoietic ablation in preparation for transplantation and after severe radiation injury generally will not respond or not respond very well no matter how much rG-CSF is administered. On the other hand, the most responsive cancer patients are those who have received modest doses of chemotherapy, but they in turn may show no benefit because they are unlikely to have severe myelosuppression. Between these extremes there are, however, many patients expected to benefit from G-CSF to ameliorate the toxicity of chemotherapy. Experts have created clinical guidelines for appropriate treatment, generally recommending G-CSF for patients at higher risk because of age, previous chemotherapy and coexisting diseases as well as the treatment regimen they are scheduled to receive. A major problem in this arena is, however, the limited clinical data on the ANC changes with specific chemotherapy regimens. Because the hematopoietic system and particularly the ANC changes are a barometer for the actual effective dose of chemotherapy administered, it may be possible to predict the effectiveness of cancer chemotherapy by obtaining serial measurements of ANC at frequent intervals after initiation of cancer treatments. New miniature devices for home monitoring of blood cell counts have the potential to make chemotherapy dosing more precise and for personalizing care.

4.2 Modeling Hematopoietic Effects of Chemotherapy and G-CSF

Given the widespread use of chemotherapy and G-CSF as an adjuvant, one might wonder if mathematical modeling might have any relevant insights into understanding how the two might be effectively combined.

In two recent modeling studies Zhuge et al. (2012) and Brooks et al. (2012) examined the predicted hematopoietic effects on neutrophil numbers when chemotherapy is administered in a series of cycles separated by a period of T days. Based on the original and somewhat simplistic initial study (Zhuge et al. 2012), it was predicted that for a hematologically normal human, there would be a significant resonance between the chemotherapy and the neutrophil regulatory system resulting in significant neutropenia if the period of the chemotherapy was about $T = 21$ days. That same paper also examined the effect of adjuvant G-CSF therapy during the chemotherapy administration. A more sophisticated model (Brooks et al. 2012) incorporated the pharmacokinetics of both the chemotherapy and G-CSF and confirmed the earlier findings. However, current work (Craig et al. 2014) that expands the physiological basis of the model considerably, by including both the pharmacokinetics of the chemotherapy and G-CSF as well as the dependence of the neutrophil maturation times on G-CSF levels, casts doubt on the predicted resonance phenomena but does offer great promise in terms of illuminating how to best administer G-CSF following chemotherapy. This is an unfolding story whose conclusion is still unknown.

5 Limitations of Data for Modeling Studies

Mathematical modeling of the regulation of hematopoiesis depends on having large sets of carefully collected data on blood and bone marrow cells. It is very difficult in human studies to obtain serial bone marrow samples. Blood can be sampled easily,

from a vein or finger prick, and there are now accurate methods to measure blood counts on extremely small samples. The challenge is to have patient participation, i.e, going to the hospital or clinical laboratory day after day to have blood cell counts done when healthy as well as when ill. So serial testing with sufficient data to permit modeling has been the limiting factor for modeling studies based on blood counts.

Researchers have recently tested a small device suitable for home monitoring of blood hematological parameters (Dale et al. 2013). Data for longitudinal measurement at daily intervals is feasible, practical and acceptable to patients. Daily systematic collection of data through home monitoring should make it possible to diagnose CN accurately, to distinguish CN (with little or no risk of evolution to leukemia) from congenital neutropenia (with a high risk of leukemia). The convenience of home counts will also help to define the clinical phenotypes for many rare hematological diseases. Often the prevalent disease description in reference texts is based on hospital data at the time of crisis, rather than the usual situation for the patients.

These home monitoring units are currently undergoing tests for FDA approval, and we are confident that they will soon be approved and widely used. When that happens, we will see a qualitative jump in the availability of hematological data for patients with a variety of diagnoses. The availability of better time resolved data will enable modelers and clinicians to test hypotheses about, e.g., the ability of a single dose of G-CSF to stop the cycling (see Sect. 3.3) or to tailor models for the hematopoietic response to chemotherapy to individuals and develop individualized therapy protocols.

6 Potential New Modeling Directions

In addition to the need for better data, modeling efforts to understand the regulation of hematopoiesis are also dependent on the discovery of new physiological mechanisms that are operative. Indeed, over the past 40 years that we have been involved in this venture our models have evolved considerably as we have learned more about the physiology of this fascinating and complicated system.

From a mathematical point of view as these discoveries are made it is a matter of translating the known physiology into a proper mathematical language and then dealing with the subsequent analysis (often new, as in the case of state dependent delays) and simulation issues. One area that has received little attention from a modeling perspective is related to what is commonly referred to as ‘noise’. In the very few studies of hematological blood cell numbers in normal humans that have been carried out, it is obvious that there are unexplained variations in blood cell numbers and the origin of these may potentially be legion. Some of the fluctuations that are seen could be of extrinsic origin from environmental influences, while others could be intrinsic to the cellular and molecular mechanisms [e.g., bursting production of mRNA in the replicating cell populations (Mackey et al. 2013)] involved in the regulation of hematopoiesis—and disentangling which is a virtually unexplored territory. There have been a few attempts to examine the implications of intrinsic noise sources in the HSCs (Lei and Mackey 2007; Lei et al. 2014) but it is clear that these are in their infancy. Further work awaits the development of new techniques to monitor the

temporal behavior of the hematopoietic system (from stem cells to mature circulating cells) for both normal humans as well as patients.

7 Discussion and Conclusions

This expository history has traced the interplay between experimental and clinical hematology on the one hand and mathematical modeling on the other in the attempt to understand the origin of cyclical neutropenia and how to best treat it. We have also touched on current research attempts to extend the modeling to considerations of chemotherapy-induced hematological damage. Our account is highly personal since one of us (Dale) has been intimately involved from the clinical and experimental side while the other (Mackey) has been involved from the mathematical modeling side.

It is clear that during our close to 40 years on again/off again collaboration the basic clinical and experimental findings have played a pivotal role in the development of the modeling and also led to the consideration of mathematical problems that were not conceived of 40 years ago. Conversely, the modeling has played a role in the thinking from the clinical side and in some cases maybe even accelerated understanding. We as players in the area are probably too close to the action to offer an unbiased answer to the question in the title of this paper ("Better medicine through mathematics?") and the definitive resolution of that issue awaits the opinion of others at a later time. However, we are both firmly of the opinion that our interactions, our false starts, and our insights have benefited enormously from the combined intellectual efforts of both of our groups.

It is a truism in science that experimental knowledge of a system is never complete, nor are mathematical models ever complete. The evolution of knowledge about the regulation of hematopoisis over the past 100 years is stark testimony to this statement, as is the evolution of mathematical model attempting to capture the essence of the clinical and laboratory knowledge. The job is not complete, nor will it be in our lifetimes.

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