

Model analysis of the contrasting effects of GM-CSF and G-CSF treatment on peripheral blood neutrophils observed in three patients with childhood-onset cyclic neutropenia

STEPHAN SCHMITZ,¹ HORST FRANKE,² MARKUS LOEFFLER,² H. ERICH WICHMANN³ AND VOLKER DIEHL¹

¹Clinic I for Internal Medicine, University of Cologne, ²Institute for Medical Information, Statistics and Epidemiology, University of Leipzig, and ³GSF Institute of Epidemiology, Neuherberg, Germany

Received 12 June 1996; accepted for publication 2 September 1996

Summary. Human cyclic neutropenia (CN) is a rare haematological disorder characterized by regular fluctuation in the serial count of blood neutrophils. The oscillations occur at subnormal levels with a stable 3-week period. To reduce the risk of serious infections during the severe neutropenic nadir phases ($<0.25 \times 10^9$ neutrophils/l) patients are usually treated with recombinant growth factors. Compared with G-CSF, which has been shown to enhance the amplitudes substantially, the response to GM-CSF is poor: neutrophil numbers are not amplified, the cycles remain unchanged or are dampened. However, two cases with a modest neutrophil increase have been reported in the literature. In a recently published clinical study the different

effects of GM-CSF and G-CSF application have been investigated in the same patients. Based on a mathematical model of CN we previously proposed, the detailed neutrophil data measured in this study are analysed by simulation. The contrasting clinical results can be quantitatively explained by the model concept of regulatory control together with possible individual feedback defects, i.e. abnormally reduced mitotic responsiveness of granulopoietic progenitor cells to GM-CSF and G-CSF.

Keywords: human cyclic neutropenia, GM-CSF, G-CSF, mathematical model.

Childhood-onset cyclic neutropenia (CN) is a rare benign haematological disorder characterized by regular oscillations in the numbers of the blood neutrophils, with a stable period of 21 d in most cases. Patients are chronically neutropenic as peak values are considerably subnormal. The nadir intervals with neutrophil counts $<0.25 \times 10^9$ /l last 4-10 d. During these phases of severe neutropenia, patients frequently develop malaise, upper respiratory tract infections, stomatitis, fever, cervical lymphadenopathy, anorexia, headache and myalgia. With increasing neutrophil levels the infections and accompanying symptoms normally disappear. (Wright *et al.* 1981; Lange, 1983; Dale & Hammond, 1988). Bone marrow examinations have revealed that cycling of peripheral blood cells is preceded by oscillations of granulopoietic marrow cells (Guerry *et al.* 1973; Brandt *et al.* 1975; Dresch *et al.* 1977).

Treatment with recombinant haemopoietic growth factors

is applied in CN to reduce the risk of serious infections during the nadir phases. Continuous G-CSF administration markedly enhances the amplitudes of the neutrophilic oscillation and shortens the period to about 14 d (Hammond *et al.* 1989; Hanada *et al.* 1990; Migliaccio *et al.* 1990; Tsunogake *et al.* 1991; Marlton *et al.* 1992). Clinical experiences with GM-CSF are somewhat ambiguous. Some authors found only a poor response: cycles remain unaffected or are dampened (Wright *et al.* 1989b; Freund *et al.* 1990). Other case reports describe a distinct neutrophil increase even during low-dose GM-CSF administration (Kurzrock *et al.* 1991; Locatelli *et al.* 1991). More recently, Wright *et al.* (1994) examined the GM-CSF and G-CSF effects in the same CN patients and documented the individually different responses they observed by detailed time courses of the blood cell counts.

Previously, we proposed a mathematical model to describe CN and the effects of G-CSF application (Schmitz *et al.* 1994, 1995). That model emerged from a model of normal human granulopoiesis modified by two defects concerning progenitor and postprogenitor cells: reduced variance of the transit

Correspondence: Dr Stephan Schmitz, Clinic I for Internal Medicine, University of Cologne, D-50924 Köln, Germany.

Table I. Individual characteristics and peak values of the neutrophil cycles before, during and after GM-CSF administration, and during subsequent G-CSF administration (Figs 5-7).

Patient	Pretreatment phase	GM-CSF phase	Post-treatment phase	G-CSF phase
1	Low-level oscillation (0.1-0.25 cells × 10 ⁹ /l)	Dampening of cycles, decreasing cell counts	Restored cycles	Enhanced oscillation (3-13 cells × 10 ⁹ /l)
2	Pronounced oscillation (2.5-3 cells × 10 ⁹ /l)	Unchanged cycles	Unchanged cycles	Markedly enhanced oscillation (3.55 cells × 10 ⁹ /l)
3	Distinct oscillation (0.6-0.8 cells × 10 ⁹ /l)	Clear increase of the cell counts	Restored cycles	Not published

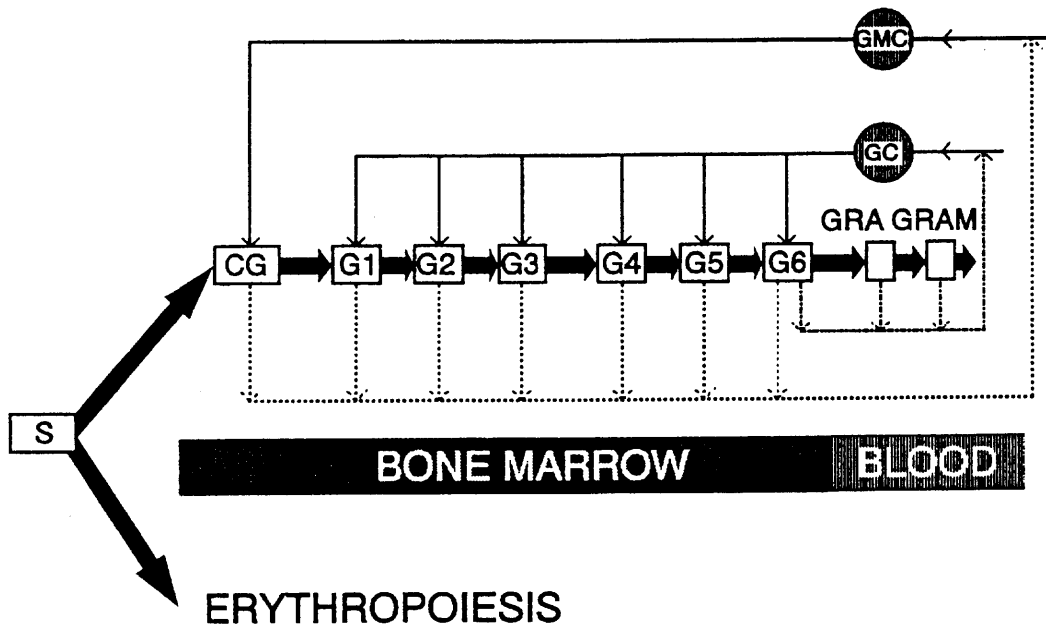


Fig 1. Model of normal granulopoiesis. Granulopoiesis and erythropoiesis (not shown) originate from the pluripotent stem cell compartment (S). Granulopoiesis is governed by two nested feedback loops related to GM-CSF (GMC) and G-CSF (GC). The factor GMC stimulates (solid lines) the mitotic activity of the CFU-GM (CG). It depends (dotted lines) on the number of CG, myeloblasts (G1), promyelocytes (G2), myelocytes (G3), metamyelocytes (G4), band (G5) and segment forms (G6). The factor GC induces (solid lines) mitotic amplification of the G1-G3 cells and early release of the postmitotic cells G4-G6 into the peripheral blood by transit time reduction. The GC level depends (dashed lines) on the number of the most mature bone marrow cells in G6 and on the number of circulating (GRA) and marginal neutrophil granulocytes (GRAM) in the blood.

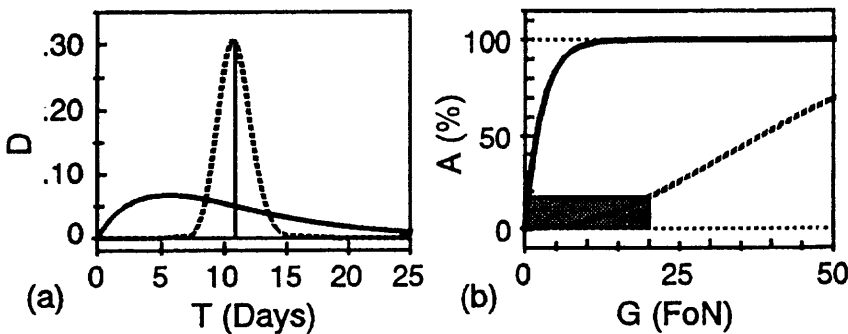


Fig 2. Defects assumed in the model of CN. (a) The variance in the distribution *D* of the transit time *T* through CG and G1-G3 stages (calculated by a Γ -distribution: Takahashi, 1966) is essentially narrowed in CN (dashed line) compared with normal granulopoiesis (solid line). (b) The amplificational response $A = 2^m$ (*m* mitoses, plotted in percent of the maximum value) to the stimulating factor *G* (GMC or GC, plotted in fraction of the normal level) is reduced in CN (dashed line) compared with normal granulopoiesis (solid line).

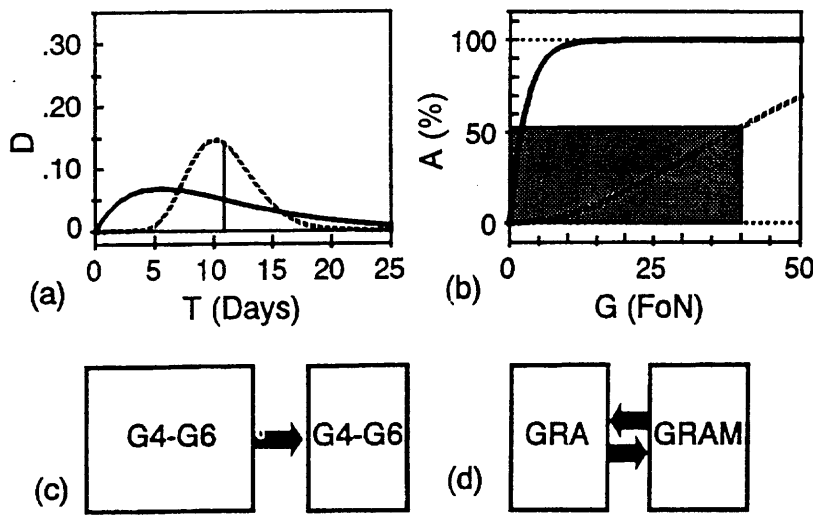


Fig 3. Effects of growth factor application in the model of CN. (a) Increase of variance: the well-peaked distribution of the transit time through CG, G1-G3 in CN is broadened (dashed line). (b) Additional amplification: higher levels of the stimulating factor enlarge the regulation window in the dose-response diagram (hatched area). (c) Early release: the mean transit time through the postmitotic compartments is reduced. (d) Demargination: peripheral neutrophils in the marginal pool GRAM return into circulation GRA and enter GRAM once again.

time (through early bone marrow stages) and abnormal dose-response relationships (regulating mitotic activity by stimulating factors). In the present work this model is used to analyse the data published by Wright *et al* (1994). The contrasting effects of G-CSF and GM-CSF on the neutrophil oscillations they found in CN patients subsequently treated with both growth factors are explained dynamically in terms of granulopoietic feedback structure and individual abnormalities of mitotic dose-response curves.

MATERIALS AND METHODS

Clinical data. The clinical data analysed in this study were published by Wright *et al* (1994). Three patients with well-characterized childhood-onset CN were treated with recombinant human GM-CSF for 6 weeks ($2.1 \mu\text{g/kg/d}$ subcutaneously). During pretreatment (6-13 weeks) and post-treatment phases (6-10 weeks) patients were also clinically observed. Subsequently, after additional therapy off phases, two of the three patients received recombinant

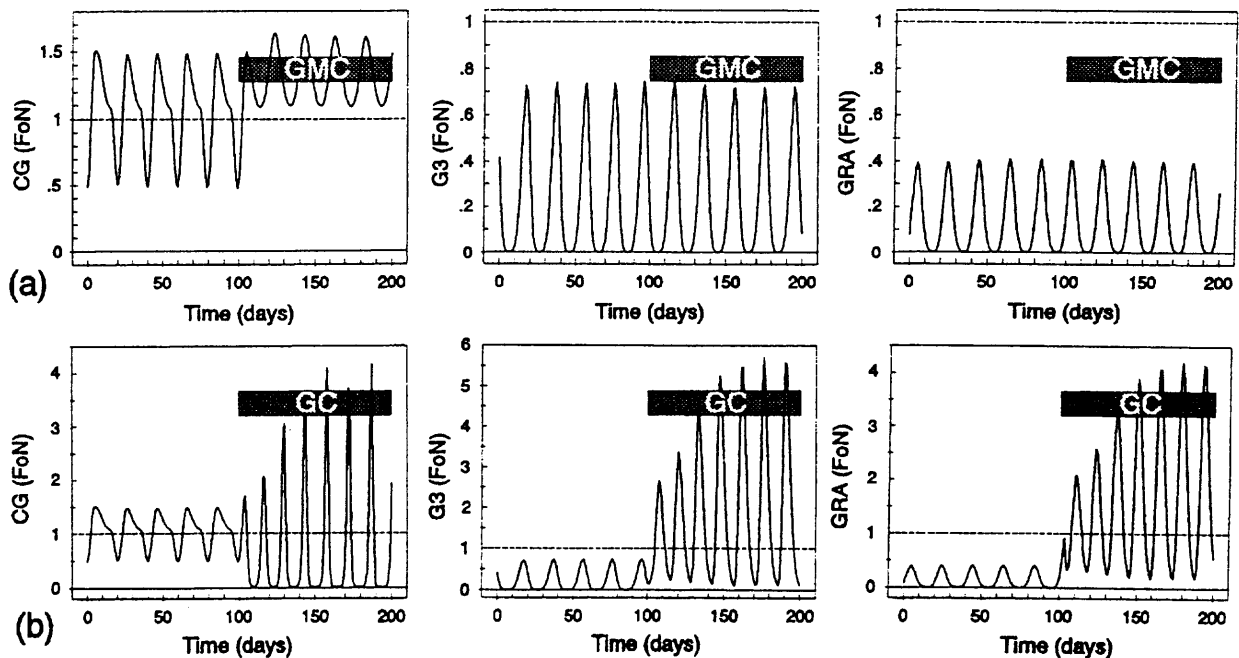


Fig 4. Simulation of growth factor application in CN. During the first 100 d untreated CN is shown. After day 100 a constant additional (exogenous) inflow into the hormone compartments is simulated (indicated by the bars). (a) GM-CSF: inflow into GMC; (b) G-CSF: inflow into GC. increased variance of the transit times through CG and G1-G3, reduced transit time through G4-G6. Cell numbers of CFU-GM (CG), myelocytes (G3) and neutrophils (GRA) are given in fraction of the normal values (FoN).

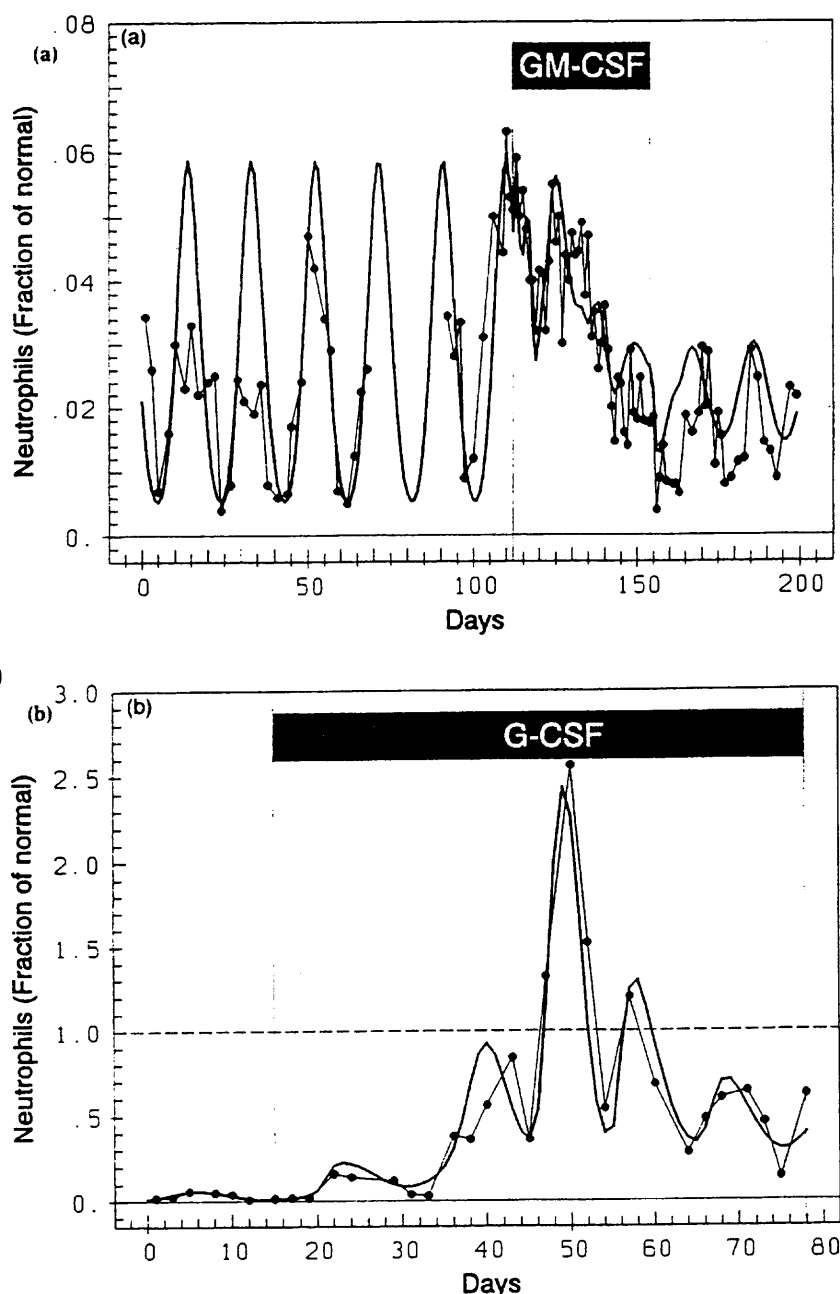


Fig 5. Clinical data of serial neutrophil counts (●) for patient 1 (Wright *et al.*, 1994) and simulation curves (broad lines): (a) treatment with GM-CSF ($2.1 \mu\text{g}/\text{kg}/\text{d}$ s.c., days 112–154); (b) treatment with G-CSF ($5 \mu\text{g}/\text{kg}/\text{d}$ s.c., days 15–78). Neutrophil numbers are given in fraction of the normal value assumed to be $5 \times 10^9/\text{l}$.

human G-CSF for up to 10 weeks ($5 \mu\text{g}/\text{kg}/\text{d}$ subcutaneously). The different individual characteristics of the cycling patterns in the serial neutrophil counts as well as changes during growth factor application are summarized in Table I.

Mathematical model: model of normal human granulopoiesis. Fig 1 shows the structure of the mathematical model. Each biological cell stage is represented by a model compartment characterized by transit time, number of mitoses, and fraction of actively proliferating cells. Succeeding compartments are connected by cell fluxes. The change of the cell counts with time in each compartment is described by a differential equation, leading to a coupled system for the

whole haemopoiesis. Erythropoiesis and granulopoiesis descend from pluripotent stem cells by differentiation into committed progenitor cells BFU-E and CFU-GM, respectively. These cells give rise to the morphologically identifiable bone marrow cells before they finally mature into functional blood elements. Self-renewal probability and proliferative fraction of the pluripotent stem cells are governed by negative feedback loops (not shown in Fig 1). Mitotic amplification and early release of granulopoietic bone marrow cells are governed by two further feedback loops (Fig 1). The regulation is achieved by sigmoidal dose-response curves depending on stimulating factors GMC and GC. It is expected that the hypothetical hormones GMC and GC reflect the role

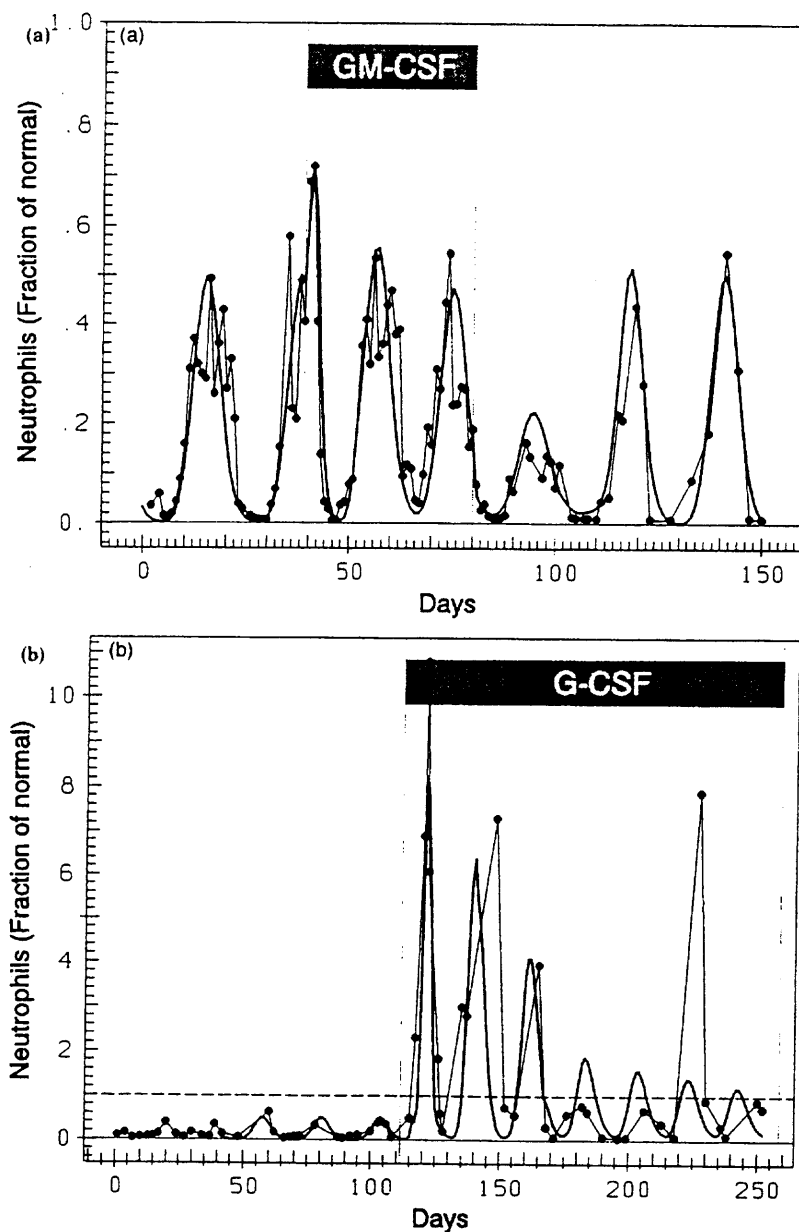


Fig 6. Clinical data of serial neutrophil counts (●) for patient 2 (Wright *et al.* 1994) and simulation curves (broad lines): (a) treatment with GM-CSF ($2.1 \mu\text{g}/\text{kg}/\text{d}$ s.c., days 39–80); (b) treatment with G-CSF ($5 \mu\text{g}/\text{kg}/\text{d}$ s.c., days 112–252). For denotation see Fig 5.

of the growth factors GM-CSF and G-CSF, respectively, in some aspects in human granulopoiesis. Model parameters providing the physiological basis of the model are taken directly from the literature or derived from published experimental data as described previously (Schmitz *et al.* 1993). Details of the model and its biomathematical realization are described elsewhere (Wichmann & Loeffler, 1985; Wichmann *et al.* 1988; Loeffler *et al.* 1989).

Model of human cyclic neutropenia. In the model of normal human granulopoiesis, two defects are assumed to describe CN (Fig 2): (1) the variance of the transit time through CG and G1–G3 cell stages is abnormally small compared with normal granulopoiesis; (2) the mitotic responsiveness of these cells to the stimulating factors GMC and GC is reduced.

The first assumption, implying a reduced dampening property of normal granulopoiesis, is essential for origin and maintenance of the cycles. The second assumption, supported by *in vitro* experiments (Wright *et al.* 1989a; Tsunogake *et al.* 1991; Hammond *et al.* 1992), is necessary to account for the subnormal neutrophil peaks observed in CN. As previously shown, this model reproduces kinetic data of marrow and blood cells that are qualitatively and quantitatively correct (Schmitz *et al.* 1994).

Model effects of GM-CSF and G-CSF treatment in CN. Continuous application of the growth factors acts via four effects in the model of CN (Fig 3): (1) the small variance of the transit time, necessary for cycling, slightly increases; this change towards normal granulopoiesis elevates nadir counts

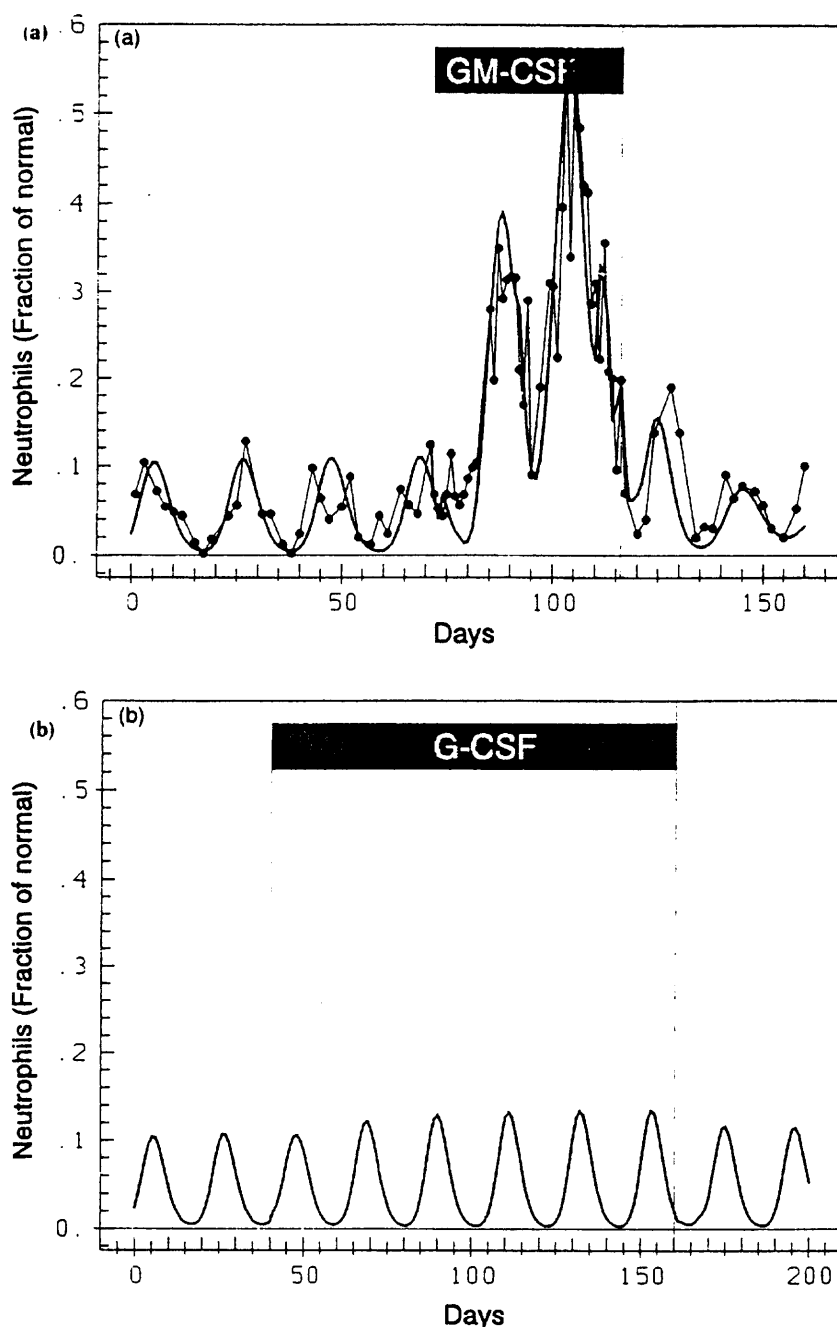


Fig 7. Clinical data of serial neutrophil counts (●) for patient 3 (Wright *et al.* 1994) and simulation curves (broad lines): (a) treatment with GM-CSF ($2.1 \mu\text{g}/\text{kg}/\text{d}$ s.c., days 72–116); (b) simulation of treatment with G-CSF during days 40–160. For denotation see Fig 5.

and dampens the oscillation; (2) an exogenous inflow into the GMC or GC compartment induces additional mitoses in the cell stages stimulated by these factors; (3) the transit time through the postmitotic stages becomes reduced; the shortened feedback pathway leads to a decrease of the cycle period; (4) peripheral neutrophils in the marginal pool demarginate; the effect enhances the number of circulating neutrophils. For details see Schmitz *et al* (1993, 1995).

Simulation of CN treated with GM-CSF and G-CSF. Fig 4 shows typical alterations of the cyclic pattern by simulation of growth factor application in the model. An exogenous inflow into the GMC compartment increases the cell

numbers in CG. Pretreatment nadir counts of about 50% of normal are elevated to levels above normal. This 'perturbation' is compensated by the GC feedback and no longer visible in G3 and the subsequent compartments. The neutrophil cycles in GRA appear therefore completely unaffected by GM-CSF administration (Fig 4a). In contrast, an inflow into the GC compartment prominently amplifies the oscillations in G1–G3. The effect propagates through all subsequent cell stages, being present also in CG by GMC feedback interaction. Elevation of the nadir counts and a shortened period length of the cycles are due to the increased variance and early release effects which are additionally assumed in this simulation (Fig 4b).

RESULTS

Figs 5–7 show model simulations of the serial blood neutrophil counts observed in three patients with CN, before, during, and after continuous administration periods of GM-CSF or G-CSF (Wright *et al.* 1994). Analysis of these data has been performed in terms of reduced variance of bone-marrow transit time, reduced mitotic responsiveness to growth factor stimulation, early release of postmitotic cells, and demargination of peripheral neutrophils. Resulting individual dose–response curves (DRCs) for the model hormones GMC and GC are shown in Fig 8. Essential model parameters for each patient are summarized in Table II.

Patient 1

The profoundly low-level fluctuations of the neutrophil counts in this patient (Fig 5a) during the pretreatment phase are reproduced by extremely reduced DRCs for GC (Fig 8b) and a slightly diminished variance of transit time through CG and G1–G3 (Table II). Amplificational effects induced at

the CG stage by exogenous GMC are abrogated in succeeding cell stages by the GC feedback. The dampening of cycling is caused by an increase of variance, early release of postmitotic cells and demargination of blood neutrophils. In the G-CSF treatment phase (Fig 5b) a distinct neutrophilic increase does not appear before day 36, i.e. 21 d after the beginning of the application. To explain this delayed response it is assumed that the highly abnormal GC DRCs partly recover under the influence of the G-CSF administration (Fig 8b).

Patient 2

In this case the pronounced (untreated) oscillation (Fig 6a) is simulated by less abnormal DRCs together with a strongly reduced variance of transit time (Fig 8 and Table II). Apart from a slightly enhanced peak at the beginning of the GM-CSF treatment phase (obtained by demargination) amplification of the peripheral neutrophils is prevented (as in patient 1) by the GC feedback mechanism. Due to the very stable cycles in this patient, dampening does not occur by enlargement of variance, early release and demargination.

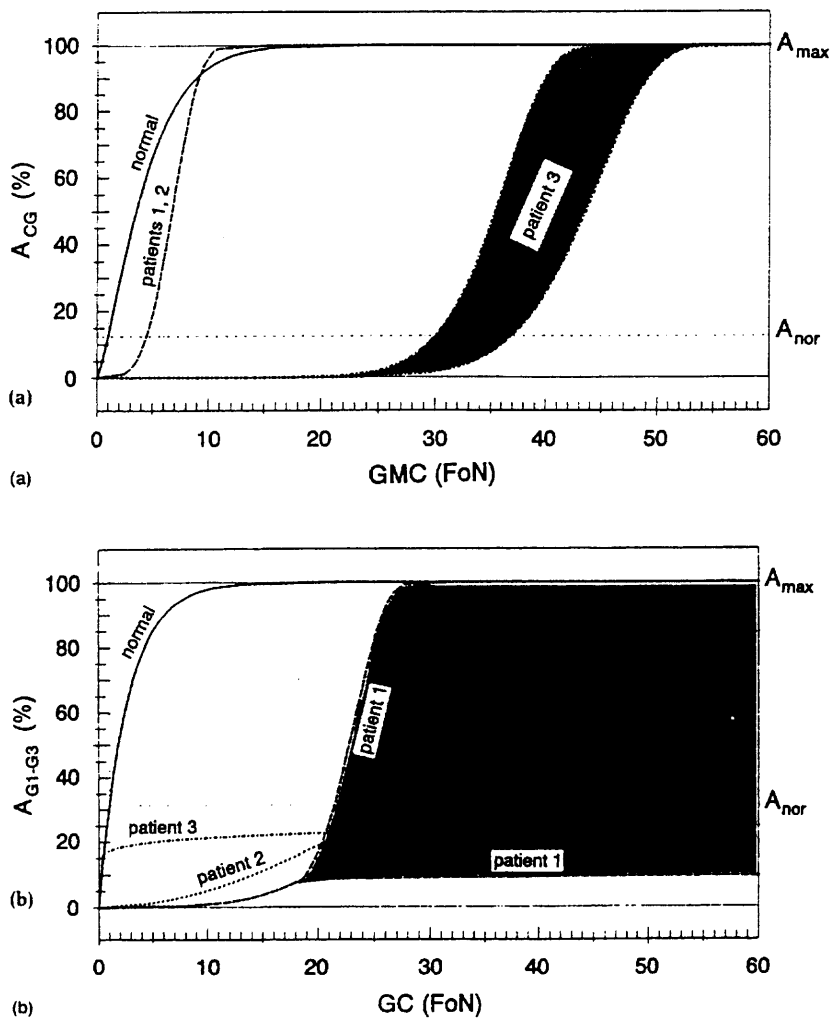


Fig 8. Dose–response curves (DRCs) for the mitotic amplification. (a) GMC DRCs: the relationship between the amplification of CG cells and the stimulating factor GMC is nearly normal for patients 1 and 2. In patient 3 a strongly reduced responsiveness is assumed, which recovers slightly during treatment with GM-CSF (shaded area). (b) GC DRCs: in patient 1 the amplificational response of the postprogenitor cells G1–G3 to GC is extremely reduced, it recovers during treatment with G-CSF (shaded area). DRCs used for patient 2 are less abnormal and unchanged by G-CSF application. In patient 3 the mitotic activity is subnormal and almost independent of the stimulating factor GC. For denotations see Fig 2.

Table II. Individual model parameters used in the simulations of the different treatment phases, and values for normal granulopoiesis: variance of transit time $\sigma_{CG,G1-G3}^2$ (through mitotic cell stages), postmitotic transit time T_{G4-G6} , demargination rate D (of peripheral neutrophils).

Patient	Parameter	Pretreatment phase	GM-CSF phase	Post-treatment phase	G-CSF phase
1	$\sigma_{CG,G1-G3}^2$ (d ²)	25.9	27.2	25.9	32.6
	T_{G4-G6} (d)	6	1.8	6	1.8
	D (%/h)	0	100	0	5
2	$\sigma_{CG,G1-G3}^2$ (d ²)	1.7	26.6	1.7	32.6
	T_{G4-G6} (d)	8.3	4.6	8.3	2.5
	D (%/h)	0	30	0	100
3	$\sigma_{CG,G1-G3}^2$ (d ²)	1.7	27.2	24.9	1.7
	T_{G4-G6} (d)	7.3	1.8	6.3	7.3
	D (%/h)	0	100	0	0
Normal	$\sigma_{CG,G1-G3}^2$ (d ²)	41.7			
	T_{G4-G6} (d)	6.3			
	D (%/h)	0			

In contrast to patient 1, the neutrophil counts increase only a few days after the beginning of the G-CSF administration (Fig 6b). The prompt huge response seen in the data can be understood without recovery of the GC DRCs (Fig 8b).

Patient 3

The modest but distinct neutrophilic response to GM-CSF found in patient 3 (Fig 7a) is incompatible with the compensatory tendency of the GC loop. To abolish this influence very flat GC DRCs are assumed (Fig 8b). Low-level cycling in the untreated phase is then driven by the remaining GMC feedback working with a strongly reduced GMC DRC (Fig 8a). Due to the ineffective GC loop the amplificational response of CG cells stimulated by exogenous GMC can now propagate to the peripheral neutrophils. The additional increase of the second peak during the GM-CSF treatment phase could be fitted by a slight recovery of the GMC DRC. According to the assumed flat GC DRCs, the model predicts no amplification by G-CSF for this patient (Fig 7b). Clinical data were not available.

DISCUSSION

Treatment with human myelopoietic growth factors has been shown to be a successful therapy to reduce the risk of serious neutropenia-related infections. In many clinical trials it has been shown that continuous G-CSF application in patients with cyclic neutropenia (CN) markedly enhances the amplitudes of the blood neutrophilic oscillations and shortens the duration of the severe nadir phases. There are fewer clinical reports concerning the use of GM-CSF. This may be due to the fact that clear effects were not observed (Wright *et al.* 1989b; Freund *et al.* 1990). Unlike G-CSF, most CN patients seem to be GM-CSF 'non-responders'. Some few responders, however, show a modest neutrophilic increase even at relatively low GM-CSF dosages (Kurzrock *et al.* 1991; Locatelli *et al.* 1991). In order to provide more

detailed information, Wright *et al.* (1994) investigated the contrasting effects of GM-CSF and G-CSF in the same patients. With respect to their cycling and response patterns they represent three different types (Table I and Figs 5–7). Patient 1: unstable low-level cycles, dampened by GM-CSF, delayed increase by G-CSF; patient 2: stable high-level cycles, unchanged by GM-CSF, undelayed pronounced increase by G-CSF; patient 3: stable cycles, GM-CSF responder.

Our model analysis shows that this variety of clinical outcome can be understood mainly from the dynamics of granulopoiesis governed by feedbacks. The sharpness of the feedback signals, emerging from the variance of the bone-marrow transit time distribution, essentially determined the stability of the oscillatory behaviour. On the other hand, dose relationships of mitoses-stimulating factors were involved in the feedback loops. This led to a characterization of the individual granulopoietic system including stability of cycling and abnormally reduced mitotic responsiveness measured by dose-response curves (DRCs). Minor individual differences can be explained additionally by early release and demargination effects.

Hence, using these model parameters, the weak cycling of patient 1 was quantified by a slightly reduced variance of the bone-marrow transit time distribution, compared to normal granulopoiesis (25.9 d^2 v 41.7 d^2 ; Table II). Due to the infirm driving mechanism, cycling was destabilized by GM-CSF administration and dampening occurred. The neutrophilic paucity is explained by highly reduced GC DRCs recovering to some extent by G-CSF (Fig 8b). In contrast, patient 2 revealed very stable cycles, arising from a strongly reduced variance (1.7 d^2), which were not disturbed by GM-CSF. Less reduced GC DRCs allowed a powerful enhancement of the neutrophilic oscillation by G-CSF. Obviously, both patients were GM-CSF non-responders.

In the model, non-responsiveness was provided by the antagonistic effect of the peripheral GC loop: the cell increase, stimulated by GMC at the CG level, was compensated in the subsequent cell stages governed by GC. This

level or the response to G-CSF application. Model predictions for all types of GM-CSF responders and non-responders are given in Table III.

In summary, model analysis of the contrasting effects of GM-CSF and G-CSF application in CN supports the assumption that granulopoiesis is regulated by two nested feedback loops related to GM-CSF and G-CSF. Abnormalities of individual DRCs, which can be examined *in vitro* by colony assays, characterize essentially childhood-onset CN patients and determine, recovery processes included, the outcome of treatment with growth factors. From the relevance of the interaction of both feedback loops, which has become obvious in this analysis, it would appear fascinating to investigate further, clinically and theoretically, the outcome of a combined application of G-CSF and GM-CSF in CN. A first modelling attempt (Fig 9) predicts elimination of the cycles with dose-dependent neutrophil counts adjustable at nearly normal levels.

ACKNOWLEDGMENT

The authors are grateful to Professor Daniel G. Wright for communication and availability of the clinical data analysed in this study.

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