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Oscillations of Granulocytic and Megakaryocytic Progenitor Cell Populations in Cyclic Neutropenia in Man

NIELS JACOBSEN & HAL E. BROXMEYER

Laboratories of Experimental Hemopoiesis,
Memorial Sloan-Kettering Institute for Cancer Research,
New York, USA

The concentration of granulocytic progenitor cells in the bone marrow of 2 patients with cyclic neutropenia was assessed at intervals during the cycle. Syncroneous oscillations were observed for cells forming neutrophilic and megakaryocytic colonies in diffusion chambers in vivo (CFU-D), suggesting a close relationship between the two cell types. However, an almost reverse relationship was observed between CFU-D and cells forming colonies in agar culture in vitro (CFU-C), which cycled out of phase with CFU-D. This supports the concept that CFU-D and CFU-C denote different cell types.

Key words: CFU-D – CFU-C – colony forming cells – cyclic neutropenia – granulopoiesis – megakaryocytopoiesis

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Correspondence to: Dr. Niels Jacobsen, Department of Medicine C, Gentofte Hospital, University Hospital of Copenhagen, DK-2900 Hellerup, Denmark

Haemopoietic progenitor cells are functionally defined by their capacity to form colonies of morphologically recognizable bone marrow or blood cells. Colony forming units in culture (CFU-C) denote populations of cells capable of forming granulocyte-macrophage aggregates in semisolid agar cultures in vitro in response to macromolecules termed colony stimulating activity (CSA) (Robinson & Pike 1970). Colony forming units in diffusion chambers (CFU-D) form neutrophilic, eosinophilic and megakaryocytic colonies in fibrin clot diffusion chambers implanted intraperitoneally into sublethally irradiated mice (Jacobsen 1975).

CFU-C and CFU-D can be partially segregated by means of velocity sedimentation (Jacobsen et al 1978) and can be distinguished on the basis of their kinetics in culture (Jacobsen et al 1979).

To provide further evidence that CFU-D and CFU-C are different cells a study of progenitor cells in cyclic neutropenia has been performed. Cyclic neutropenia is a disease characterized by periodic changes in the peripheral blood neutrophil count. In addition, oscillations of eosinophils, reticulocytes, platelets and monocytes have been reported (Guerry et al 1974). Indirect and direct experimental evidence obtained from studies of human and canine cyclic

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neutropenia supports the contention that the disorder is due to stem cell or progenitor cell defects, resulting in periodic failure of haemopoiesis (Dale & Graw 1974, Weiden et al 1974, Jones et al 1975, Greenberg et al 1976).

There is a well-known cycling in the number of CFU-C relative to total cells in the bone marrow of patients with cyclic neutropenia (Greenberg & Schrier 1973).

The present study shows that granulocytic and megakaryocytic CFU-D oscillate out of phase with CFU-C.

MATERIALS AND METHODS

2 patients with well documented cyclic neutropenia were studied. Both had peripheral blood neutrophil counts which varied cyclically within 21-day intervals. Informed consents to perform bone marrow punctures for experimental purposes were obtained in each case.

Bone marrow cells were aspirated from the posterior superior iliac spine and the cells were mixed with heparin as an anticoagulant.

Density cut separation. Buffy coat cells from the marrow aspirates were separated by a bovine

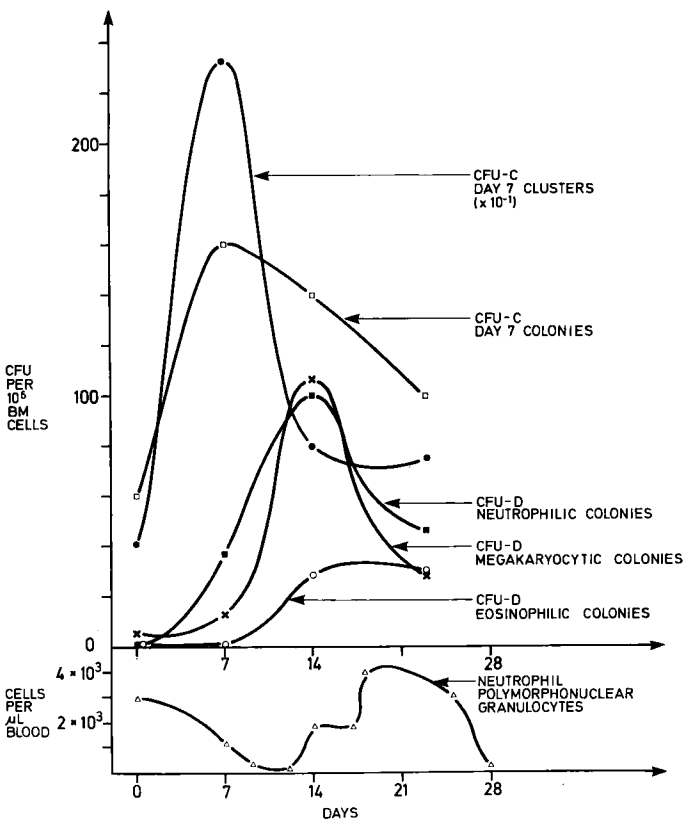

Figure 1. Oscillations of progenitor cell concentrations in the bone marrow in one patient with cyclic neutropenia. Number of neutrophilic (■), megakaryocytic (×) and eosinophilic (○) CFU-D and CFU-C forming granulocytic colonies (□) and clusters (●) per 10^6 low density cells. Each point represents the mean of an average of 8 diffusion chambers or 4 agar cultures. Absolute neutrophil count per μl of peripheral blood as a function of time is shown at the bottom.

serum albumin density cut procedure as previously described (Broxmeyer et al 1977). The collected buoyant fraction (density $<$ 1.070 g/ml) contained the vast majority of CFU in the buffy coat layer (Jacobsen et al 1978) and was depleted of polymorphonuclear granulocytes. The cells were washed twice prior to culture.

Assays for CFU

1) *CFU-C.* Cells were suspended in modified McCoy's 5A medium containing 10 % fetal calf serum (FCS) and 0.3 % Bacto Agar (Difco) and plated on top of feeder layers, containing 10^6 normal human peripheral blood leukocytes, in 35 mm Petri dishes (Robinson & Pike 1970). 2×10^5 low density cells were plated per dish. The cultures were incubated at 37° in a humidified atmosphere of 7.5 % CO_2 in air and scored for clusters (3–50 cells per aggregate) and colonies ($>$ 50 cells) after 7 d of incubation. 3 to 5 plates were scored per point.

2) *CFU-D.* Fibrin clot diffusion chambers (Millipore GS membranes, pore size 0.22 μm) were inoculated with cells in modified McCoy's 5A medium, 20 % FCS and 0.5 % human fibrinogen. Thrombin was added to induce coagulation of the fibrinogen suspension. Each chamber received 2×10^5 low density bone marrow cells. The chambers were implanted i.p. into 5–7 week old female Swiss mice (CDl, Charles River, Wilmington, Mass., USA), two chambers into each mouse. All mice received 600 r total body irradiation from a ^{137}Cs source 2–4 h before chamber implantation. After 7 d, the chambers were removed, cleaned on the outer surface and reimplanted into newly irradiated mice for a further 7 d of culture. The cultures were harvested after a total of 14 d, stained with May-Grünwald Giemsa stain and scored for neutrophilic and eosinophilic colonies ($>$ 30 cells) and megakaryocytic aggregates ($\geq$ 2 cells) (Jacobsen 1975, Jacobsen et al 1978). An average of 8 chambers were scored per point.

RESULTS AND DISCUSSION

The results obtained in one of two similar studies are presented in Figure 1, which shows the variations in peripheral blood neutrophil count and bone marrow progenitor cell concentrations as a function of time. This case was representative of both patients.

Sequential determinations of CFU-D and CFU-C during one complete cycle revealed large variations in the concentration of progenitor cells in the bone marrow (Figure 1). Identical oscillation curves were obtained for neutrophilic and megakaryocytic CFU-D, suggesting a close relationship between the two cell types. However, an almost inverse relationship was observed between the concentration of CFU-C and CFU-D, supporting the contention that these cells were different. Cells that formed clusters on day 7 in agar oscillated slightly out of phase with CFU-C which gave rise to the colonies. The cluster forming cells may in part be identical with cells that form colonies on day 14 in agar (Jacobsen et al 1978). This latter cell type was, however, not assessed in the present series of experiments. It is unlikely that the CFU-C fluctuation was related to the use of different feeder layers, since identical cyclic variations have been observed using human placental conditioned medium as a constant source of CSA (Broxmeyer, unpublished).

Cells that gave rise to eosinophil colonies varied cyclically, but possibly slightly out of phase with neutrophilic and megakaryocytic CFU-D.

The present results support the conclusion that the in vivo diffusion chamber technique detects a family of cells that differ from the cells measured on day 7 in agar culture in vitro.

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