Hemopoietin-Initiated Changes in Differential Retransplantability of Mouse Femoral Marrow-Derived Colony-Forming Units (CFU)[1] (36747)

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(Introduced by W. J. Fallberg)
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The spleen colony method (1) has been widely used to study the effects of hemopoietins and host microenvironment on the proliferation and differentiation of hemopoietic progenitor cells (colony-forming units, CFU). The majority of transplantable CFU are thought to be uncommitted, but to become committed, upon seeding, by the hosts' hemopoietic microenvironments (2). Hemopoietins are essential for the proliferation, differentiation and maturation of CFU. Posttreatment of hemopoietically reconstituted supralethally irradiated hosts with hemopoietin does not affect the total or differential colony forming potential of the transplanted CFU (3, 4). However, pretreatment of donors with cell line-specific hemopoietins such as renal granulopoietic factor (GPF) or erythrocyte stimulating factor (ESF) does increase the total CFU number and shifts the differential exocolonizing potential of a measurable proportion of the CFU in favor of the hemopoietin-specific cell line (5).

The purpose of the present study was to determine whether donor treatment with hemopoietins (GPF and ESF) would increase the size of a pool of microenvironment-independent, irreversibly committed CFU and/or a pool of microenvironment-dependent. reversibly committed CFU. And furthermore the study attempted to determine whether such a pool of CFU consists predominantly of finitely and/or "infinitely" mitotable progenitor cells.

Materials and Methods. Bioassay mice. A total of 315 A/J males aged 8–10 wk—35 donors, 145 primary hosts and 135 secondary hosts—were used for this study. Mice were housed, fed and watered as described elsewhere (5).

Experimental design (*Fig. 1.*) Intact donor mice were injected with either 3.26 mg of calf renal GPF (6) in 0.5 ml isotonic saline, or 0.5 ml ESF-rich, dialyzed, anemic horse plasma, or 0.5 ml of pyrogen-free isotonic saline. Femoral marrow cells were harvested at times where peak hemopoietin-specific CFU responses had previously been shown to occur (5)—30 hr following GPF and 24 hr following ESF treatment.

Hosts received 750 R whole body irradiation ($LD_{99/30}$) 3–5 hr before being subjected to marrow transplantation. The radiation was delivered at a rate of 130 R/min in air from a G-E Maxitron source (250 kVp, 30 mA, Th III filter with 1 mm Al and 0.5 mm Cu, HVL 3.0 mm in air).

Pooled donor cell suspensions were prepared for each experimental group. Primary hosts were injected intravenously with either 25×10^3 or 5×10^6 viable nucleated donor cells. The low-dose hosts were sacrificed 9 days later to evaluate the CFU content of the cell sample. The high-dose hosts were sacrificed 24 hr or 8 days after transplantation, and used as donors for the secondary hosts.

During the first 24 hr following primary host injection, the donor CFU had had time to come into contact with the host spleens' environment, but had not as yet begun to divide (7). By 8 days, however, the CFU had had time to seed on the splenic microenvironment and also to self-replicate and dif-

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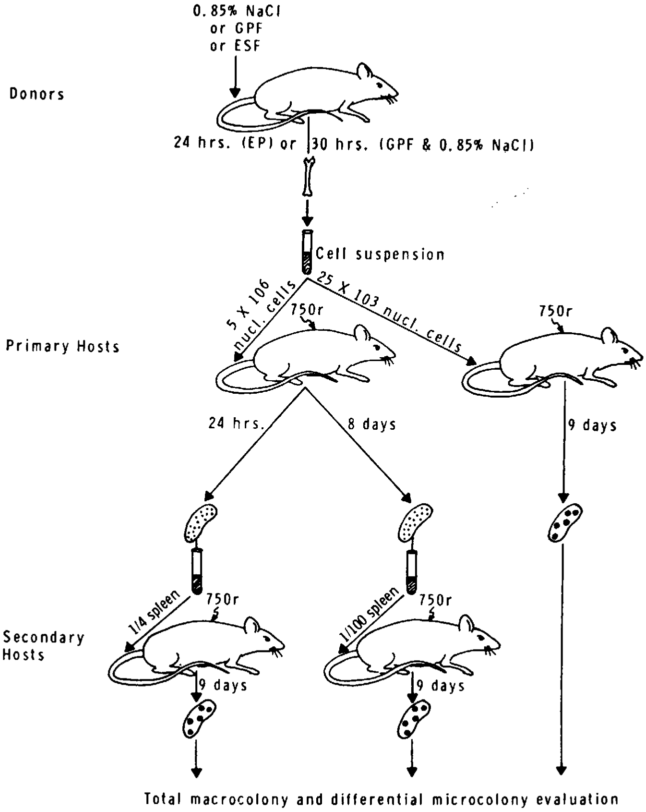

Fig. 1. Experimental protocol.

ferentiate into clones. Therefore secondary hosts were given an intravenous cell dose equivalent to 1/4 of a 24-hr or 1/100 of an 8-day high-dose primary host spleen. These cell doses were based on the premise (a) that approximately 1/6 of the injected CFU would settle in the host spleens (8) and (b) that an estimated 25–30-fold CFU increase could be expected to occur within a given primary host spleen by 8 days posttransplantation (9). The f-fraction (cloning efficiency) estimate was not corrected for variations such as those described by Fred and Smith (10) following different types of donor treatment.

Secondary spleens were harvested 9 days after transplantation.

Five replicate runs were performed with GPF and saline; two with ESF.

Harvested spleens were fixed in Bouin's solution. All surface colonies were counted at 9× magnification with a dissection microscope.

Spleen colonies were typed differentially from hematoxylin- and eosin-stained, 5 μ thick, longitudinal sections taken at 200 μ intervals throughout the entire spleen. Clones with a 15-cell or greater diameter were scored as colonies. Erythrocytic:granulocytic (E/G) colony ratios were calculated from the differential colony counts.

The f-fraction of transplanted cells was estimated by the method of Siminovitch, McCulloch and Till (8).

Results. The data indicate (Table I; Fig. 2) that 30 and 24 hr after a single dose of GPF or ESF, respectively, the donor marrow's exocolonizing potential was almost doubled and the differential CFU potential had shifted in favor of the hemopoietin-specific cell line.

When donor marrow-derived CFU remained in the primary host only 24 hr, the f-value of CFU retransplanted into secondary hosts remained within normal limits for A/J mice (.112–.125) and the transplanted CFU retained their hemopoietin-initated cell line-dependent differential colony forming potential (Table I; Fig. 2): E/G ratios were 1.0–1.2 for GPF-treated and 4.2–5.4 for ESF-treated donor marrow-derived primary and secondary host spleen colonies, as opposed to E/G ratios of 2.5–2.8 in controls.

When donor marrow-derived CFU were allowed to seed and proliferate within the primary hosts' splenic microenvironment for 8 days, the transplanted CFU still retained their hemopoietin-initiated cell line-specific differential colony-forming potential (Table I; Fig. 2): E/G ratios were 1.2 for GPF-treated and 5.4 for ESF-treated donor marrow-derived secondary host spleen colonies, as opposed to 2.5 in controls.

The proliferation-dependent increases in f-values for transplantable CFU in saline-treated and ESF-treated donor-derived marrow hosts was indentical—3.530 and 3.340, respectively (Table I). However, the f-value increase in transplantable CFU from GPF-treated donor-derived marrow was only 2/3 as great as normal—2.020 instead of 3.530 (Table I).

The nucleated cellularity of the femoral marrow remained identical in all 3 donor groups. The splenic cellularity of the primary hosts was not determined in these preliminary studies.

Discussion. The present studies confirmed our previously published findings (5) that humoral stimulation of donors with exogenous hemopoietins (GPF or ESF) increased the size of (a) hemopoietin-specific committed cell pool(s). The findings furthermore suggested that for the majority of *affected* CFU this commitment was irreversible, since a large proportion of the progeny of these CFU appeared to express their hemopoietin-initiated, cell line-specific commitment, apparently regardless of the microenvironment on which they seeded, in both primary and secondary hosts.

The absolute increase (Fig. 2) in transplantable granulocytic CFU within 30 hrs of GPF and in erythrocytic CFU within 24 hrs of ESF administration may have been due to

TABLE I. Differential Retransplantation Potential of GPF- and ESF-treated Donor Marrow-Derived CFU in Primary and Secondary Hosts.

Treatment	Donors		Cell source	Size cell inoculum	Host spleens			
	Dose/20 g body wt	No. mice			No.	No. surface colonies ($M \pm SE$)	f-fraction	E/G ratio
0.85% NaCl	0.5 ml	14	30-hr Donor BM	25×10^3 nucl. cells	41	6.5 ± 0.5		2.6
			24-hr prim host spl.	1/4 spl.	27	6.9 ± 0.5	0.122	2.8
			8-day prim host spl.	1/100 spl.	30	7.7 ± 1.3	3.530	2.5
GPF	3.26 mg	15	30-hr Donor BM	25×10^3 nucl. cells	25	11.9 ± 0.9		1.0
			24-hr prim host spl.	1/4 spl.	23	11.3 ± 0.7	0.112	1.0
			8-day prim host spl.	1/100 spl.	25	8.2 ± 0.4	2.020	1.2
ESF	0.5 ml	6	24-hr Donor BM	25×10^3 nucl. cells	17	10.9 ± 0.9		4.2
			24-hr prim host spl.	1/4 spl.	17	10.5 ± 0.7	0.125	5.0
			8-day prim host spl.	1/100 spl.	13	10.5 ± 0.7	3.340	5.4

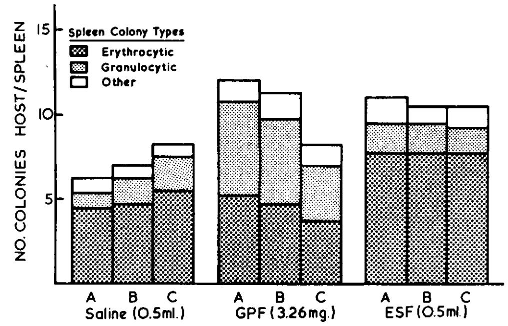

FIG. 2. Differential counts of GPF- and ESF-treated donor marrow-derived spleen colonies in primary and secondary hosts. (A) 30-hr donor marrow-derived colonies; (B) 24-hr primary host spleen-derived colonies; (C) 8-day primary host spleen-derived colonies.

the following: (a) mobilization of hemopoietin-specific committed CFU out of a reserve pool into the transplantable committed CFU pool and/or (b) preferential shunting of uncommitted CFU into the hemopoietin-depleted committed CFU pool followed by proliferation in both CFU pools.

The 24-hr and 8-day residence periods allowed the donor cells in the primary hosts was designed to compare the effects of the splenic microenvironment on nondividing (24-hr) and exponentially proliferating (8-day) CFU.

It is obvious from the data that neither the short-term (24-hr) residence in the primary host hemopoietic microenvironment, nor circulation of CFU between hemopoietic tissues during the first few days posttransplantation (11), nor long-term (8-day) residence, seeding and proliferation in the splenic microenvironment affected the hemopoietin-initiated commitment of CFU.

This indicated that the CFU whose numbers increased following hemopoietin treatment were predominantly irreversibly committed cells. For if the microenvironment were a commitment modulating factor for these hemopoietin-stimulated CFU, the latter would not have retained their original hemopoietin-initiated cell line-specific commitment through two transplant generations. Rather they would have yielded a normal E/G ratio, as has been shown to occur (2, 3) upon retransplantation of monocommitted colonies from untreated donor-derived marrow CFU.

An interesting finding was that GPF-treated donor marrow-derived CFU had only 2/3 as great a retransplantation potential as did the saline- and ESF-treated donor marrow-derived CFU. One possible explanation is that the cell cycle time of the granulocytic series is about 50% longer than that reported (7) for erythrocytic and uncommitted progenitor cells during the exponential growth, and that this is only detectable when the predominant CFU in the system are granulocytically rather than erythrocytically committed. Another possibility is that after undergoing a few cell divisions even irreversibly committed CFU need the appropriate hemopoietic microenvironment to further respond to their cell line-specific hemopoietin. Such a hypothesis would be in line with Curry, Trentin and Wolf's theory (2) that there are 2–3 times as many erythrocytic as there are granulocytic hemopoietic inductive microenvironments in the mouse spleen.

As has been suggested by Bennett and Cudkowicz (12), committed hemopoietic progenitor cells can be "infinitely" self-replicating—capable of producing colonies in both primary and secondary hosts—and finitely self-replicating—capable of producing colonies in primary hosts only. From the present data, it appeared that the hemopoietin-stimulated donor marrow-derived committed cells belonged primarily to the "infinitely" mitotable progenitor compartment, retaining their self-replicatory potential through at least two transplant generations. However, any conclusions regarding the extent of the committed CFU self-replicatory potential must be held in abeyance pending studies of multiple serial retransplantation of hemopoietin-stimulated CFU present in entire colonized spleens and/or in individual monocommitted colonies.

Summary. The spleen colony system was used to test *in vivo* effects of hemopoietins on the total and differential exocolonizing potential of A/J mouse marrow colony-forming units (CFU) transplanted serially into primary and secondary, supralethally irradiated, isogeneic hosts. The hemopoietins used were GPF (calf renal granulopoietic factor) and

ESF (erythrocyte stimulating factor-rich anemic horse plasma).

As measured by total and differential primary host spleen colony counts, GPF and ESF evoked a time-dependent increase in a pool of committed donor CFU. These CFU were transplantable from primary into secondary hosts without losing their hemopoietin-initiated, cell line-specific commitment.

The f-fraction remained within normal limits (0.112–0.125) in all experimental groups when primary host spleen cells were harvested at 24 hr and transplanted into secondary hosts. When the donor CFU were allowed to reside and replicate in the primary host spleen for 8 days before retransplantation into the secondary hosts, the f-fraction manifested a 27–29-fold increase for saline- and ESF-treated marrow-derived CFU, but only an 19-fold increase for GPF-treated marrow-derived CFU. Theoretical explanations for this difference in CFU seeding efficiency are discussed.

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