Induced Changes in Transplantability of Hemopoietic Colony Forming Cells (33015)

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The assay for colony forming units (CFU) described by Till and McCulloch (1) has been used in studies of effects of a variety of conditions which influence hemopoiesis. These assay values represent the fraction of injected colony forming cells (CFC) lodging and making grossly visible colonies in the spleen. Assay of the colony forming cell population requires a knowledge of this transplantation fraction. Determinations of the fraction have given essentially the same results for several strains of mice (2, 3) and for cells grown 3–20 days in an irradiated recipient (4). It seemed possible, however, that treatments such as irradiation and injection of endotoxin or vinblastine, which are known to affect CFU (5–7) might alter the transplantation fraction.

Methods. The mice used were (BALB/c × DBA/$_2$) F_1 females 12–16 weeks old, housed individually. Cell suspensions were prepared as previously described (6). Transplantation fractions were obtained essentially as described by Siminovitch *et al.* (2). Briefly, suitable aliquots of marrow cells from treated and untreated donor mice were injected i.v. into 2 groups of recipient mice given 900 rads of ^{60}Co irradiation less than an hour before. One group of recipients served for assay of the CFU injected. The other group was injected with an appropriate aliquot of the cells, sacrificed after a given interval, and an assay was made of the CFU recovered from the spleen. The interval between injection and extraction of cells from the spleen was 3 hours unless otherwise specified. CFU recovered/CFU injected is the fraction, f_2, for this second transplantation. It has been assumed that f_2 is equal to f_1, the fraction for the first transplantation (which cannot be measured by this method), and that CFC = CFU/f_2. The method of estimating means of fractions and their standard errors, reflecting variation both within and between experiments, is that previously used for ratios (8). The ^{60}Co irradiation was given at a rate of 120–150 rads/min. *S. typhosa* endotoxin (Difco, Detroit) was given as an i.p. injection of 25 μg/mouse. Vinblastine sulfate (VLB, Eli Lilly and Co., Indianapolis) was given as an i.p. injection of 4 μg/gm.

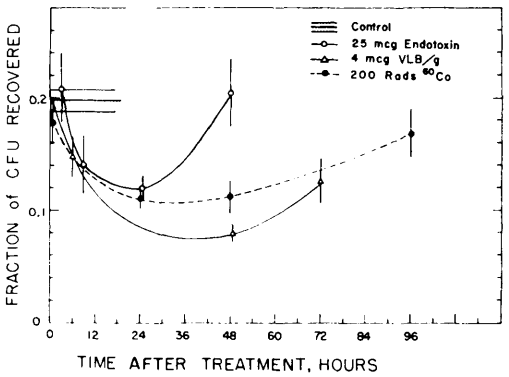

FIG. 1. Transplantation fractions for femoral CFU of mice given 25 μg of endotoxin, 4 μg/gm of vinblastine, or 200 rads of ^{60}Co irradiation. Number of experiments per point: control, 18; endotoxin 9 hours, 2; endotoxin 24 hours, 5; 200 rads 24 hours, 4; VLB 48 hours, 3. The other points represent single experiments.

Results. Transplantation fractions for marrow cells from normal donors and donors given endotoxin, VLB or 200 rad irradiation are shown in Fig. 1. The mean fraction for normal cells was 0.20 ± 0.01, not importantly different from the fraction 0.17 obtained by Siminovitch *et al.* (2) or 0.24 obtained by Schooley (3). One day after the injection of endotoxin the fraction had dropped to 0.12, 2 days after the VLB injection the fraction was 0.08, and both 1 and 2 days after 200 rads the fraction was 0.11. After endotoxin the fraction had returned to normal in 2 days, while after VLB or irra-

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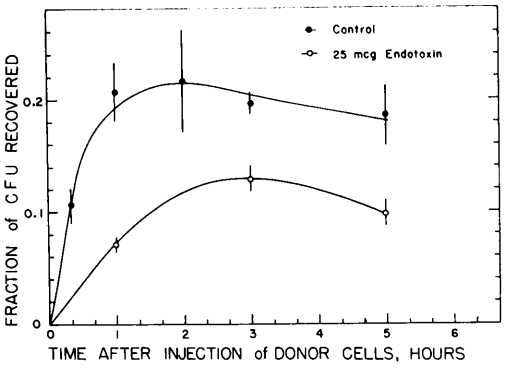

FIG. 2. Transplantation fractions for femoral CFU of mice given no treatment or 25 μg of endotoxin 1 day before, estimated from assays made 20 min to 5 hours after injection of donor cells. Number of experiments per point: control 2 hours, 2; 3 hours, 18; endotoxin treated 3 hours, 5. Other points represent single experiments.

diation it remained below normal for several days.

These differences in transplantation fraction might be explained in part by differences in the time required for the cells to settle in the recipient spleen. Effects of varying the interval between injection and sacrifice for untreated and endotoxin treated donors are illustrated in Fig. 2. Cells from normal donors showed no significant difference between intervals of 1, 2, 3, and 5 hours. For endotoxin treated donors, although 3 hours appears to be sufficient for transplantation, the fraction at 1 hour was lower, indicating that these cells did not lodge in the recipient spleen as quickly as the cells from controls.

Estimates of the number of CFC in the inoculum are based on the fraction of CFU obtained from a second transplantation, since the fraction of colony forming *cells* transplanted cannot be measured as such. Confidence that these fractions are equal would be strengthened if a third transplantation should give the same fraction. In two experiments with normal mice the fraction, f_3, was 0.20, the same as the mean f_2 value for 18 experiments. In a single experiment with cells from mice given endotoxin a day earlier, the fraction, f_3, was 0.15 compared to a mean f_2 of 0.12 for 5 experiments (range, 0.09–0.16).

In irradiated mice CFU values for cells assayed 1 day after exposure are lower than values for cells assayed a few minutes after exposure (6–9), a drop which has not been satisfactorily explained. As seen in Table I, this drop was less for CFC than for CFU because of the lower transplantation fraction observed 1 day after irradiation. Between minutes and 1 day after 200 rads there was a 70% loss in CFU compared to a 50% loss in CFC.

Pretreatment with endotoxin or VLB has been shown to reduce or eliminate this post-irradiation drop (6, 7). Thus, CFU values 1 day after irradiation were higher in treated mice than in controls. Transplantation fractions were lower for treated mice, resulting in even greater differences in CFC than in CFU (Table I). For mice given endotoxin this was more pronounced after 300 rads than after 200 rads; CFC were 2.8 times control and CFU 1.8 times control. For mice given VLB and 200 rads, CFC were 6.6 times control compared to 2.4 times control for CFU.

TABLE I. Colony Forming Cells per Femur Estimated from Mean CFU and Observed Transplantation Fraction for Irradiated Donors and Donors Given 25 μg of Endotoxin 1 Day Before or 4 μg of VLB/gm 2 Days Before Irradiation.

Treatment	CFU/femur	Transplantation fraction	CFC/femur
200 rads (min)	807	0.18	4483
200 rads (1 day)	240	0.11	2182
Endotoxin, 200 rads (1 day)	312	0.09	3467
VLB, 200 rads (1 day)	579	0.04	14,475
300 rads (1 day)	55	0.08	688
Endotoxin, 300 rads (1 day)	97	0.05	1940

Discussion. Changes in donor cells must have been responsible for the observed differences in transplantation fraction since all recipient mice in these experiments were given the same treatment, namely, 900 rad irradiation. It is not known which characteristics of the cells determine transplantability or how these are affected by treatment with endotoxin, VLB, or radiation. If fragility is greater in the cells from treated mice one might suppose that its effects would be lost in the first transplantation, and that subsequent fractions would be more nearly normal. Changes in cell configuration and adhesiveness are also possibilities to be considered. Although the mechanisms responsible remain obscure, it is important to be aware of changes in transplantability which may be associated with treatment of the donor mouse.

Summary. Hemopoietic colony forming cells can be estimated from the assay of CFU, provided the transplantation fraction is known. The fraction for first transplantation cannot be determined by the present methods and has been assumed to be the same as the measurable fraction obtained from a second transplantation. Fractions of CFU recovered on third transplantation were found to be the same as on second, supporting this assumption. Transplantability was altered by treatment of donor mice with irradiation, endotoxin, and VLB. Thus, the loss in CFC between minutes after and 1 day after irradiation was less than the loss in CFU. Pretreatment of irradiated mice with endotoxin or VLB caused a greater reduction in the transplantation fraction than radiation alone. Thus, 1 day after irradiation, differences in CFC values between control and treated mice were even greater than the differences in CFU values previously reported. Clearly, changes in transplantability can be induced and this must be taken into account in the interpretation of effects of various treatments on hemopoietic colony forming cell populations.

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Protein Synthesis in Frog Embryos and Frog Liver Preparations, and Its Inhibition by Embryo Components* (33016)

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In the course of frog development, the total protein content of the organism remains constant or decreases slightly during embryonic development and in the first days after hatching, and a net increase of total protein is not seen until some days after the larvae begin active feeding (1). The factors that control the pattern of protein turnover and net synthesis of protein during amphibian development have not been defined, and the detailed characteristics of the protein-synthesizing system in amphibians have

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