Cells Capable of Colony Formation in the Peripheral Blood of Man

Abstract. *Colony-forming cells have been found in the peripheral blood of man and have been grown in vitro by use of a soft agar gel technique. It has been possible to collect these cells with a blood-cell separator in numbers similar to those found in the peripheral circulation. Repeat leukapheresis of the same donor does not reduce the number of circulating colony-forming cells.*

Colonies of proliferating leukocytes can be grown from suspensions of mouse (*1*) and human (*2*) marrow that were cultured in soft semisolid agar. The number and size of these colonies is increased by the incorporation of certain serum or urine specimens into the agar (*3, 4*). Optimum colony growth from human marrow occurs after 10 to 15 days of incubation (*5*). The cells that make up these colonies are granulocytes and macrophages, and it has been hypothesized that they develop from stem cells committed to granulocytic differentiation (*3*). Using a similar technique, we have found that such cells are present in the peripheral blood of man and can be collected in large numbers by leukapheresis with the use of a blood-cell separator (*6*).

Allogeneic marrow engraftment has been observed after the transfusion of peripheral blood leukocytes from patients with chronic myelogenous leukemia to patients with myelosuppression (*7*), and also has been demonstrated in cross-circulation experiments between lethally irradiated and normal dogs (*8*). In addition, the transfusion of peripheral blood leukocytes, from syngeneic or allogeneic donors, collected with the blood-cell separator can initiate early bone marrow recovery after a course of myelosuppressive chemotherapy (*9*). These findings suggest that bone marrow stem cells exist in the peripheral blood and that it may therefore be possible to achieve bone marrow repopulation by transfusion of peripheral blood leukocytes.

Samples of bone marrow, peripheral blood leukocytes (BPL), and leukocytes collected by leukapheresis (LL) were obtained from nine normal individuals and one hematologically normal patient with cancer. Additional marrow and blood samples were obtained from four hematologically normal patients with cancer. Bone marrow (5 ml) was aspirated in heparin from the posterior iliac crest; peripheral blood was collected and defibrinated; and leukocytes collected by leukapheresis were anticoagulated with acid-citrate-dextrose-A solution. All specimens were prepared for culture by washing three times with tris-buffered ammonium chloride (*p*H 7.2) to lyse the red blood cells and were resuspended in CMRL 1066 medium (Grand Island Biological Co.) enriched with (per milliliter) 20 μg of L-asparagine, 75 μg of diethylaminoethyl dextran, 100 units of penicillin, and 100 μg of streptomycin, and with 0.2 m*M* L-glutamine. Specimens were then diluted five to one with pooled human serum to a concentration of 2×10^5 nucleated cells per milliliter. Each culture consisted of 1 ml of this suspension, 1 ml of a 0.3 percent solution of agar, and 0.15 ml of either a concentrated urine known to stimulate mouse marrow to form colonies in vitro or normal saline. In this procedure, 2×10^5 cells were used because in preliminary studies this concentration gave the maximum number of colonies and was on the linear portion of the inoculum response curve. All cultures were set up in duplicate in 3.5-cm petri plates and incubated at 37°C in a moist atmosphere of 5 percent CO_2 with air. Plates were examined by phase-contrast microscopy on day 1 to rule out cell clumping and on day 7 to check cell viability and rule out contamination; on day 14 the colonies on each plate were counted. A colony was considered to be any cluster of ten or more cells which was not present on day 1 and which had shown evidence of cell multiplication between days 7 and 14. The number of cells in each colony ranged from 10 to 250, with the majority of the colonies containing between 20 and 150 cells at 14 days.

More than 50 percent of the samples that we studied grew colonies. The colonies grew more regularly in the stimulated samples and more regularly from bone marrow than from PBL or LL samples. The median number of colonies per plate was also higher from bone marrow than from PBL or LL (Table 1). On the nine occasions that bone marrow, PBL, and LL were cultured simultaneously, the number of colonies from bone marrow was higher than or equal to the number from PBL on seven occasions. The number of samples showing colony formation and the number of colonies per plate were slightly higher for the LL than for the PBL, possibly indicating a concentration of colony-forming cells (CFC) by the blood-cell separator.

Seven patients had more than one leukapheresis and two of these had a total of seven procedures each. There were not more than 2 days between each procedure. Simultaneous cultures were made with PBL and LL and the results (Table 2) again indicated a concentration of CFC with the blood-cell separator. There was no evidence of increase or decrease in the number of circulating CFC in the peripheral blood after repeated leukaphereses. On

Table 1. Colony-forming cells per 2×10^5 nucleated cells cultured in vitro for 14 days. Abbreviations: Unstim., unstimulated; Stim., stimulated with 0.15 ml of concentrated human urine.

Source of inoculum	Type of culture	No. of subjects	Positive cultures (%)	Mean No. of colonies in positive cultures	Median No. of colonies in positive cultures	Range of all cultures
Bone marrow	Unstim.	15	80	44	45	0–106
	Stim.		87	63	57	0–152
Peripheral blood leukocytes	Unstim.	34	50	28	24	0–122
	Stim.		56	32	30	0–164
Leukapheresis leukocytes	Unstim.	38	66	29	12	0–164
	Stim.		71	39	17	0–232

Table 2. Number of colonies per plate in stimulated cultures of peripheral blood and leukapheresis leukocytes collected and cultured simultaneously at 2×10^5 nucleated cells per plate for 14 days.

Source of inoculum	No. of subjects	Colonies				Positive colonies (%)
		Mean No.	Median No.	Mean No. in positive cultures	Median No. in positive cultures	
Peripheral blood	29	18	9	30	28	58
Leukapheresis leukocytes	29	23	11	34	21	65

six occasions no CFC were found in the peripheral blood when colonies were grown from LL, and on three occasions CFC were grown from PBL and not from LL. The inability to grow colonies on these occasions may have been due either to the absence of CFC or to difficulties with the culture technique. On nine occasions bone marrow was aspirated at the same time the leukapheresis was performed, and the median number of CFC in the bone marrow, PBL, and LL did not differ from the overall results in Table 1.

Bone marrow was aspirated from three patients prior to and after a series of leukaphereses; in two of these cases the number of CFC was higher on the second aspirate than it was on the first.

In conclusion, CFC have been demonstrated in the peripheral blood in a concentration of approximately 1 per 10,000 nucleated cells. The presence of CFC in the peripheral blood has also been demonstrated recently by others but in lower concentration (*10*), possibly because the investigators were not working in the linear portion of the inoculum response curve. The colonies consisted predominantly of granulocytic cells and appear to be similar to those colonies that were previously demonstrated in the bone marrow of mice (*1*) and man (*2*) and that are derived from committed stem cells. The CFC circulating in the peripheral blood can be collected in large quantities by leukapheresis with a blood-cell separator and in numbers similar to those found in the peripheral blood. Since the blood-cell separator collects 20 to 30 percent of the circulating leukocytes, of which 60 to 90 percent are large mononuclear cells (*11*), this would suggest that the CFC are present in the large mononuclear cell fraction of the peripheral leukocytes.

In a series of five leukaphereses, in which a total of 50 liters of whole blood can be processed, 5×10^{10} nucleated cells containing a total of 4×10^7 CFC (calculated from the median CFC of 17 in the LL) can be collected. If one assumes an average nucleated cell count in the bone marrow of 15×10^3, there would be 2.0×10^7 CFC (calculated from the median of 57 CFC in the marrow) in 500 ml of bone marrow. This suggests that the transfusion of sufficient quantities of peripheral leukocytes might be as effective as bone marrow in the granulocytic repopulation of patients with severe myelosuppression. Evidence of this has been shown in identical twins (*9*). Uncommitted stem cells capable of differentiation to all myeloid cell lines have not been demonstrated with this system. This does not preclude their presence in the peripheral blood and their potential value in marrow repopulation. For instance there is evidence, from circulation experiments in dogs (*8*) and in those patients who develop myeloid grafts after leukocyte transfusions from donors with chronic myelogenous leukemia (*7*), that these cells do exist in the peripheral circulation.

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Increasing the Multiplicity of Ribosomal RNA Genes in Drosophila melanogaster

Abstract. *In wild-type* Drosophila melanogaster *females there are about 250 ribosomal RNA genes in each nucleolus organizer region of the two X chromosomes. When this same nucleolus organizer region is present in flies in only a single dose, the number of ribosomal RNA genes increases to approximately 400. This increase is most easily explained by disproportionate replication of these genes.*

In the eucaryotes thus far studied the multiple copies of the 18*S* and 28*S* RNA genes are clustered at the nucleolus organizer (NO) locus (*1–3*). Experiments with both *Xenopus* and *Drosophila* have shown that the amount of 18*S* and 28*S* ribosomal RNA (rRNA) hybridizable per unit DNA is directly proportional to the dosage of the NO segment per genome (*2, 3*). However, in the course of investigating the redundancy of the rRNA genes in *Drosophila melanogaster*, I have discovered several cases which contradict this observation. When flies carry only one NO region (Fig. 1A), on their X chromosome, there are approximately 150 more rRNA genes per X as compared to the value when the same wild-type X chromosome is present as part of the usual two doses in X/X females. This striking increase occurs during the development of these individuals, probably involves more than one cell type, and cannot be due to gene recombination between homologous chromosomes.