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The Spleen as a Site of Colony-Forming Cell Production in Myelofibrosis (40971)[1]

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Abstract. Increased numbers of colony-forming cells (CFU-C) were found in peripheral blood of 11 patients with myelofibrosis. CFU-C determinations were performed on blood from the splenic vein and artery and peripheral blood of two of these patients at the time of splenectomy. There was a significantly greater concentration of CFU-C in the splenic venous blood as opposed to splenic arterial or peripheral blood. In a patient with pathologic stage IIA Hodgkin's disease, CFU-C concentrations in splenic venous and arterial blood were not statistically different and were similar to those in peripheral blood. These findings suggest that the spleen may be an important site of CFU-C production in some patients with myelofibrosis.

Colony-forming cells (CFU-C) are often increased in the peripheral blood of patients with chronic granulocytic leukemia (CGL) (1, 2) and myelofibrosis (3). It has been suggested that the spleen may be a major source of CFU-C in these disorders (4–7). In order to investigate this point further, we performed CFU-C determinations on splenic venous and arterial blood obtained during splenectomy from two patients with myelofibrosis. The findings support the role of the spleen as an important site of CFU-C production in myelofibrosis.

Materials and methods. Two patients with a diagnosis of myelofibrosis (one primary and one secondary to polycythemia vera) were followed at the Syracuse Veterans Administration Hematology Clinic on Polycythemia Vera Study Group protocols and are presented in the following case reports (Patients 1 and 2). Informed consent was obtained from all patients and the research was carried out according to the Declaration of Helsinki.

CFU-C determinations were performed on unfractionated peripheral blood obtained from 20 normal volunteers and 11 patients with myelofibrosis (including the two patients presented in case reports). All blood samples were collected in a syringe rinsed with preservative-free heparin. Red cells were sedimented by inverting the syringe for 1 hr and the leukocyte-rich plasma was removed. CFU-C determinations were performed on simultaneously obtained samples from the splenic artery and vein and peripheral blood of Patients 1 and 2 and a patient with pathologic Stage IIA Hodgkin's disease. The CFU-C assay was performed using the standard double-layer agar culture technique as described by Pike and Robinson (8). The underlayer contained 1×10^6 peripheral blood leukocytes/ml from the same normal donor. The overlayer consisted of $1-5 \times 10^5$ nucleated cells/ml. The plates were incubated in quadruplicate at 37°C in a humidified atmosphere of 5% CO_2–95% air. After 14 days the cultures were examined with an inverted microscope. Aggregates of greater than 40 cells were counted as colonies. Results were expressed as colonies (mean ± SD for the quadruplicate samples) per 5×10^5 nucleated cells.

Patient 1. Myelofibrosis was diagnosed by bone marrow biopsy in a 60-year-old male in March 1976. Therapy with melphalan, prednisone, and testosterone enanthate was unsuccessful. Management consisted of transfusions of packed red cells. In an attempt to decrease transfusion requirements, 250 rad (over five sessions) was ad-

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ministered to the spleen, in March 1977. Because of progressive splenomegaly and further increasing transfusion requirements, splenectomy was performed 1 month later.

At the time of splenectomy, WBC was 1400/μl (with 40% granulocytes). Peripheral blood smear revealed leukoerythroblastosis, marked anisocytosis, poikilocytosis, teardrop forms, and thrombocytopenia. A 2272-g, spleen revealed hemosiderosis, fibrosis, and extramedullary hematopoiesis, with prominent megakaryocytic and erythroid elements and a paucity of mature granulocytes. Postoperatively, the pancytopenia failed to improve and the patient died secondary to sepsis.

Patient 2. Polycythemia vera was diagnosed in a 30-year-old male in 1968. Management consisted of phlebotomies and various myelosuppressive agents. In April 1979, bone marrow biopsy revealed areas of fibrosis interspersed with areas of hypercellularity. Hemoglobin was 12.2 g/dl, WBC 24,500/ml, and platelets 375,000/μl. Because of severe abdominal discomfort, splenectomy was performed in May 1979.

The spleen weighed 2160 g. Histological examination of the spleen and liver biopsy revealed extramedullary hematopoiesis, consisting of proliferation of erythroid, megakaryocytic, and myeloid elements including mature leukocytes. Postoperatively, the patient developed leukocytosis (WBC greater than 90,000/μl with 90% granulocytes) and thrombocytosis (platelets greater than 1,000,000/μl). Both have resolved after treatment with melphalan and the patient is currently asymptomatic.

Results. CFU-C recovered from the peripheral blood of 20 normal volunteers ranged from 0 to 10 colonies (mean 4 ± 4) per 5 × 10^5 nucleated cells plated (Table I). In seven patients with primary myelofibrosis, including Patient 1, circulating CFU-C ranged from 108 to 585 (mean 220 ± 161). In one of these patients, determinations a year apart revealed 585 and 530 colonies/5 × 10^5 cells (17,433 and 22,366 colonies/ml peripheral blood). In a second patient, determinations 1 month apart revealed 118 and 102 colonies/5 × 10^5 cells (6042 and 5304 colonies/ml peripheral blood). In two patients with myelofibrosis following polycythemia vera (including Patient 2), the numbers of CFU-C in peripheral blood were 110 and 155. Two patients with myelofibrosis secondary to metastatic breast cancer had 92 and 99 circulating CFU-C (Table I).

CFU-C determinations on blood collected simultaneously at the time of splenectomy in Patient 1 revealed identical numbers in the peripheral blood and splenic arterial blood (3 ± 1 colonies/5 × 10^5 nucleated cells) (Table II). The number of CFU-C in splenic venous blood (23 ± 4) was significantly increased ($P < 0.001$) over peripheral and splenic arterial CFU-C. In Patient 2, there were similar numbers of CFU-C in peripheral blood (155 ± 22) and splenic arterial blood (162 ± 44) (Table II). The number of CFU-C in splenic venous blood (292 ± 63) was significantly higher

TABLE I. CFU-C IN PERIPHERAL BLOOD OF NORMAL CONTROLS AND PATIENTS WITH MYELOFIBROSIS (PRIOR TO THERAPY)

	Colonies/5 × 10^5 nucleated cells		Colonies/ml peripheral blood	
	Mean ± SD	Range	Mean ± SD	Range
Normals (N = 20)	4 ± 4	0–10	56 ± 6	0–140
Primary myelofibrosis (N = 7)	220 ± 161	108–585	8217 ± 6884	2,160–20,160
Myelofibrosis following polycythemia vera (N = 2)	133	110–155	5437	3,278–7,595
Myelofibrosis secondary to metastatic breast cancer (N = 2)	96	92–99	2117	1,841–2,392

TABLE II. CFU-C DETERMINATIONS IN PATIENTS 1 AND 2

	CFU-C (colonies/5 × 10^5 nucleated cells)			
	Peripheral blood	Splenic artery	Splenic vein	P value[a]
Patient 1				
Prior to splenectomy	130 ± 22[b]			
At splenectomy (s/p splenic irradiation)	3 ± 1	3 ± 1	23 ± 4	<0.001
Days s/p splenectomy	0			
Patient 2				
At splenectomy	155 ± 22	162 ± 44	292 ± 63	<0.05
5 months s/p splenectomy	96 ± 36			
Patient with stage IIA Hodgkin's disease				
At splenectomy	9 ± 3	7 ± 2	6 ± 3	N.S.

[a] Between splenic artery and vein.
[b] Mean ± SD for quadruplicate samples.

than the number of CFU-C in the splenic artery ($P < 0.05$). In a patient with pathological stage IIA Hodgkin's disease, CFU-C assay revealed similar numbers in peripheral blood (9 ± 3), splenic arterial blood (7 ± 2), and splenic venous blood (6 ± 3) (Table II).

Discussion. It is well established that patients with CGL have increased numbers of CFU-C in the peripheral blood (1, 2). Chervenick (3) has also demonstrated elevated numbers of circulating CFU-C in patients with myelofibrosis. Data from our laboratory (Table I) are in agreement and extend the observation to patients with myelofibrosis secondary to metastatic breast cancer.

A decrease in circulating neutrophils has often been observed following splenic irradiation in patients with CGL (9, 10). A decrease in circulating CFU-C has been demonstrated following splenic irradiation in a patient with CGL (10) and in a patient with myelofibrosis (6). Through a series of blood volume calculations, Koeffler *et al.* (6) have shown that there were not enough CFU-C circulating through the spleen at the time of irradiation to account for the fall in total number of CFU-C in their patient. They were unable to demonstrate any radiation-induced humoral inhibitors of granulopoiesis. In addition, the CFU-C in their patient did not manifest increased radiosensitivity. They thus concluded that the probable mechanism for the radiation-induced granulocytopenia was destruction of proliferating precursor cells in the spleen.

Bagby (5) has provided more direct evidence to support the role of the spleen as a source of CFU-C in CGL. CFU-C was assayed at the time of splenectomy in a patient with CGL. Splenic venous blood CFU-C was twice that of peripheral venous blood. Postsplenectomy the number of circulating CFU-C fell dramatically. By the second week postsplenectomy, the number of CFU-C in the peripheral blood returned to the high preoperative levels, suggesting the presence of another source of CFU-C in addition to the spleen.

In the present study, the findings suggest that in myelofibrosis, the spleen is also a significant source of CFU-C. In both patients studied, the concentration of CFU-C was significantly greater in splenic venous blood than in splenic arterial blood. In contrast, in a control patient undergoing splenectomy as a staging procedure for Hodgkin's disease, similar concentrations of CFU-C were found in the splenic vein and artery. These results confirm the con-

clusions of Koeffler *et al.* (6) and extend the finding of Bagby (5) to patients with myelofibrosis.

There appear to be various sources of CFU-C in patients with myelofibrosis, with the spleen, bone marrow, and liver contributing variably in any one patient to circulating CFU-C and to subsequent granulocyte production. In patients in whom the bone marrow and liver may contribute significantly to CFU-C production, as in Patient 2, high levels of CFU-C would continue after splenic irradiation or splenectomy. In those in whom the spleen may be virtually the sole source of CFU-C, such as Patient 1, splenic irradiation and/or splenectomy may result in profound granulocytopenia (6, 10). Thus, a more complete understanding of CFU-C production in myelofibrosis would be helpful in planning more rational treatment regimens.

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1. Moore, M. A. S., Williams, N., and Metcalf, D., J. Nat. Cancer Inst. **50**, 603 (1973).
2. Goldman, J. M., Th'ng, K. H., and Lowenthal, R. M., Brit. J. Cancer **30**, 1 (1974).
3. Chervenick, P. A., Blood **41**, 67 (1973).
4. Adler, S. S., Scand. J. Haematol. **17**, 153 (1976).
5. Bagby, G. C. Jr, Scand. J. Haematol. **20**, 193 (1978).
6. Koeffler, H. P., Cline, M. J., and Golde, D. W., Brit. J. Haematol. **43**, 69 (1979).
7. Goldberg, J., Tice, D., Nelson, D., Landaw, S. A., and Gottlieb, A. J., Blood **50**, 221A (1977) (Abstract).
8. Pike, B. L., and Robinson, W. A., J. Cell Physiol. **76**, 77 (1970).
9. Li, J. G., Radiology **80**, 471 (1963).
10. Barrett, A. J., Longhurst, P., Humble, J. G., Newton, K. A., Brit. Med. J. **1**, 1259 (1977).

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