

Demonstration of Hemopoietic Stem Cells in the Peripheral Blood of Baboons by Cross Circulation

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Baboons were given 1200 R total body irradiation from two opposing ^{60}Co sources. Three animals were given supportive therapy only and died, as expected, within 8 days of irradiation with profound marrow hypoplasia. Five baboons were cross-circulated with unirradiated partners and died within 14 days with evidence suggestive of graft-versus-host disease. Their marrows were repopulated with hemopoietic precursor cells, and three of the five had rises in peripheral white blood cell counts to more than 1500/cu mm before death. These results are compatible with the presence of hemopoietic stem cells in the peripheral blood of a nonhuman primate, the baboon.

HEMOPOIETIC STEM CELLS capable of regenerating irradiated marrow parenchyma after autologous or syngeneic transplantation have been found in the peripheral blood of rodents^{1,2} and dogs.³⁻⁵ Evidence that such cells exist in man has remained in doubt⁶⁻⁸ although more differentiated cells (i.e., granulocytic and erythrocytic progenitor cells) have been cultured in vitro from human peripheral blood.⁹⁻¹⁴ The present study was undertaken to test for the presence of hemopoietic stem cells in the peripheral blood of a nonhuman primate, the baboon. To this purpose baboons were lethally irradiated with 1200 R total-body irradiation (TBI), then cross-circulated with a random, unirradiated partner and observed for hemopoietic engraftment.

MATERIALS AND METHODS

Thirteen baboons (*Papio papio*) (dewormed and free of tuberculosis) weighing 7-13 kg were observed for disease for at least 6 wk before use. They were then acclimated to restraining chairs for 2 wk and were maintained in these chairs throughout the study.¹⁵ Cross-circulation partners were matched for baboon secretory red blood cell antigen A.

On the day preceding TBI, baboons were anesthetized with pentobarbital sodium, and femoral arteriovenous shunts were established.^{16,17} Movement of the leg bearing the shunt was limited by a leg restrainer. Irradiated baboons were given 1200 R TBI (midline air exposure) from two opposing ^{60}Co sources at an exposure rate of 9.2 R/min and with a source-target distance of 180 cm. This dose corresponded to approximately 900-1000 rads midline tissue exposure.¹⁸ The radiation exposure was monitored by a Victoreen R meter and by radioluminescence dosimetry. After TBI, all baboons were given ampicillin, 250-500 mg twice daily intravenously (i.v.), and they were supported with parenteral fluids and electrolytes as needed.

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Submitted October 19, 1976; accepted April 26, 1977.

Supported by Grants CA 18047 and CA 15704 from the National Cancer Institute and Grant RR 00166 from the National Institutes of Health. Dr. Sale is supported in part by a Fellowship from the American Cancer Society. Dr. Thomas is a recipient of Research Career Award A1 02425 from the National Institute of Allergy and Infectious Diseases.

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Table 1. Data on Baboons Given 1200 R.

Baboon No. (Group)*	Survival (Days)	Rise in WBC†	Last Peripheral Blood Counts Before Death			Marrow Histology Differential (%)								
			Granulocytes/ cu mm	Platelets/ cu mm	Reticulocytes‡ (%)	Cellularity§	Myeloid	Erythroid	Megakaryocytes	Lymphocytes	Plasma Cells	Stroma Cells and Histiocytes		
66343(I)	8	No	35	27,000	0	Decreased	0	0	0	0	0	10	10	80
66777(I)	8	No	20	3,000	0	10	0	0	0	0	0	10	20	70
66347(I)	8	No	15	30,000	0	<10	0	0	0	0	0	15	20	65
66196(II)	8	No	10	36,000	0.4	20	10	5	Present	Present	Present	20	50	15
66144(II)	9	Yes	320	37,000	1.4	10	10	10	Present	Present	Present	15	5	60
66181(II)	9	No	126	46,000	1.2	30-40	30	20	Present	Present	Present	5	5	40
66180(II)	11	Yes	110	1,000	0.2	50	40	20	Present	Present	Present	5	5	30
66268(II)	13	Yes	1,200	28,000	Not done	10	20	10	Present	Present	Present	25	5	40

* Group I, supportive therapy only; group II, cross-circulated with an unirradiated partner.

† After the postirradiation nadir.

‡ Corrected for hematocrit changes.

§ Cell/fat ratio.

|| Estimate based on marrow smears only.

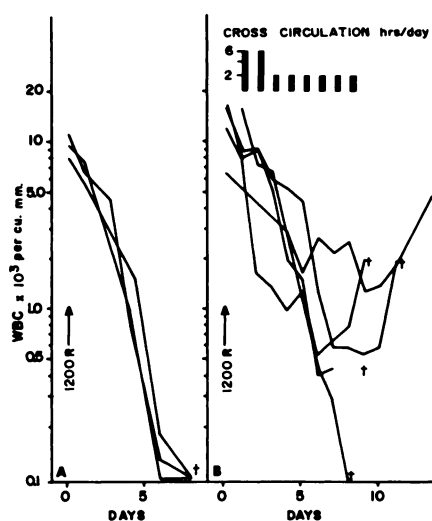


Fig. 1. WBC changes in lethally irradiated baboons (B) without and (A) with subsequent cross circulation with an unirradiated partner.

Two groups of baboons were studied. Group I consisted of three baboons given 1200 R without subsequent cross circulation. Group II consisted of five baboons given 1200 R followed by cross circulation with an unirradiated partner. The technique of cross circulation has been described in detail elsewhere.¹⁷ Briefly, the arterial cannula of each baboon was connected with the venous cannula of the partner by a piece of silastic tubing 30 cm long and 2.6 mm in internal diameter. Flow rates through the shunts ranged from 100 to 250 ml/min. At the end of each cross circulation, the arterial and venous cannulae of each baboon were connected by a small piece of Teflon. Cross circulation was carried out for 6 hr daily on days 1 and 2 after TBI and for 2 hr daily on days 3-8. Cross circulation in baboon 66196 was terminated on day 5. Both cross-circulation partners were treated with the immunosuppressive drug methotrexate (MTX), 0.25 mg/kg body weight i.v. on days 1, 3, 5, and 7 after TBI in an attempt at delaying the onset of graft-versus-host disease (GVHD).^{19,20}

White blood cell (WBC) counts, platelet counts, and hematocrits were performed daily before cross circulation. Criteria for marrow engraftment were a spontaneous rise in WBC after the post-irradiation nadir and the presence of hemopoietic precursor cells in smears and sections of marrow from ribs, femoral head, and iliac crest. Gross and histologic signs of GVHD at autopsy^{21,22} were also evaluated.

RESULTS

Table 1 summarizes the results. The three baboons in group I that were not cross-circulated died on day 8 with pneumonia. All three developed a profound leukopenia by day 5 which persisted (Fig. 1 and Table 1). The last granulocyte counts before death were 15-35/cu mm, and none of the three baboons had reticulocytes. The platelet counts were 233,000-295,000/cu mm on the day of irradiation and declined to 3000-30,000/cu mm on the day of death without evidence of hemorrhage. Their marrows at autopsy were hypoplastic (Fig. 2) and failed to show myeloid or erythroid precursors or megakaryocytes (Table 1). Lymph node and spleen histology showed severe depletion of lymphocytes and sinus histiocytosis.

The five baboons that were cross-circulated with unirradiated partners survived between 8 and 13 days. Three of the five showed spontaneous rises in WBC before death to more than 1500/cu mm. The last granulocyte counts before death were 10-1200/cu mm, and reticulocyte levels were 0.2%-1.4% (Ta-

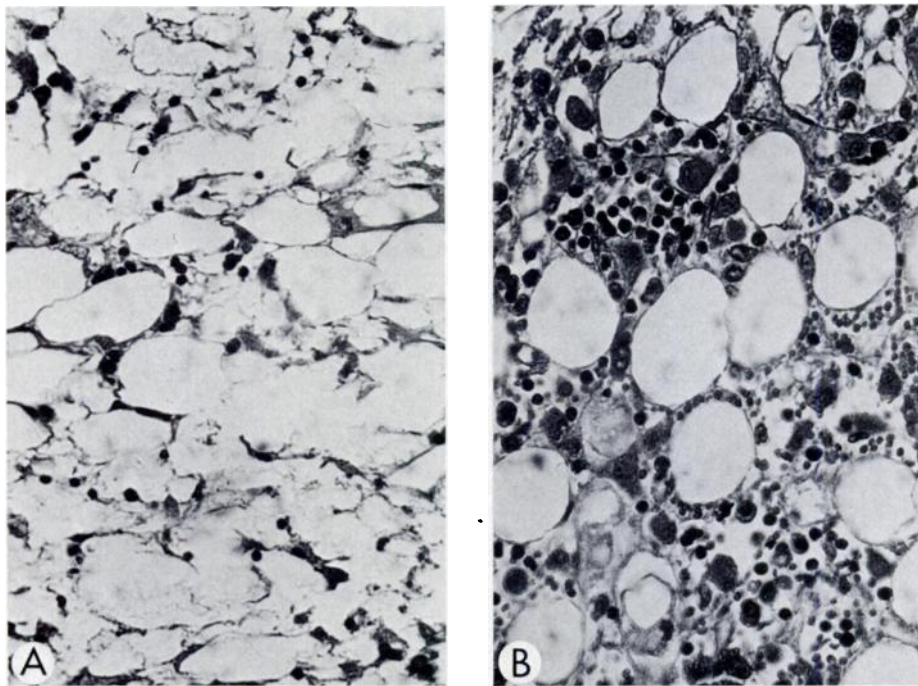


Fig. 2. Marrow histology at autopsy in two lethally irradiated baboons. (A) Baboon 6677 was not cross-circulated. (B) Baboon 66180 was cross-circulated with an unirradiated partner. Hematoxylin-eosin. $\times 250$.

ble 1). The platelet counts were 252,000–523,000/cu mm on the day of irradiation. They declined to 29,000–181,000/cu mm at the end of the cross-circulation period and were 1000–46,000/cu mm immediately before death.

The five baboons died after a brief period of anorexia and diarrhea with infection. All five showed clear evidence of regeneration of the hemopoietic parenchyma at autopsy (Table 1, Fig. 2). Baboons 66144, 66180, and 66268 had histologic evidence of GVHD in the liver. In baboon 66144, this evidence consisted of bile duct necrosis, hepatic cell necrosis, and portal infiltrates with mononuclear cells. Baboons 66180 and 66268 showed portal infiltrates and mild hepatocellular necrosis. All but baboon 66196 had changes consistent with GVHD of the gut. Baboons 66144 and 66181 had distinctive dilated atypical crypt abscesses similar to those described by de Vries²¹ and Lerner et al.²² Baboons 66180 and 66268 had mucosal denudation consistent with but not diagnostic of gut GVHD. Lymph nodes and spleens were generally depleted of lymphocytes, with only baboon 66144 showing residual germinal centers. All baboons showed sinus histiocytosis and all showed an immunoblastic reaction and plasmacytosis which ranged from minimal and focal (baboon 66181) to diffuse and moderate (baboon 66180).

DISCUSSION

This report has described evidence for the presence of circulating hemopoietic stem cells in the blood of baboons. Animals were given 1200 R TBI to provide

a setting in which spontaneous recovery of their own hemopoiesis was not likely to occur. It is therefore reasonable to assume that the regeneration of the hemopoietic parenchyma seen in all five irradiated and cross-circulated baboons was due to engraftment of stem cells derived from the blood of the unirradiated cross-circulation partner. Karyotype analyses of marrow cells from baboons with cross-circulation partners of the opposite sex, which would have provided further support for allogeneic engraftment, were not carried out. Whether the hemopoietic regeneration observed was due to engraftment of "pluripotent" or "committed" stem cells could not be determined with the present study. Long-term observations in dogs, however, have shown sustained engraftment of autologous and allogeneic cells for periods measured in months and even years, indicating that pluripotent stem cells were present among the infused peripheral blood leukocytes.^{4,23}

Despite the use of MTX, cross-circulated baboons with engraftment died within 14 days of TBI. Similar early death, presumably related to acute GVHD, has been seen in irradiated, cross-circulated dogs,¹⁹ dogs given WBC infusions from random donors,²⁴ and rhesus monkeys given marrow grafts from random donors.^{25,26} Whether acute GVHD in baboons can be modified by the use of sibling donors matched at the major histocompatibility complex, as is the case in dogs,²⁷ remains to be tested. It may also be possible to reduce the incidence and severity of GVHD by separating the hemopoietic stem cells from the immunologically active cells present in the peripheral blood.^{28,29}

Circulating pluripotent hemopoietic stem cells could be used instead of marrow or as a supplement to marrow for allogeneic hemopoietic transplantation. This possibility has been explored in dogs irradiated with 1200 R TBI. When these dogs were cross-circulated with unirradiated partners¹⁹ or given infusions of large numbers of peripheral WBC alone or in combination with marrow,^{20,23,24,27} consistent hemopoietic grafts were achieved.

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