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The Size of the Bone Marrow Erythropoietic Compartment in Splenectomized Mice

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The spleen has an important erythropoietic function in normal adult mice. Experiences were made to evaluate bone marrow erythropoiesis in splenectomized animals.

Data show that 120 days after splenectomy a mild anemia is still present and that boht the stem cell and the differentiated compartments are unable to expand. Exposure to chronic hypoxia does not change these results.

It is inferred that bone marrow does not increase its erythropoietic rate despite the absence of the spleen, and that the relationship between these two organs in regard to the control of red blood cells production differs from that observed in other mammals including man.

Spleen is an active erythropoietic organ in normal adult mice (2, 6, 7, 11), in contrast with other mammals, including man, that show splenic erythropoiesis only during their embryonic development (13, 24). Moreover, evidence has been presented which indicates that the spleen may function as the primary organ of erythropoietic homeostasis in mice (4, 12). A marked increase in splenic erythropoiesis occurs in mice once they are bled, made hypoxic or injected with erythropoietin (4), while bone marrow not only shows failure to participate in erythroid response to increased demands (6, 18) but seems also unable to attend ordinary requirements. Splenectomized animals show a moderate anemia and their response to erythropoietic stimulus is smaller than that observed in normal ones (2, 6).

It is commonly accepted the fact that physiological deficit created by injury or excision of tissues is followed by processes of hypertrophy and hyperplasia which tend to normalize the impaired function (1). This work attempts to establish if after ablation of the spleen, the erythropoietic bone marrow compartment is able to expand in order to compensate the lack of splenic erythropoiesis. Studies were made to evaluate both the stem cell and the differentiated compartments.

Materials and Methods

Mice. The mice were adult males of the BALB/ep strain (from Atomic Energy Commission, Argentina) fed a standard diet. Irradiated receptors were females. Each group of experimental animals con-

tained 8-10 individuals. Animals did not receive additional iron since in previous experiments the examination of peripheral blood and bone marrow smears (stained with Pearl's stain) revealed normal iron stores.

Splenectomy. Was performed under ether anesthesia through a flank incision. No special sterile precautions were taken. During the experiments the animals gained weight normally and never showed signs of infectious or any other disease. Sham splenectomy consisted of exteriorizing the spleen, replacing it and closing the wound.

Blood volume (BV) and red cell volume (RCV). Was measured by the Fe^{59} labeled red cells dilution technique (10). Labeled erythrocytes were obtained from donor mice injected with 2 μc Fe^{59} and bled 7 days later. An amount of 0.2 ml of those packed cells previously washed three times with saline was injected intravenously into the experimental animals. They were bled out via cardiac puncture 30 minutes later. BV was calculated according to the usual formulae (10), and RCV obtained by means of BV and hematocrit data. The results are expressed as per cent of body weight.

Hypoxia. Animals were exposed for 26 consecutive days to a simulated altitude of 6,500 m, 12 hours a day. Such schedule is able to produce and maintain a polycythemic status. Decompression was obtained in a chamber similar to that described by WRIGHT (26).

Radioiron distribution studies. Individual mice received via the tail vein 0.2 μc of Fe^{59} as ferric citrate in a volume of 0.2 ml saline. Three hours later the animals were anesthetized, bled as much as possible via cardiac puncture, and eviscerated. Spleen was removed, washed with saline, radioiron incorporation measured and smears made immediately. Both femurs were removed, cleaned from soft tissues, radioactivity measured, then opened and a portion of the marrow flushed out and smeared. The three hours interval was determined in previous experiences as the maximal uptake for both bone marrow and spleen. Total bone marrow uptake was calculated according to the formulae: Fe^{59} uptake in both femurs $\times$ 9 (3). Radioactivity was measured in individual specimens a well type scintillation counter and expressed as percentage of the injected dose.

Differential counts. Were made from smears stained with May Grünwald-Giemsa. One to three thousand cells were classified by the usual morphologic criteria and the percentage of erythroblasts determined. Hematocrits were done by a micromethod.

Stem cell compartment studies. The erythrocytic progeny of hematopoietic stem cells was measured by the method described by HODGSON (15). To obtain irradiated receptors, radiation was generated by a 250 kV Siemens Stabilipan machine operating at 20 mA and 200 kV. A 0.5 mm copper filter was used with 2.0 mm of aluminium, and the exposures were measured in air with a Philips universal dosimeter at the position occupied by the center of the animal's body. The dose-rate averaged 37.2 R per min at 70 cm. Total exposure time to the X-ray beam was 22 min and 48 sec: hence a total of 850 R was delivered to the entire body. Bone marrow and splenic suspensions were prepared as described by KUBANEK *et al.* (18) using Hank's solution as a diluent. After cells were counted by usual hemacytometric techniques, suspensions were adjusted to the desired cell concentration with the same medium. Cells were injected intravenously within three hours after irradiation, and 0.2 μc Fe^{59} were adminstered 7 days after. Blood (0.2 ml) was drawn from the heart 24

hours after tracer injection, and radioactivity measured. The percentage uptake of radioiron in erythrocytes was calculated assuming the blood volume of the mice to be 7 ml/100 g. Evaluation of the relative number of stem cells present in a cell suspension can be made indirectly by measuring the Fe^{59} uptake by erythroid cells derived from them.

Statistical analysis. Results were analysed for statistical significance using the Student *t* test.

Results

BV and RCV. Table I shows data corresponding to mice of different ages at the moment of splenectomy. It can be seen that a mild anemia develops which does not recuperate after 120 days. Furthermore, the age of the mice at the moment of the ablation of the spleen does not influence the recovery of the bone marrow.

Differentiated compartment. Radioiron uptake and percent erythroid cells in bone marrow at different times after splenectomy are detailed in Table II. Results does not show any difference after 120 days. Chronic hypoxia, able to increase BV as judged by the hematocrit value, does not increase the bone marrow erythropoietic activity. In all cases iron was injected 4 hours after cessation of hypoxia.

Table I. *Blood volume (BV, red cell volume (RCV) and hematocrit values in normal and splenectomized mice.*
Values are means±Standard deviation.

Group	Hematocrit	BV	RCV	% Decrease in RCV
Control	50.1±0.2	7.3±0.5	3.6±0.2	—
Splenectomized (Age 1 week) 120 days after splenectomy	44.4±0.4	5.7±0.2	2.6±0.1 *	27.8
Splenectomized (Age 4 weeks) 120 days after splenectomy	48.2±0.2	5.7±0.1	2.7±0.05 *	24.9
Splenectomized (Age 10 weeks) 40 days after splenectomy	48.2±0.4	6.3±0.4	3.0±0.2 *	16.7
Splenectomized (Age 10 weeks) 120 days after splenectomy	48.4±0.4	6.3±0.2	3.0±0.1 *	16.7

* $P < 0.02$ when compared with controls.

Table II. *Radioiron uptake and percent erythroblasts in normal and splenectomized mice.*
Values are means±Standard deviation.

Group	Hematocrit	% Fe uptake spleen	% Fe uptake bone marrow	% Erithroblasts bone marrow
Control	50.1±0.2	12.8±1.2	25.0±2.9	22.2±2.4
40 days after splenectomy	48.2±0.4	—	26.8±2.6	24.7±2.2
120 days after splenectomy	48.4±0.4	—	27.3±1.7	25.7±2.7
120 days after splenectomy+hypoxia	60.7±1.4	—	29.8±2.4	29.5±1.4

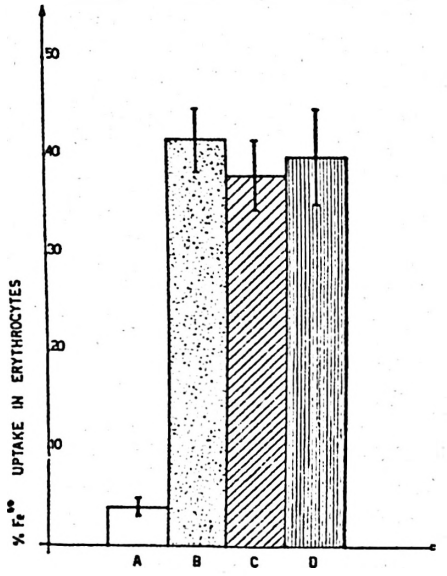

Fig. 1. *Effect of splenectomy on the stem cell compartment of the bone marrow in adult mice.*
A: Controls (Hank's solution); B: Normal bone marrow; C: 60 days after splenectomy; D: 120 days after splenectomy. Each mice was injected with 0.36×10^5 bone marrow cells. Vertical bars delineate ± 1 Standard Deviation.

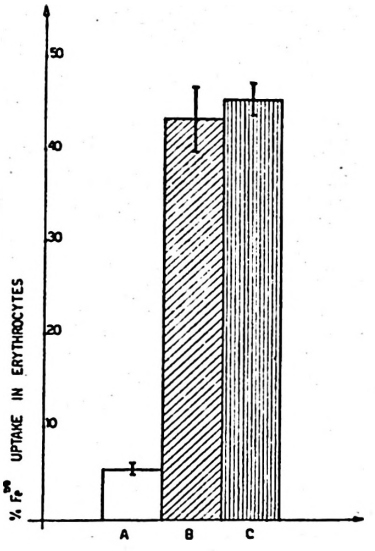

Fig. 2. *Effect of splenectomy and chronic hypoxia on the stem cell compartment of the bone marrow in adult mice.*
Animals were sacrificed 4 days after cessation of hypoxia. A: Controls (Hank's solution); B: Normal bone marrow; C: 120 days after splenectomy + hypoxia. Each mice was injected with 0.30×10^5 bone marrow cells. Vertical bars delineate ± 1 Standard Deviation.

Stem cell compartment. Figue 1 shows results obtained with bone marrow suspensions from mice at different times after splenectomy. Figure 2 details data obtained with bone marrow suspensions from mice subjected to chronic hypoxia (4 days after cessation of the stimulus) compared with normal controls. Absence of expansion is also observed in this compartment in all cases.

Discussion

This work deals with the evaluation of the size of the bone marrow erythropoietic compartment after splenectomy. Since it is generally accepted that erythropoiesis involves the constant entry of undifferentiated stem cells into the pathway for erythroblastic differentiation (23), it seems reasonable to look for the size of both compartments, the undifferentiated one and the so called madurative-proliferative, which is composed by the semimature erythroid cells.

The results obtained in this investigation agree with data available in the open literature in that splenectomy produces a mild anemia which is apparent at least 120 days after splenectomy. The moment when ablation is performed does not change these results, suggesting that the distribution of the erythropoietic tissue does not depend on the presence of the spleen in that period of the development of hematopoiesis in which erythropoiesis is partially shifted from the spleen to the bone marrow (3).

The erythropoietic rate of the bone marrow does not appear to be affected by splenectomy. This functional relationship between bone marrow and spleen in regard to erythropoiesis is quite different from that observed in other mammals,

where it is generally accepted that spleen may exert an inhibitory effect upon bone marrow activity (9). After splenectomy in normal individuals there is no anemia and moderate polycythemia may develop (25). Moreover, hypersplenism is manifested by a lack of circulating blood cells including erythrocytes (8).

Evidence is given for the hypothesis that a prolonged hypoxic stimulation may increase the size of the hematopoietic stem cell compartment (16, 21, 22). This compartment must continually expand to meet the depopulation of its number as a consequence of their differentiation. When the stimulating factor is removed, the relative size of the precursor population continues to increase for a period of 3-6 days. In such conditions, mice bone marrow does not show any capacity of expansion, showing unability to grow despite the increased demands maintained for a prolonged period of time. In other mammals, including man, splenic erythropoiesis is confined to the fetus and bone marrow is able to increase its production several times if neccesary (14, 17). The slight response observed in splenectomized mice seems to be attributable to extramedullary erythropoietic foci (6).

The reason for the failure of the bone marrow to participate to a greater extent in mice erythropoiesis remains unanswered (18). It has been suggested (5, 11) that, as there is no place to accomodate more differentiated erythroid cells, the bone marrow in mice produces erythrocytes at its maximal rate, and in presence of an increased demand it becomes neccesary the assistance of the spleen. Moreover, an increase in myelopoiesis causes a «crowding out» of erythroblasts from the bone marrow to the spleen (11).

The importance of the microenvironment for effective bone marrow function is well established: hemopoietic stem cells must be provided with sufficient concentrations of local factors essential to them to assume a hemopoietic role (20), thus an adequate blood flow is obviously a prerequisite for cellular proliferation (19).

Perhaps the erytropoietic behaviour of mice bone marrow is regulated through aspects of cellular environment which are consequence of its microcirculation.

Resumen

El bazo tiene una importante actividad eritropoyética en el ratón adulto normal. Se efectuaron experiencias tendientes a evaluar la producción medular de hematíes en animales esplenectomizados.

Se pudo comprobar que 120 días después de efectuada la operación persiste una anemia moderada y que tanto el compartimiento de células indiferenciadas como el formado por células eritroides inmaduras no han modificado su capacidad funcional. La exposición a hipoxia prolongada no modifica estos resultados.

Se concluye que la medula en esta especie no incrementa la producción de hematíes a pesar de la ausencia del bazo, y que la relación funcional que existe entre ambos órganos con respecto a la eritropoyesis difiere de la que se observa en otros mamíferos entre los que se incluye el hombre.

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