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The Production of Megakaryocytic Macrocytosis by Systemic Factors in S1/S1^d Mice[1] (39782)

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Introduction. The megakaryocytes of S1/S1^d mice are reduced in number and macrocytic (1). The platelet counts of these mice are normal (1), so macromegakaryocytosis cannot be attributed to feedback stimulation from peripheral thrombocytopenia (2). S1/S1^d mice have an hereditary anemia that appears to be due to abnormality of the stromal elements of hematopoietic tissues (3, 4). The present experiments, involving cross transplantation of bone marrow fragments between S1/S1^d mice and their normal +/+ littermates, were done to test the hypothesis that the macromegakaryocytosis of S1/S1^d mice was the consequence of a direct local effect of the abnormal stroma on cells of the megakaryocytic system.

When marrow tissue is transplanted to sites that are normally nonhematopoietic, necrosis, vascularization, and regeneration of hematopoiesis occur sequentially (5). It is thought that regeneration of essential stromal elements occurs from cells within the implant, because regeneration may be prevented by exposure of the tissue to cytotoxic agents before implantation (6, 7). Hematopoietic cells that populate the graft have been shown to derive totally or in part from stem cells that migrate into the graft (7-10).

Materials and Methods. Female mice of the S1/S1^d strain and their homozygous +/+ (normal) littermates were purchased from the Jackson Laboratories, Bar Harbor, Maine, at about 5 weeks of age. They were fed Charles River rat, mouse, hamster formula, and drinking water was supplemented with neomycin and modified Dakin's solution. Experiments were begun when mice were 12-14 weeks old.

Donor mice were killed by cervical dislocation after cardiac puncture under ether anesthesia. Femurs were removed and prepared for implantation by a modification of the technique that Dorr *et al.* used for tibiae (11). Both ends of the femurs were shaved off with a razor blade and about one-third of the bone cortex was removed longitudinally, so that marrow tissue was exposed. Femurs thus prepared were implanted subcutaneously in the abdominal wall (12) of normal and S1/S1^d recipient mice that had been anesthetized with Nembutal given intraperitoneally. Each recipient was implanted with two femurs from one S1/S1^d donor on one side of the abdomen and with two femurs from one +/+ donor on the other side of the abdomen. Six weeks after implanation, recipient mice were killed and the tissues were studied.

Blood counts were done on cardiac blood anticoagulated with dry K_2EDTA. Platelet counts were done by phase microscopy (13), hematocrits with a Drummond microhematocrit machine, and red cell counts by Coulter counter.

Megakaryocyte sizes were estimated from bone marrow smears. Smears were made from one humerus from each donor, one femur from each recipient, and one of the two regenerated implants from each donor. The photomicrographic technique has been described (14), and cell size (area) was arbitrarily designated as planimeter units. Megakaryocyte morphology was evaluated as described (15). Size determinations were made on stage III, mature megakaryocytes with fully granular cytoplasm and not on less mature cells. Megakaryocytes normally increase in size with maturation, and since their age distribution may have differed in the different tissues studied, only one maturation stage was evaluated.

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The second donor humerus, recipient femur, and implant from each donor were fixed in formalin, decalcified, sectioned longitudinally, and stained with hematoxylin and eosin for microscopic evaluation of cellularity. Cellularity was scored from 0 to 1; a score of 1 was the estimated cellularity of the humeral marrow from the +/+ donor for each recipient.

Results. The sizes of megakaryocytes in donors, hosts, and regenerated implants are shown in Figs. 1 and 2. Increased size of S1/S1^d megakaryocytes was confirmed in marrow from donors and hosts. The ratio of size for S1/S1^d: +/+ megakaryocytes for donors and hosts was 1.6–1.8, values comparable to the 1.6 previously reported (1). The size of megakaryocytes in the implants was the same as that in the host animals regardless of the genotype of the donor.

The relative cellularity of marrow growing *in situ* in donors and hosts and in implants is shown in Table I. Humeral marrow from donors of both genotypes was more cellular that the respective femoral marrow from hosts, because femoral marrow tended to be fatty at both ends. S1/S1^d marrow *in situ* was judged to be 75–80% as cellular as +/+ marrow; reduction in cellularity in S1/S1^d mice was associated with an increase in the proportion of the marrow sections that was occupied by sinusoids. By comparison

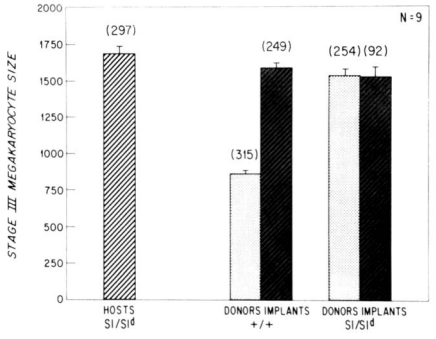

FIG. 1. Megakaryocyte size is expressed in planimeter units on the ordinate for cells from bone marrow of S1/S1^d hosts, their +/+ and S1/S1^d implants, and the donors of the implants. In the double columns, the dotted bars represent megakaryocyte size in donor marrow; the striped bars show size after regeneration of implants of marrow from the same donors. Each value is the average (±SEM) for the number of megakaryocytes shown in parentheses from nine mice.

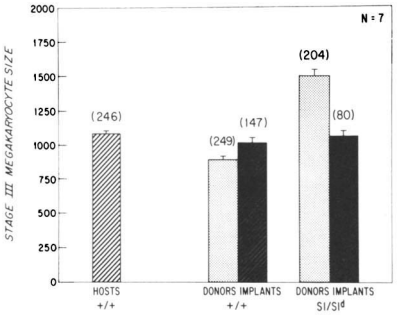

FIG. 2. Megakaryocyte size is expressed in planimeter units on the ordinate for cells from bone marrow of +/+ hosts, their +/+ and S1/S1^d implants, and the donors of the implants. In the double columns, the dotted bars represent megakaryocyte size in donor marrow; the striped bars show size after regeneration of implants of marrow from the same donors. Each value is the average (±SEM) for the number of megakaryocytes shown in parentheses from seven mice.

TABLE I. RELATIVE CELLULARITY OF BONE MARROW SECTIONS.

Bone	*N*	Score
+/+		
Donor humerus	15	1.00 ± 0[a]
Host femur	7	0.84 ± 0.04
Implant in +/+ host	6	0.47 ± 0.06
Implant in S1/S1^d host	9	0.91 ± 0.03
S1/S1[a]		
Donor humerus	16	0.82 ± 0.02
Host femur	9	0.59 ± 0.03
Implant in +/+ host	7	0.33 ± 0.08
Implant in S1/S1^d host	9	0.40 ± 0.06

[a] Average ± SEM.

to genotypically similar marrow growing *in situ*, S1/S1^d marrow implants appeared to regenerate less well than +/+. This was especially pronounced in S1/S1^d hosts where +/+ implants were normally cellular and S1/S1^d implants were only half as cellular as donor humeri.

Blood platelet counts, hematocrits, and red cell MCV for donor and host mice of both genotypes are shown in Table II. S1/S1^d mice had macrocytic anemia, and their platelet counts were not different from those of +/+ mice. The hematocrits of S1/S1^d hosts were slightly, but significantly ($P < 0.01$), higher than those of donors.

Discussion. It was found that the size of megakaryocytes in bone marrow implants reflected the genotype of the host animal rather than that of the donor. From this

TABLE II. BLOOD COUNTS OF DONOR AND HOST MICE.

Parameter measured	S1/S1^d		+/+	
	Donors	Hosts	Donors	Hosts
Platelets/mm^3 ($\times 10^6$)	1.516 ± 0.062 (16)[a]	1.508 ± 0.086 (9)	1.449 ± 0.033 (15)	1.458 ± 0.074 (8)
Hematocrit	29 ± 0.8 (15)	32 ± 0.7 (9)	47 ± 0.4 (15)	46 ± 0.6 (8)
MCV	75 ± 3.2 (15)	72 ± 0.7 (9)	45 ± 0.7 (15)	45 ± 0.8 (8)

[a] Each value is the average ± SEM for the number of mice shown in parentheses.

observation it could be concluded that megakaryocytic macrocytosis in S1/S1^d mice did not totally result from direct interaction between megakaryocytes or their precursors and an abnormal stromal component which regenerated from cells of donor origin in the implant.

Macromegakaryocytosis is a characteristic of the response to feedback stimulation of platelet production in the presence of experimentally induced thrombocytopenia (2). Thus it could be postulated that chronic stimulation of megakaryocytopoiesis occurred in S1/S1^d mice by a humoral mechanism similar to that which may be generated in the presence of thrombocytopenia. In the absence of thrombocytopenia, however, it would be necessary to also make additional postulates. S1/S1^d platelets may be intrinsically defective in a function that is monitored in the regulation of the level of a thrombopoietin. Alternatively, the demand for platelets could be increased by the hereditary tissue defect which is known not to be limited to hematopoietic tissues (16). However, a difference from the thrombocytopenic stimulation of megakaryocytopoiesis is shown by the absence of macrocytic platelets in the blood of S1/S1^d mice (1, 17). DNA content of the macromegakaryocytes in S1/S1^d mice has not been measured, so it is not known whether their ploidy is increased as it is in stimulated megakaryocytes (18).

The findings do not exclude the possibility that an abnormal stromal element may be locally responsible for the large size of megakaryocytes both *in situ* and in marrow implants in S1/S1^d mice. The sinusoids are generally considered to be stromal, but, during the regeneration of transplanted marrow fragments, capillary penetration from surrounding tissue occurs (5). Host vascular cells may then populate the sinusoids in the regenerating graft, and the close physical relationship between megakaryocytes and vascular walls (19) might indicate that there are direct interactions between megakaryocytes and vascular cells. Essential stromal cells might also be migratory, as are the hematopoietic stem cells, so host participation in implant regeneration could be proposed to occur by influx of a stromal precursor. Others (7, 20) have reported that implants of S1/S1^d marrow have less erythropoietic activity than implants of normal marrow, and the substantially greater cellularity of +/+ implants in S1/S1^d hosts was confirmatory. These findings indicate that elements derived from the donor influenced hematopoiesis even though host factors determined the final size of megakaryocytes.

S1/S1^d marrow *in situ* was judged microscopically to be about 75–80% as cellular as +/+ and to have more sinusoids. Their splenic tissue has also been reported to have an increased number of sinusoids (21). Counts of tibial marrow suspensions showed a greater deficit of cells in S1/S1^d mice (1) suggesting that factors other than cell density within the marrow may have contributed to the reduced numbers of hematopoietic cells. For example, the W/W^v mouse, with an hereditary anemia of stem cell origin, has also been reported to have a reduced volume of its medullary cavity. (22)

The anemia of S1/S1^d mice is improved by implantation of normal splenic tissue. (23) The presence of +/+ marrow implants may have accounted for the slightly higher hematocrits in S1/S1^d hosts than in donors.

The results of these experiments indicate that the regulation of megakaryocytopoiesis in the S1/S1^d mouse is abnormal; the abnormality may be different from the stromal defect that affects erythropoiesis. Identification of the humoral or cellular factor that induces macromegakaryocytosis in these

mice may partially clarify normal homeostatic mechanisms.

Summary. Bone marrow tissue was cross-transplanted subcutaneously between S1/S1^d mice and their normal +/+ littermates. Megakaryocyte size was evaluated in bone marrow from donors, hosts, and regenerated implants. Macromegakaryocytosis was found in S1/S1^d marrow *in situ* and in all implants in S1/S1^d hosts. Megakaryocytes were of normal size in +/+ marrow and in all implants in +/+ hosts. The macrocytosis of megakaryocytes in S1/S1^d mice appeared to be due to systemic factors or host elements that were incorporated into the regenerated implants rather than to a stromal abnormality that persisted with graft regeneration after transplantation.

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