The origin of hemopoietic cells in ectopic implants of spleen and marrow[1]

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Summary. Spleen and marrow tissues were cross-transplanted between CBA and CBA/HT6 mice. The majority of karyotypes in the regenerated implants were of recipient origin indicating that these implants are chimeric structures with the stroma of donor origin providing a framework for the proliferation of the recipient's hemopoietic stem cell.

The understanding of the nature of hemopoiesis has been advanced by the recognition that hemopoietic tissues are made of 2 components: proliferating hemopoietic cells and the supporting stroma which provides a suitable milieu conducive to the proliferation of hemopoietic cells[2,3].

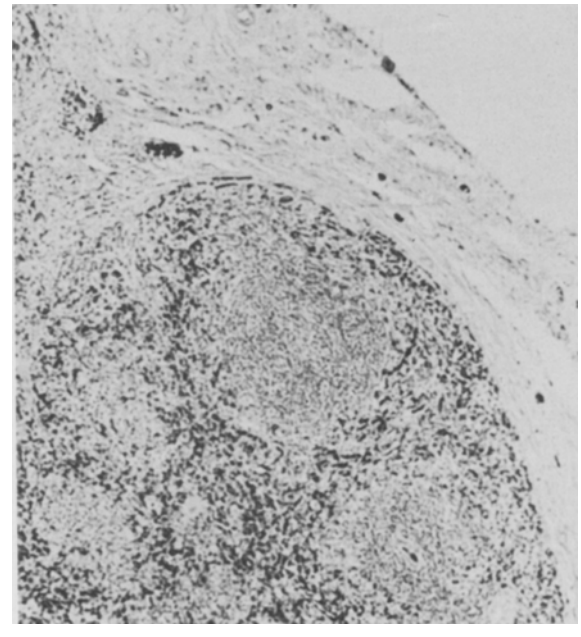
When bits of hemopoietic organs (spleen, bone marrow) are transplanted autologously or isologously in ectopic sites, they regenerate into new organs. During this regenerative process, the supporting stroma is first reconstituted and is then repopulated by proliferating hemopoietic cells[4,5]. Experimental evidence indicates that the stroma of these regenerated organs are of donor origin[6–8]. We now provide direct evidence, from chromosome studies, for the recipient origin of hemopoietic cells in spleen and bone marrow tissues cross-transplanted in mice.

Materials and methods. Female mice of CBA and CBA/HT6 strain were used. Animals were splenectomized and wedge-shaped pieces of the tissue were implanted into a pocket in the s.c. tissue of the abdomen as previously described[5,8]. For marrow tissue implantation, both femurs were removed and both their ends were broken. A large needle, attached to an air-containing syringe, was inserted into one end of the tubular bone and the marrow tissue was forced out into a pocket incised into the s.c. tissue of the abdomen, as previously described[8]. All implants were cross-transplants so that the T6 chromosome marker could be employed for identification of hemopoietic cells.

Some implants were removed sequentially at daily intervals, and formalin-fixed, paraffin-embedded sections were studied histologically. For chromosome studies 35-day implants were used. Animals were given 0.5 ml of 0.25% aqueous colchicine i.p. 90 min at 37 °C before study. Implants were then removed, minced in 1.12% sodium citrate buffer and incubated for 15 min. Chromosome preparations were made according to the method of Bunker[9].

Results and discussion. The pattern of spleen regeneration in mice was similar to what has been described in rats[5]. Almost the entire implant became necrotic for lack of circulation except for a thin peripheral shell wherein a circulation was established from the adjacent s.c. tissue. This shell served as a regenerative zone growing inward and reconstituting the structure of splenic red pulp. This was followed by its repopulation with proliferating hemopoietic cells with a proponderance of erythroid elements. Repopulation by lymphocytes, predominantly in the periarterial zones, followed and the structure of white pulp gradually became evident (figure). The process was completed within 10–15 days.

The pattern of marrow regeneration was also similar to what has been described in other species[4]. Within 24 h an intense circulation was established in the implant. This rapid establishment of a circulation is the result of considerable potential of the marrow tissue (in contrast to spleen or liver) to stimulate endothelial proliferation and angiogenesis in the surrounding tissue[10,11]. The newly established circulation carried most hempoietic cells present within the tissue, so that after 24 h only a meshwork of marrow stroma was discernible. The hemopoietic stroma began to proliferate and partly differentiated into osteoblasts leading to the laydown of osteoid tissue within the interstices of which the organization of perimordial marrow cavity appeared and was repopulated by hemopoietic cells. This was associated



Reconstituted splenic implant. The surrounding s.c. connective tissue is seen on the top, forming a thick capsule for the implant. The white pulp is seen as multiple nodules. In the interstices of these nodules, the red pulp contains hemopoietic cells which appear as dark-staining cells. HE × 150.

Origin of cells populating the regenerated, cross-transplanted spleen and bone marrow

Implanted tissue	Donor animal	Recipient animal	No. of metaphase plates studied	No. of recipient markers	Percent of recipient cells
Spleen	CBA	CBA/T6	45	42	86
	CBA/T6	CBA	39	38	97
		Total	84	80	95
Bone marrow	CBA	CBA/T6	14	14	100
	CBA/T6	CBA	10	8	80
		Total	22	24	91

with bone resorption in the central part of the implant, so that the well-established implant, by day 15–20, consisted of a hemopoetic nodules surrounded by a thin shell of bone.

The results of chromosome studies are shown in the table and indicate that 5 weeks after implantation the majority of cell karyotypes found in implants of both marrow and spleen tissues were of recipient origin. Because both the proportion and the turnover rate of cells in hemopoietic stroma is rather low, in contrast to that of hemopoietic cells[12], one may make the reasonable assumption that these karyotypes were derived from proliferating hemopoietic cells. Furthermore the donor origin of stromal cells in similar systems, has been established by using radiation studies[13,14] or chromosome marker[6,15]. Hence, the cells of the recipient origin identified in these implants must have been different from stromal cells and were probably proliferating hemopoietic cells.

These results are in agreement with those in several other experimental systems[8,16-18], and are consistent with the view that the hemopoietic stem cell circulates but it is selectively trapped by hemopoietic stroma which provides it with a suitable milieu for proliferation and differentiation. Therefore, ectopic implants of spleen and marrow tissue may be considered as chimeric structures with the stroma of donor origin providing a framework upon which the hemopoietic stem cell of recipient origin can proliferate and differentiate[19].

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Plasma cyclic 3′,5′-guanosine monophosphate and cyclic 3′,5′-adenosine monophosphate response to methacholine in man

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Summary. Cholinergic agents are known to induce increases in tissue and plasma levels of cyclic GMP in experimental animals. We observed that i.m. injection of methacholine, a cholinergic agent, caused significant increases in plasma cyclic GMP and cyclic AMP in man.

George et al.[1] found that acetylcholine caused an increase in the level of cyclic 3′,5′-guanosine monophosphate (cyclic GMP), accompanied by no change or a slight decrease in cyclic 3′,5′-adenosine monophosphate (cyclic AMP), in the isolated rat heart. Several reports have been made of similar observations in vitro and in vivo[2-4]. Honma and Ui[5] reported that s.c. injection of cholinergic agents such as carbachol, methacholine, and bethanechol in rats caused a significant increase in plasma cyclic GMP with a slight increase in plasma cyclic AMP. Therefore the purpose of the present study is to extend from rat to man the observation that methacholine increases plasma cyclic GMP and cyclic AMP.

14 healthy males in excellent physical condition between the ages 18 and 42 were selected as subjects for this study. After overnight fasting and in a resting state in the supine position, all subjects received an i.m. injection of 10 mg of methacholine. Blood samples for the determination of plasma cyclic GMP and cyclic AMP were obtained via an indwelling i.v. cannula before and after the injection of methacholine at 2-min intervals for a period of 26 min. The concentrations of plasma cyclic GMP and cyclic AMP were measured by the ultrasensitive radioimmunoassay method described in a previous publication[6]. Statistical significance was determined by means of Student's t-test.

In this study in 14 normal subjects, the mean basal level of plasma cyclic GMP was 5.2 ± 0.3 pmoles/ml (mean $\pm$ SE) and the significant increases were observed between 4 min and 26 min with a peak at 12 min after the injection of methacholine. The mean basal level of plasma cyclic AMP was 20.6 ± 1.1 pmoles/ml (mean $\pm$ SE). Not only plasma cyclic GMP but also plasma cyclic AMP increased in response to methacholine and reached a peak at 12 min. After the injection of methacholine all the subjects had the symptoms referable to cholinergic stimulation such as facial flushing, salivation, sweating, palpitation, cough and a desire to urinate. The increase in plasma cyclic GMP was greater than the increase in plasma cyclic AMP, and the plasma cyclic GMP was still elevated at 26 min after the injection of methacholine, while the plasma cyclic AMP almost declined to basal level at 26 min after the injection of methacholine.

The data for the basal levels of plasma cyclic GMP and cyclic AMP in man in this study are similar to those obtained by Steiner et al.[7] using a radioimmunoassay technique.