ABSENCE OF STIMULATION OF COLONY-FORMING ACTIVITY OF SPLENIC HEMATOPOIETIC STEM CELLS BY T LYMPHOCYTES

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Much experimental evidence has recently been obtained that migration, proliferation, and differentiation of polypotent hematopoietic stem cells (PHSC) of the bone marrow are under thymic control [2, 5, 13]. Nevertheless, testing the effect of interaction between PHSC and T lymphocytes in a syngeneic system did not show an increase in the number of colonies in the recipients' spleens [11], although this does not rule out the possibility of interaction demonstrable in a syngeneic combination of thymus and irradiated bone marrow cells [4] or bone marrow treated with anti-θ-serum [3], and also with rabbit antiserum against mouse brain (RAMBS) [11]. These results suggest that thymus cells interact with a small population of colony-forming cells and evidently increase their proliferative activity, as a result of which the number of CFUs in the recipients' spleens may remain unchanged, whereas the number of PHSC within the colonies increase [1, 6]. This can be demonstrated in a model using an intermediate recipient, by transferring spleen cells into secondary recipients.

The object of this investigation was to compare the sensitivity of PHSC from bone marrow and spleen to the stimulating action of T cells.

EXPERIMENTAL METHOD

Experiments were carried out on C57BL/6 mice and on (CBA × C57BL)F_1 hybrids obtained from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR. Primary lethally irradiated recipients (R_1) were injected with thymus cells (4×10^7) together with bone marrow (10^6) or spleen (2×10^7) cells in optimal proportions [1]. On the 8th day after transplantation spleen cells from R_1 in a dose of 0.2×10^6 were transplanted into secondary recipients (R_2). The number of CFUs, determined by the method of cloning in the R_2 spleen on the 9th day [12], reflected the character of interaction between the test cell populations.

The method of estimation of PHSC by the number of exogenous colonies in the spleen [12] also was used to assess the effect of interaction of bone marrow or spleen cells with thymocytes in a semiallogeneic parent—hybrid system.

EXPERIMENTAL RESULTS

Table 1 gives the results of a study of interaction between thymocytes and PHSC from spleen and bone marrow in a syngeneic combination, with the use of an intermediate recipient. As Table 1 shows, thymus cells stimulated the colony-forming activity of the bone marrow PHSC, for both the relative and absolute numbers of exogenous CFU in spleens of R_1 mice into which bone marrow cells were transplanted together with thymocytes were 20% greater than the corresponding values for R_1 mice, which received bone marrow cells only. Meanwhile, transplantation of a combination of spleen and thymus cells not only did not increase the number of exogenous CFU, but reduced it almost by half.

The absence of a stimulating effect of the thymocytes on the colony-forming activity of PHSC also was found in a semiallogeneic system. As Fig. 1 shows, transplantation of a combination of different doses of thymus cells from mice of the parental (C57BL/6) genotype with syngeneic bone marrow cells into first generation hybrids (F_1) increased the number of

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with Splenic (S) and Bone Marrow (BM) Hematopoietic Stem Cells of Donor (CBA × C57BL)F_1 Mice in Spleen of Primary Syngeneic Recipients (M ± m)

Donors' cells injected	Number of nucleated cells in spleen of primary recipients, $\cdot 10^6$	Number of CFU in spleen of primary recipients	
		relative ($\cdot 0{,}2 \cdot 10^6$)	absolute
BM	40,6±5,5	17,8±0,8	3618,2±164,4
BM*+T	42,5±3,9	26,1±2,2	5582±183,2
S	46,3±3,9	14,0±1,1	3241±193,7
S+T	46,9±8,5	8,2±0,3	1922,9±118,2

*BM cells in a dose of 1×10^6, S cells in a dose of 20×10^6, and T cells in a dose of 40×10^6 were injected into primary lethally irradiated recipients.

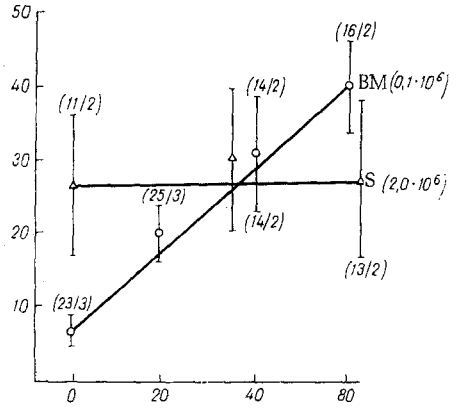

Fig. 1. Effect of thymus cells of C57BL mice on number of exogenous splenic colonies formed by bone marrow (BM) or splenic (S) hematopoietic stem cells of syngeneic animals when transplanted together into (CBA × C57BL)F_1 recipients. Abscissa, dose of thymus cells ($\times 10^6$); ordinate, number of exogenous colonies of hematopoietic cells in the spleen. Numerator — number of animals, denominator — number of experiments.

colonies of hematopoietic cells in the hybrids' spleen by 3, 5, and 6 times for doses of thymus cells of 20×10^6, 40×10^6, and 80×10^6 respectively. However, none of the above doses of thymocytes led to an increase in the number of CFU when they were transplanted together with spleen cells of the parental genotype. A difference in principle was thus found between PHSC of bone marrow and spleen, with respect to the regulatory role of T cells for hematopoietic stem cells.

Difficulties in the study of mechanisms controlling the proliferative and differentiating activity of PHSC are due to the extreme heterogeneity of the stem cell pool, which includes both the least mature precursors, with the greatest ability of self-maintenance, and also their progenies, which differ in various functional characteristics but which are not committed in the generally accepted meaning of the term, i.e., they are unipotent. It has been shown, for example, that when bone marrow cells are fractionated in a BSA gradient, not all CFU-containing fractions, but only CFUs with a density of more than 1.073 g/cm^3 are stimulated by the addition of thymus cells [11], further evidence of a definite stage of differentiation and (or) a definite phase of the cell cycle of PHSC interacting with T cells. Splenic PHSC are known to differ from bone marrow PHSC in a number of functional characteristics [7, 8, 13]

and they are probably a more mature population of stem cells than the bone marrow PHSC, of which they are the progenies [9]. The present investigation showed that the colony-forming ability of splenic PHSC, unlike that of bone marrow PHSC, does not increase during interaction with thymus cells. Since the increase in colony formation is not in this case the result of increased migration of CFU into the spleen, for in that case an increase in the number of exogenous colonies would be expected in R_1 spleens but not in R_2, it can be tentatively suggested that the effect of thymocytes is linked with their action on proliferative activity of PSHC. Probably in the course of their ontogeny hematopoietic stem cells lose their ability to respond to the proliferative stimulus arising from T cells. It may be that the higher proliferative activity of splenic than of bone marrow PHSC is an obstacle to their interaction with T cells.

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PROPERTIES OF BONE TISSUE INDUCED BY TRANSITIONAL EPITHELIUM

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The transitional epithelium of the urinary tract possesses marked osteogenic activity: in adult mammals bone tissue is formed around grafts of this tissue and in the pelvis of the kidneys after ligation of its blood vessels, and ectopic bone marrow organs appear [2]. If the epithelium is transplanted in diffusion chambers bone is formed on the outer surface of the millipore filters, direct proof that it is formed by inducible osteogenic precursor cells (IOPC) of the recipient [1, 4].

Bone is formed in guinea pigs after both autografting and homografting of epithelium. In the first case, however, it persists for many months, but in the second case only for a few weeks. It has accordingly been postulated that transitional epithelium evokes the formation of a nonself-supporting, inductor-dependent bone which exists only while the inductor continues to act [3, 4]. The aim of this investigation was to test whether the cause of resorption of ectopic bone tissue is in fact immunologic rejection of the allogeneic epithelium.

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