AGE-RELATED CHANGES IN THE CONTENT OF CLONOGENIC STROMAL PRECURSOR CELLS IN THE HEMATOPOIETIC ORGANS OF GUINEA PIGS

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The population of stromal precursors in the femoral marrow, spleen, and thymus was determined by monolayer culture of hematopoietic and lymphoid tissue in guinea pigs of different ages. The number of clonogenic stromal precursor cells was found to depend on the animals' age. It reached a maximum in the femoral marrow and spleen after 2 months, thereafter it remained unchanged with age in the spleen, but decreased significantly in the bone marrow. In the period of active function of the thymus the population of its stromal precursors remained relatively constant, but in old guinea pigs no stromal precursors were present.

KEY WORDS: hematopoietic and lymphopoietic organs; monolayer culture; stromal precursors; age changes.

Investigations have shown that proliferation and differentiation of hematopoietic cells and lymphocytes are determined by the microenvironment of the hemato- and lymphopoietic organs [1-3]. Stromal mechanocytes of the hematopoietic organs, the clonogenic precursors of which give rise to colonies of fibroblasts when cultured in vitro [4], participate in the formation of the specific microenvironment. Considering the effect of the microenvironment on hemato- and lymphopoiesis, it was decided to determine the number of stromal precursors contained in the femoral marrow, spleen, and thymus of guinea pigs of different ages. The method of monolayer culture of cells from hematopoietic and lymphoid tissue was used, for it enables the number of stromal precursor cells to be determined by counting the number of growing fibroblast colonies [5].

EXPERIMENTAL METHOD

Guinea pigs of three age groups – 10 days, 2 months, and 1.5 years – were used as donors of hematopoietic organs. A cell suspension was obtained by the method described previously [4] and the total number of nucleated cells was counted in the femoral marrow, spleen, and thymus. Bone marrow (2×10^6-5×10^6), spleen (5×10^6-15×10^6), and thymus (20×10^6-40×10^6) cells were explanted in 100-ml flasks. The culture medium consisted of medium 199 with 10% embryonic calf serum and the gaseous phase consisted of a mixture of air with 5% CO_2. On the 10th day the cultures were fixed with ethyl alcohol, stained with azure-eosin solution by the Romanovski–Giemsa method, and the number of fibroblast colonies containing no fewer than 50 cells was counted, from which the number of colony-forming cells (CFU-F) in the explanted suspension was determined.

EXPERIMENTAL RESULTS

The total number of nucleated cells in the femoral marrow rose sharply from 13×10^6 in the 10-day-old guinea pigs to 120×10^6 in guinea pigs aged 2 months, after which it remained almost unchanged (Table 1). The concentration of CFU-F in the bone marrow fell from 1×10^4 cells in the young guinea pigs to 1×10^5 cells in the old. The number of CFU-F in the 10-day-old guinea pigs was about 4000 per femur, whereas in guinea pigs aged 2 months it was 5 times greater, and in old guinea pigs it fell again to 2000. The total number of nucleated cells and the number of stromal precursors in the femoral marrow during growth of the animal thus increased, to reach a maximum by 2 months; by 1.5 years the number of hematopoietic cells remained at the maximal level, but the number of stromal precursor cells fell sharply.

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TABLE 1. Number of Stromal Precursor Cells in Hematopoietic Tissue of Guinea Pigs of Different Ages

Age of animals	Femoral marrow		Spleen		Thymus	
	number of nucleated cells $\times 10^7$	number of CFU-F $\times 10^3$	number of nucleated cells $\times 10^7$	number of CFU-F $\times 10^3$	number of nucleated cells $\times 10^7$	number of CFU-F $\times 10^3$
10 days	1,3±0,2	4,1±2,0	4,3±3,4	0,7±0,1	22,5±0,5	0,6±0,4
2 months	11,9±2,2	19,2±4,4	18,9±3,3	2,7±0,9	47,3±12,9	0,4±0,1
1½ years	9,8±2,2	2,0±0,6	41,0±9.0	2,3±0,9	0,4±0,1	0

The number of cells in the spleen increased with age from 40×10^6 in the 10-day-old animals to 400×10^6 in animals aged 1.5 years. The number of CFU-F in the spleen reached a maximum (about 3000) by the age of 2 months, and thereafter remained unchanged until 1.5 years. However, the concentration of CFU-F among spleen cells in the old animals was between one-third and one-quarter of that found in animals aged 2 months.

The CFU-F concentration in the spleen remained several times lower than in the bone marrow throughout life.

The total number of cells in the thymus of the animals aged 10 days was 225×10^6, it rose to 470×10^6 in the animals aged 2 months, and in the old animals only about 4×10^6 nucleated cells could be liberated from the thymus, which showed evidence of fatty degeneration. The concentration of CFU-F in the thymus did not exceed 1 per 10^6 cells, and it remained lower than in the spleen and bone marrow throughout life. The total number of stromal precursor cells in the thymus in the postnatal period was a few hundred, and in the thymus of old animals no CFU-F could be found.

The number of clonogenic stromal precursors in the hematopoietic organs thus changed with age. By 2 months it had reached a maximum in the bone marrow and spleen of guinea pigs. During aging the number of precursors in the spleen is unchanged, whereas in the femoral marrow it is significantly reduced. In the period of active functioning of the thymus the number of stromal precursors in it is relatively constant, whereas in old guinea pigs no stromal precursors are present. No direct relationship can be observed between the change in the number of hematopoietic cells and in the number of stromal precursor cells, although age involution of the thymus correlates with disappearance of clonogenic stromal precursors.

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