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MIGRATION AND DIFFERENTIATION OF HEMATOPOIETIC STEM CELLS IN AUTOIMMUNE MICE OF DIFFERENT AGES

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Migration and differentiation of hematopoietic stem cells were studied in autoimmune (NZB × NZW)F_1 mice of different ages. Migration of stem cells was shown to be reduced in old (NZB × NZW)F_1 mice. Irrespective of age, inhibition of differentiation of stem cells along the granuloid path of development was observed in (NZB × NZW)F_1 mice. It is suggested that in (NZB × NZW)F_1 mice there is either a defect of development of the T-lymphocyte subpopulation influencing differentiation of stem cells along the granuloid pathway or a genetic defect at the level of precursors of the granulocyte series (CFU_c).

KEY WORDS: stem cell; hematopoietic colony; differentiation.

It was shown previously that during aging migration of hematopoietic stem cells from the bone marrow diminishes in normal mice of several lines [4]. In old animals inhibition of differentiation of stem cells along the granuloid path is observed under these circumstances [5].

Inhibition of migration of granuloid differentiation of stem cells has also been observed in thymectomized mice [2, 8]. Since these processes are restored to normal by injection of thymocytes or peripheral T-cells from young donors into thymectomized (artificial T-deficiency) or old (age T-deficiency) mice, it has been suggested that thymus-dependent lymphocytes have a determining (inducing) effect on differentiation of the stem cell along the granuloid pathway.

In the investigation described below migration and differentiation of hematopoietic stem cells were studied in (NZB × NZW)F_1 mice, which are used as a model of systemic lupus erythematosus in man. As a rule an autoimmune state, associated with a deficiency of the T-system of lymphocytes, develops in (NZB × NZW)F_1 mice at the age of 4-5 months.

EXPERIMENTAL METHOD

(NZB × NZW)F_1 mice of different age groups were used in the experiments. The number of hematopoietic colonies in the spleen was counted by the method in [10]. Migration of stem cells was assessed by the method described previously [3]. Suspensions of bone marrow cells were prepared in the usual way and injected intravenously into lethally irradiated syngeneic recipients aged 2-2.5 months in a dose of $1 \cdot 10^5$ karyocytes. For the histological study of the character and number of colonies, spleens fixed in Bouin's fluid were embedded in paraffin wax and used for cutting series of sections 5-7 μ thick. The sections were stained with hematoxylin-eosin. The numerical results were subjected to statistical analysis in the usual way.

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TABLE 1. Migration and Character of Stem Cell Differentiation in (NZB × NZW)F_1 Mice of Different Ages

Age of mice, months	Number of colonies in spleen on microscopic analysis	Type of hematopoietic colonies					Ratio of number of erythroid colonies to number of granuloid
		erythroid	granuloid	megakaryocytic	undifferentiated	mixed	
1½–2	16,7±3,3 (15)	11,87±2,1	1,57±0,7	2,8±0,8	0,23±0,51	0,27±0,2	7,6
4	29,3±2,7 (13)	23,75±4,2	2,5±1,1	1,65±0,65	0,73±0,3	0,65±0,4	9,5
5–6	4,4±1,5 (5)	4,0±1,1	0,2	0,2	0	0	20,0
8	3,8±2,3 (5)	1,4±0,25	0	2,4±1,1	0	0	—

Legend. Here and in Tables 2 and 3: number of mice given in parentheses.

TABLE 2. Number and Histological Type of Splenic Colonies in (NZB × NZW)F_1 Mice in Exogenous Cloning System

Age of mice, months	Number of colonies in spleen on microscopic analysis	Type of hematopoietic colonies					Ratio of number of erythroid colonies to number of granuloid
		erythroid	granuloid	megakaryocytic	undifferentiated	mixed	
2	35,0±3,7 (6)	16,0±1,08	4,16±2,2	12,3±1,6	2,3±0,04	0,17	3,8
5	12,4±3,3 (5)	6,6±1,9	1,8±1,3	2,6±0,2	1,6±0,22	—	3,7
8	16,6±2,1 (9)	5,5±0,7	0	11,2±1,1	0	0,18±0,1	—

TABLE 3. Number and Histological Type of Endogenous Splenic Colonies in (NZB × NZW)F_1 Mice

Age of mice, months	Number of colonies in spleen on microscopic analysis	Type of hematopoietic colonies					Ratio of number of erythroid colonies to number of granuloid
		erythroid	granuloid	megakaryocytic	undifferentiated	mixed	
2	22,5±15,0 (3)	9,0±6,7	0	12,0±10,0	0	1,5±0,43	—
5	14,1±5,02 (7)	3,57±1,0	0,3±0,14	10,7±3,6	0	0,14±0,1	12
8–9	6,2±0,8 (12)	2,8±0,5	0	2,6±0,7	0,8±0,2	0,22±0,1	—

EXPERIMENTAL RESULTS

To study migration of stem cells, (NZB × NZW)F_1 mice of different ages were irradiated with x-rays in a lethal dose of 850 R, with the hind limb screened up to mid-calf level, and the number and type of hematopoietic colonies in the spleen were determined 7 days later. It was shown previously that each colony is formed from a single stem cell which has migrated from the screened part of the bone marrow. It will be clear from Table 1 that migration of stem cells from the bone marrow in (NZB × NZW)F_1 mice aged 4 months was more intensive than that in mice aged 1.5-2 months. However, at ages of 5-6 and 8 months migration was sharply reduced: the number of colonies was reduced by 4-7 times compared with mice aged 1.5, 2, and 4 months. Determination of the relative (calculated per 10^5 cells) and absolute (calculated per cell population of the bone) number of stem cells in the bone marrow of the screened limb of mice aged 2 months (38.1 ± 3.2 and 3560 colony-forming units – CFU – respectively) and aged 8 months (21.6 ± 2.7 and 2000 CFU) showed that their number diminished by 33-50% with age. Consequently, besides a decrease in the number of stem cells migrating from the bone marrow there was a true decrease in migration of stem cells associated with the development of autoimmune pathology.

Histological analysis of the splenic colonies showed that in mice of all age groups studied the ratio between erythroid and granuloid colonies was sharply displaced in favor of erythroid. The ratio of the number of erythroid colonies to the number of myeloid increased with age from 7.6 to 20.0 (Table 1). In normal mice of nonautoimmune lines the ratio of erythroid to granuloid colonies averaged 2 [2, 5, 6, 7].

Data on the number and differentiation of bone marrow stem cells during their transplantation into lethally irradiated recipients' are given in Table 2. In the experiments of this series bone marrow cells from the test mice were transplanted into lethally irradiated (850 R) syngeneic recipients and the number and type of the colonies in the recipients' spleen were determined. It will be clear from Table 2 that in mice aged 5 months the number of CFU in the bone marrow was reduced by almost two-thirds compared with that in mice aged 2 months. The number of erythroid colonies exceeded the number of granuloid in the spleen of both groups of mice (the ratios were 3.8 and 3.7).

Finally, in the last series of investigations the number and type of endogenous colonies in the spleen of sublethally (600 R) irradiated mice were studied. Just as in the exogenous cloning system (Table 2), these experiments (Table 3) showed that granuloid differentiation of stem cells in the (NZB × NZW)F_1 mice of the age groups studied was depressed or completely absent. A decrease also was observed in the number of colonies with age.

Hence, irrespective of age, inhibition of stem cell differentiation along the granuloid path of development is observed in autoimmune (NZB × NZW)F_1 mice. The view has already been expressed that differentiation of stem cells along the granuloid path is under the influence of T-lymphocytes. The existence of a special subpopulation of T-cells, known as T-differentiating (TD) cells, has been postulated [1]. It can be tentatively suggested that inhibition of granuloid differentiation in autoimmune (NZB × NZW)F_1 mice is connected with a defect of development of the TD-cell subpopulation. On the other hand, the possibility likewise cannot be ruled out that in (NZB × NZW)F_1 mice there is a genetic defect at the level of the precursor of the granulocyte series (CFU_c). The changes found in the types of colonies may also find other explanations: changes in the microenvironment, inhibition of mitosis in the granulocyte series, increased mobility of granulocytic cells. These problems will be analyzed in subsequent investigations.

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