DIFFERENTIATION OF HEMATOPOIETIC PRECURSOR CELLS IN MICE WITH EXPERIMENTAL T IMMUNODEFICIENCY CORRECTED BY MYELOPEPTIDES

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A T-cell immunodeficiency develops in the late stages after thymectomy on sexually mature mice (≥2 months), and one of its manifestations is a shift of differentiation of hematopoietic precursor cells toward erythropoiesis as a result of elimination of T-differentiating lymphocytes [1, 2]. Normalization of stem-cell differentiation can be achieved by injection of syngeneic lymphocytes or of thymus hormones [1, 2, 7]. The important role not only of the thymus and thymus factors, but also of bone marrow, in the regulation of hematopoiesis has been demonstrated. Immunoregulatory molecules of peptide nature (myelopeptides), produced by bone-marrow cells, stimulate differentiation of bone marrow cells in vitro and endogenous colony formation and have a therapeutic action in some forms of leukemias, by increasing the fraction of differentiated cells in the patients' blood [3-6]. We therefore decided to study correction by myelopeptides of disturbed differentiation of hematopoietic precursor cells in the presence of a T-cell immunodeficiency induced by thymectomy in sexually mature mice.

EXPERIMENTAL METHOD

Mature CBA mice, either intact (control) or thymectomized at the age of 6 weeks, were used as bone marrow donors. At least 2 months after the operation the mice were given an injection of "myelopide" (a preparation of myelopeptides) in a dose of 100 μg per mouse in 0.2 ml physiological saline, intravenously or subcutaneously, once, three times, or repeatedly (up to 10 times). The preparation was kept in solution at −18°C and thawed once immediately before injection. The bone marrow was removed from the mice 24 h after receiving the last injection of myelopide, a suspension was prepared in medium No. 199, and it was injected intravenously in a dose of $5 \cdot 10^4$ nucleated cells into (CBA × C57BL)F_1 recipients, irradiated in an absorbed dose of 8.5 Gy. The recipients were irradiated 4-6 h before the experiment on the "Igur" apparatus with ^{137}Ce γ-rays in a dose rate of 1.78 Gy/min. On the 7th day after injection of the cells the spleens were removed from the recipients, fixed in Bouin's fluid, and the number of macrocolonies was counted. The fixed spleens were then embedded in paraffin wax and serial sections were cut and stained with hematoxylin. The number of microcolonies was counted and their type determined in the sections under the microscope (magnification 400). The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Table 1 gives data on the effect of the myelopeptides on differentiation of CBA mouse bone marrow cells in the spleen of lethally irradiated (CBA × C57BL)F_1 recipients. Table 1 shows that thymectomy performed on the bone marrow donors led to a shift of differentiation of the hematopoietic precursor cells toward erythropoiesis. The number of erythroid colonies increased from 17.3 to 20.7 and the number of granulocytic colonies fell from 8.4 to 4.8 (groups 1 and 3). The ratio between erythroid and granulocytic colonies (E/G) changed from 2.0 and 4.3. Injection of myelopide into the thymectomized donors (group 4) caused a

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TABLE 1. Effect of Myelopeptides on Differentiation of Hematopoietic Precursor Cells of Thymectomized and Intact Mice

Group No.	Translated cells	Number of mice	Number of colonies		Type of hematopoietic colonies					E/G
			macro-colonies	micro-colonies	erythroid (E)	granulo-cytic (G)	megakary-ocytic	mixed	undifferentiated	
1	BM	26	14,4±1,0	26,5±1,3	17,3±0,9	8,4±0,4	0,3±0,1	0,6±0,1	0	2,0
2	BM MP	26	18,8±1,1	28,8±1,4	17,5±1,0	9,8±0,7	0,2±0,1	1,6±0,3	0	1,8
3	BM TE	27	23,1±1,1	26,8±1,8	20,7±1,1	4,8±0,4	0,2±0,1	0,9±0,2	0,3±0,1	4,3
4	BM TE MP	27	15,7±0,9	30,7±1,4	16,5±1,2	12,4±1,0	0,4±0,1	1,3±0,3	0,03±0,03	1,3

Legend. BM) Bone marrow of intact mice; BM MP) bone marrow of intract mice receiving myelopide; BM TE) bone marrow of thymectomized mice; BM TE MP) bone marrow of thymectomized mice receiving myelopide.

decrease in the number of erythroid colonies from 20.7 and 16.5 and an increase in the number of granulocytic colonies from 4.8 to 12.4. The ratio E/G fell from 4.3 to 1.3, i.e., the normal type of hematopoiesis was established. Injection of myelopeptides into mice with normal hematopoiesis (group 2) had no effect on the character of differentiation of the hematopoietic precursor cells. The E/G ratio remained at the normal level of 1.8. The total number of microcolonies in all groups remained at virtually the same level irrespective of the injection of myelopide. Myelopeptides evidently can act simultaneously on erythroid and granulocytic branches of hematopoiesis, inhibiting one and stimulating the other, or on their common early precursor. The number of macrocolonies varied, being equal to the number of erythroid colonies in the given group. The reason was that macrocolonies were counted visually on the surface of the whole spleen, whereas granulocytic colonies, located in the thickness of the spleen, were not counted under those circumstances. The absence of action of myelopeptides on normal hematopoiesis shows that myelopeptides evidently possess not so much a stimulating action, but rather a normalizing action which is manifested during various disturbances of normal physiological processes, including differentiation of stem cells. It must be pointed out that myelopide acted in the same way irrespective of the schedule of its intravenous or subcutaneous injection, or whether it was injected once, three times, or more often. There are two possible explanations: either myelopeptides have a brief action lasting not more than 1 day, after which the action of all previous injections is abolished, or a single injection of the preparation is sufficient for manifestation of its effect.

It can be concluded from these results that under the experimental conditions used the myelopeptides had a correcting action on differentiation of hematopoietic stem cells in T-cell immunodeficiency, similar to the action of T-differentiating lymphocytes. Myelopeptides do not affect the differentiation of hematopoietic cells in normal animals. It can accordingly be postulated that thymectomy induces a deficiency not only of T cells but also of endogenous myelopeptides. How myelopeptides correct hematopoiesis – at the level of committed precursor cells or of polypotent stem cells – future research will show.

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