INTERACTION BETWEEN SYNGENEIC HEMATOPOIETIC STEM CELLS AND LYMPHOCYTES IN LEUKEMIC AKR MICE

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From its early stages leukemia is accompanied by marked immunosuppression [5, 7]. Disturbances in the complex chain of immunopoiesis are reckoned with effect from its initial point, namely the hematopoietic stem cell. Differentiation of hematopoietic cells is shifted [3] and their pool in the bone marrow and spleen is modified [3, 4]. Hence it has been postulated that there must be a certain factor which limits the proliferative and differentiating power of hematopoietic stem cells during the development of leukemia. Meanwhile migration, growth, and maturation of hematopoietic cells are thymus-dependent processes [2, 8], and it was this which served as the basis for the investigation described below, which was devoted to a study of the effect of T lymphocytes on colony formation and differentiation of hematopoietic cells during spontaneous leukemia developing in AKR mice [6].

EXPERIMENTAL METHOD

Experiments were carried out on female AKR mice obtained from the Stolbovaya nursery Academy of Medical Sciences of the USSR. The colony-forming ability of the hematopoietic stem cells was studied on a syngeneic donor—recipient model by the method of Till and McCulloch [10], by transplanting $1 \cdot 10^5$ bone marrow cells, either alone or together with lymphocytes or thymocytes in a ratio of bone marrow cells to lymphocytes of 1:5 and 1:20 into lethally irradiated (850 R) syngeneic recipients. To study the effect of T cells on endogenous colony formation, $1 \cdot 10^6$ thymus or lymph nodes lymphocytes were injected into sublethally irradiated (650 R) healthy syngeneic recipients. On the 8th day after injection of the cell suspensions the mice were killed by cervical dislocation, the spleen was removed and fixed in Bouin's fluid, and the number of macrocolonies, corresponding to the number of colony-forming units, was counted. To determine differentiation of hematopoietic cells, the fixed spleen was embedded in paraffin wax and sections were cut to a thickness of 5-7 μ. The histological preparations were stained with hematoxylin and eosin. Leukemia was diagnosed in the animals by determining the cell composition of the peripheral blood. Mice with identical values of the blood formula, leukocyte count, and hemoglobin concentration were selected for the experimental group. The type of leukemia was confirmed by histological investigation of the internal organs (thymus, lymph nodes, spleen, liver, kidneys) and the cytological picture of the bone marrow. The presence of septicemia as a complication of leukemia in the late stages was established by cultures of peripheral blood, liver, kidney, thymus, lymph nodes, and spleen on nutrient media, followed by identification of the microorganisms on the basis of their cultural and morphological features.

EXPERIMENTAL RESULTS

The strongly inhibitory effect of thymus and lymph node cells on colony formation of syngeneic healthy and autologous bone marrow was established in mice aged 8-10 months after the beginning and development of leukemia diagnosed as being of lymphoblastic type and with a course characterized by a thymoma with severe anemia. Depression was assessed histologically as inactivation of colony-forming units relative to all three branches of hematopoiesis: erythroid, myeloid, and megakaryocytic (Table 1).

The ratio between the number of "surviving" erythroid and myeloid colonies under these circumstances was practically the same as in the control. In the case of 100% inactivation,

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TABLE 1. Quantitative Histological Analysis of Hematopoietic Colonies in Recipients' Spleens after Transplantation of Bone Marrow and Lymphocytes of Healthy and Leukemic AKR Mice

Donors of bone marrow	Transplanted cells	Ratio of bone marrow cells to lymphocytes	Number of microcolonies per spleen (M ± m)	Ratio of erythroid to granuloid colonies	Number of animals
Healthy mice	Bone marrow	—	9,0±0,2	3:1	10
	Bone marrow + lymph nodes	1:5	9,0±0,3	2,6:1	10
		1:20	10,0±0,2	3:1	10
	Bone marrow + thymus	1:5	8,5±0,4	3:1	8
		1:20	10,0±0,3	3,6:1	10
	Bone marrow + leukemic lymph nodes	1:5	0,0	—	8
		1:20	0,0	—	9
	Bone marrow + leukemic thymus	1:5	2,8±0,1	2,5:1	10
		1:20	0,0	—	8
Leukemic mice	Leukemic bone marrow	1:5	9,8±0,6	3,6:1	10
	Leukemic bone marrow + lymph nodes	1:5	9,7±0,2	3:1	7
		1:20	10,0±0,5	3:1	7
	Leukemic bone marrow + thymus	1:5	13,0±0,3	3,8:1	8
		1:20	11,0±0,9	4:1	10
	Leukemic bone marrow + leukemic lymph nodes	1:5	1,0±0,1	2:1	8
		1:20	0,7±0,2	2,5:1	10
	Leukemic bone marrow + leukemic thymus	1:5	1,5±0,3	5:1	10
		1:20	0,0	—	7

TABLE 2. Stimulation of Growth of Exocolonies of Granuloid Type by Syngeneic T Lymphocytes from AKR Mice in Late Stages of Spontaneous Leukemia Complicated by Septicemia

Donors of bone marrow	Transplanted cells	Ratio of bone marrow cells to lymphocytes	Number of microcolonies per spleen (M ± m)	Ratio of erythroid to granuloid colonies	Number of animals
Healthy mice	Bone marrow	—	15,0±1,0	3,3:1	9
	Bone marrow + lymph nodes	1:5	12,0±1,0	2,7:1	7
		1:20	13,0±0,9	3:1	10
	Bone marrow + thymus	1:5	12,6±0,7	3,4:1	10
		1:20	14,0±1,0	3,2:1	10
	Bone marrow + leukemic lymph nodes	1:5	41,0±2,0	1:2,7	8
		1:20	51,1±1,4	1:2,1	10
	Bone marrow + leukemic thymus	1:5	22,5±0,7	1:1	10
		1:20	33,1±1,9	1:1	10
Leukemic mice	Leukemic bone marrow	—	21,0±1,4	3:1	10
	Leukemic bone marrow + lymph nodes	1:5	18,0±0,9	3,2:1	8
		1:20	22,0±1,0	3:1	9
	Leukemic bone marrow + thymus	1:5	19,0±1,2	3,2:1	9
		1:20	20,1±0,9	3,2:1	10
	Leukemic bone marrow + leukemic lymph nodes	1:5	54,0±2,3	1:1,5	8
		1:20	43,0±2,0	1:2	9
	Leukemic bone marrow + leukemic thymus	1:5	40,0±2,5	1:2,5	8
		1:20	34,0±1,9	1:1	10

TABLE 3. Stimulation of Growth of Endogenous Colonies of Granuloid Type by Syngeneic T Lymphocytes of AKR Mice in Late Stages of Spontaneous Leukemia Complicated by Septicemia

Transplanted cells	Number of microcolonies per spleen (M ± m)	Ratio of erythroid to granuloid colonies	Number of animals
—	20,4±0,6	3,2:1	10
Intact lymph nodes	22,8±1,0	4:1	10
Intact thymus	23,0±1,2	3:1	9
Leukemic lymph nodes	30,2±1,2	1:1	10
Leukemic thymus	29,5±0,8	1:1	10

considerable splenomegaly was observed, evidently arising because of migration of leukemic lymphoblasts. Thymocytes and lymphocytes of healthy mice did not change the proliferation and direction of differentiation of the stem cells of either healthy or leukemic bone marrow. In similar experiments in the late stages of leukemia, complicated by generalized staphylococcal infection, T cells were found to have a marked stimulating effect on exogenous colony formation of syngeneic hematopoietic cells. Histological analysis of serial sections through the spleen showed an increase in the number of granuloid colonies, resulting in a change in the ratio of erythroid to myeloid colonies to 1:1-1:2.7 (Table 2). The stimulating effect of the T lymphocytes also extended to growth of endogenous colonies: Transplantation of $1 \cdot 10^6$ leukemic cells of the thymus or lymph nodes into syngeneic healthy sublethally irradiated recipients increased by 50% the proliferative power of their splenic colony-forming units, mainly on account of granuloid colonies. The erythroid-myeloid ratio in this case was changed to 1:1 (Fig. 3).

The ability of lymphocytes to stimulate granulopoiesis was combined with a peripheral blood picture uncharacteristic of lymphoblastic leukemia: Besides immature lymphocytes, atypical monocytes (up to 20%) and neutrophils (40-50%) also were present in large numbers. Monocytes, metamyelocytes, and myelocytes, and sometimes lymphocytes were carrying out phagocytosis of bacterial cells. The cytological picture of the bone marrow, and also histological investigation of the internal organs (lymph nodes, thymus, spleen, liver, kidneys) nevertheless were evidence of typical lymphoid infiltration.

Interaction between hematopoietic stem cells and lymphocytes, leading at the stage of development of the disease to inhibition of hematopoiesis, can thus take place in AKR mice with spontaneous leukemia, and it is evidently due either to transformation of thymus-dependent lymphocytes, induced by leukemogenic virus, and subsequent inactivation of the animal's own hematopoietic cells in the manner of allogeneic cells [1], or by inhibition of the hematopoietic cells by some humoral factor present in leukemic cells. At the septicemic stage the stimulating effect of lymphocytes may perhaps be connected with their activation by staphylococci and subsequent realization of the colony-stimulating factor, the positive stimulus to monocytopoiesis and granulopoiesis [9], as is confirmed by the intensified growth of myeloid colonies and the discharge of numerous neutrophils and monocytes, which play an important role in the defense against infection, into the peripheral blood.

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