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STATE OF LYMPHOPOIESIS IN MICE WITH ALLOXAN DIABETES

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Diabetes mellitus is one of the commonest diseases of the endocrine system in man. Among the many structural and functional disturbances observed in this diseases, changes in the immunity system are not the least important [1, 5-7]. Lowering of the resistance of the body to infectious diseases, and the associated development of angiopathies [3], etc. are a characteristic feature of diabetes [9]. Experimental observations have shown that lymphopoiesis may be disturbed in this disease. For instance, marked inhibition of lymphophoiesis has been found in the thymus of pancreatectomized rats [8] and of mice with alloxan diabetes [4].

Accordingly, in the investigation described below, the state of lymphopoiesis was studied in animals with alloxan diabetes.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino mice weighing 20-25 g, in some of which alloxan diabetes was induced by a single subcutaneous injection of a 4% solution of alloxan hydrate (from Lachema-Chemapol, Czechoslovakia), in a dose of 400 mg/kg on an empty stomach. The group of diabetic animals consisted of mice in which the blood sugar concentration determined by the orthotoluidin method standardized in the USSR, when tested twice on the 3rd and 14th days after injection of alloxan, was not below 14 mM (250 mg%). To rule out any possible toxic effect of alloxan itself on lymphopoiesis, a control group of 13 mice was formed, into which the compound was injected in the same dose as into the diabetic mice, but these mice did not develop diabetes because of their individual resistance. The blood sugar concentration in these animals on the 14th day after injection of alloxan did not differ significantly from that in intact healthy mice.

The total leukocyte count, the absolute and relative lymphocyte counts, and the number of T-, B-, and O-cells separately, identified by the cytochemical reaction for acid phosphatase, detected by the method of Goldberg and Barka [2], were determined in the peripheral blood of all the mice 2 weeks after injection of alloxan. After blood analysis, all the animals were given an intraperitoneal injection of ^{3}H-thymidine (All-Union "Izotop" Combine, specific activity 925 GBq/mmole) in a dose of 40 MBq/kg, 1 h before the animals were killed by cervical dislocation. The weight of the lymphoid organs, their relative weights, and their cell composition, and the number of myelokaryocytes in the femoral diaphysis were determined

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in the sacrificed mice, and films of bone marrow and squash preparations from the thymus, spleen, and lymph nodes of the ileocecal complex were prepared. Some of these preparations were fixed in methanol, stained with azure II-eosin, and used to obtain cell counts and to calculate mitotic indices, whereas others were coated with type M photographic emulsion (Moscow Technical Photographic Plate Factory) and incubated in darkness at 4°C for 20 days, after which they were developed, counterstained with azure II-eosin, and used to calculate labeling indices of the lymphoid cells. Cell counts were based on 500 cells, mitotic indices and total labeling indices of the lymphoid cells were determined on 2000 cells, labeling indices of lymphoblasts on 100 cells, and of prolymphocytes on 200 cells. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Three weeks after injection of alloxan the diabetic mice developed considerable lymphocytopenia, associated with a fall in the numbers of both T- and B-lymphocytes in the blood (by 30 and 27%, respectively; Table 1). These data agree with those of clinical investigations, evidence of a significant fall in the number of T- and B-cells in the blood of patients with diabetes [1, 5, 7]. The investigations showed that lymphocytopenia in mice with severe diabetes is usually accompanied by the appearance of degeneratively changed microforms of lymphocytes in the bloodstream.

Besides the hematologic changes mentioned above, definite changes also were found in the organs of lymphopoiesis in the diabetic mice. These changes were most marked in the thymus and bone marrow. Hypoplastic changes were discovered in the thymus of diabetic mice: a marked decrease in the number of cells in the organ, a decrease in the relative number of young proliferating cells (lymphoblasts and prolymphocytes), and a reduction of their proliferative activity (Table 2).

The absolute and relative weight of the thymus in the diabetic mice also was reduced ($P < 0.001$) to mean values of 22.2 mg and 1.04 mg/g, respectively (41.8 mg and 1.86 mg/g in healthy mice). The total number of myelokaryocytes in the diabetic mice showed no significant change. Meanwhile, there was a significant decrease in the relative and absolute numbers of lymphoid cells (prolymphocytes and lymphocytes) in them, and a decrease in their labeling index (Table 2), which indicated hypoplasia of the lymphoid branch of the bone marrow. Changes discovered in the lymph nodes of the ileocecal complex in diabetic mice were less marked and were confined to a significant decrease (on average by 30%) in their weight, relative weight, and total cell content. No significant changes in the parameters studied were to be found in the spleen of the diabetic mice.

It can be concluded from the results of this investigation that the state of alloxan diabetes is characterized by marked disturbances of lymphopoiesis. Lymphopoiesis in the thymus and bone marrow is most substantially affected, and it is probably this which ultimately determines the lymphocytopenia and the decrease in the number of T- and B-lymphocytes in the bloodstream developing in association with diabetes. Lymphopoiesis in the lymph nodes and spleen is evidently virtually not depressed under these conditions. The disturbances found in diabetic mice could hardly be attributable to the toxic effect of alloxan on the lymphoid cells, be-

TABLE 1. Total Number of Leukocytes and Absolute Number of Lymphocytes in Blood of Healthy and Diabetic Mice ($\bar{X} \pm m$)

Parameter	Healthy mice (n = 21)	Diabetic mice (n = 13)	P
Total number of leukocytes, 10^9/liter	11,15±0,49	11,53±0,53	>0,05
Absolute number, 10^9/liter			
of all lymphocytes	7,29±0,10	5,40±0,42	<0,001
of T-lymphocytes	4,00±0,08	2,80±0,22	<0,001
of B-lymphocytes	2,76±0,06	2,02±0,16	<0,001
of O-lymphocytes	0,55±0,01	0,55±0,04	>0,05

TABLE 2. Cytological Parameters Characterizing State of Lymphopoiesis in Thymus and Bone Marrow of Healthy and Diabetic Mice

Parameter	Healthy mice (n = 18)	Diabetic mice (n=16)	P
Thymus			
Number of karyocytes, millions	125,10±14,11	33,29±7,67	<0,001
Relative number, percent, of:			
Lymphoblasts	2,4±0,2	1,2±0,2	<0,001
Prolymphocytes	7,7±0,6	3,9±0,4	<0,001
Lymphocytes	89,1±0,6	9461±0,6	<0,001
Mitotic index of all lymphoid cells percent, of:	0,80±0,14	0.47±0,06	<0,01
Labeling index, percent, of:			
Lymphoblasts	64,9±2,6	40,3±3,3	<0,001
Prolymphocytes	36,6±2,9	24,9±3,2	<0,001
All lymphoid cells	6,05±0,48	3,00±0,30	<0,001
Bone marrow			
Number of myelokarocytes (per femur), millions	22,7±1,0	23,0±1,1	>0,05
Lymphoid cells, %	9,90±0,4	8,0±0,7	<0,05
Lymphoid cells, millions	2,14±0,10	1,75±0,15	<0,05
Labeling index, %			
Prolymphocytes	18,30±0,16	8,00±0,90	<0,001
All lymphoid cells	2,87±0,29	1,64±0,17	<0,01

cause a single injection of alloxan in a diabetogenic dose, if it did not cause diabetes, had no significant effect on the peripheral blood parameters or the state of lymphopoiesis in the bone marrow and lymphoid organs studied in control mice. The absolute number of lymphocytes in their blood, for instance, averaged $8.08 \cdot 10^9$, and the labeling indices of prolymphocytes in the thymus and bone marrow were 36.3 and 16.3%, respectively ($P < 0.05$). Probably the main role in the pathogenesis of the disturbances revealed by this investigation may be played either by the insulin deficiency directly or, as some workers [7] consider, by secondary endocrine-humoral disorders arising in diabetes and, in particular, elevation of the blood levels of unsaturated fatty acids and glucocorticoids.

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