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# LYMPHOCYTE KINETICS IN MICE WITH ALLOXAN DIABETES

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Among the many different hematologic disturbances developing in diabetes a special place is occupied by the marked inhibition of cellular immunity [3, 4, 8]. Insulin is known to possess the properties of a general paleohematopoietim in mammals and man: proliferation of the lymphoid cells of the thymus and bone marrow and also the function of cytotoxic T lymphocytes are known to depend to the greatest degree on it, whereas the formation and function of phylogenetically younger B lymphocytes are not disturbed as a general rule if this hormone is deficient, and indeed, they may even be enhanced.

The aim of this investigation was to study the kinetics of the lymphoid cells of the thymus, lymph nodes, and blood in mice with alloxan diabetes.

## EXPERIMENTAL METHOD

Experiments were carried out on 100 male BALB/c mice (from the "Rassvet" Nursery, Tomsk) weighing 18-20 g, in some of which alloxan diabetes was induced by the method described previously [3]. The blood sugar level of the diabetic animals was not lower than 14 mM. The lifespan of the peripheral blood lymphocytes was determined autoradiographically, with the use of $^3$H-thymidine [6]. 5-methyl-$^3$H-thymidine ("Izotop" Production Combine, specific radioactivity 925 GBq/mmole) was injected intraperitoneally once daily in a dose of 40 MBq/kg for 14 days, after which all the animals were given unlabeled thymidine ("Fluka," Swizerland) from the 15th through the 30th day of the experiment in order to block reutilization of the radionuclide (the unlabeled thymidine was added to the drinking water in a concentration of 100 mg/liter). To study the dynamics of the appearance and disappearance of labeled lymphocytes, blood films were obtained throughout the experiment at intervals of 1-2 days, and subsequently used for autoradiograhic investigation [3]. Growth fractions and temporal parameters of the mitotic cycle of the lymphoblasts and prolymphocytes in the thymus and ileocecal lymph nodes were determined by the method of saturation with $^3$H-thymidine [2, 5]. The radionuclide was injected intraperitoneally in a dose of 20 MBq/kg seven times in the course of 24 h at intervals of 4 h, or twice in the same dose at intervals of 1 and 2 h, or

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TABLE 1. Kinetics of Lymphocytes in Normal and Diabetic Mice

| Parameter | Normal | Diabetic | p |
|---|---|---|---|
| | Thymus | | |
| Lympgoblasts: | | | |
| Growth fraction, percent | 96,3±1,1 | 85,6±1,7 | <0,01 |
| Mitotic cycle, h | | | |
| T | 9,7±0,3 | 12,2±0,5 | <0,001 |
| Phase $G_1$ | 1,1±0,06 | 3,4±0,2 | <0,001 |
| Phase S | 6,2±0,1 | 6,0±0,3 | >0,05 |
| Phase $G_2$ | 1,2±0,03 | 1,4±0,06 | <0,01 |
| Phase M | 1,1±0,04 | 1,4±0,04 | <0,001 |
| Prolymphocytes | | | |
| Growth fraction, percent | 71,6±2,5 | 52,4±4,7 | <0,01 |
| Mitotic cycle, h | | | |
| T | 14,9±0,6 | 17,4±1,3 | >0,05 |
| Phase $G_1$ | 4,2±0,1 | 6,3±0,3 | <0,001 |
| Phase S | 8,3±0,6 | 7,2±0,6 | >0,05 |
| Phase $G_2$ | 1,3±0,07 | 2,0±0,3 | <0,05 |
| Phase M | 1,1±0,03 | 1,1±0,04 | >0,05 |
| | Lymph nodes | | |
| Lymphblasts | | | |
| Growth fraction, percent | 96,0±1,3 | 95,4±1,0 | >0,05 |
| Mitotic cycle, h | | | |
| T | 12,2±0,1 | 12,6±1,4 | >0,05 |
| Phase $G_1$ | 1,1±0,2 | 1,2±0,3 | >0,05 |
| Phase S | 9,4±1,5 | 7,9±0,7 | >0,05 |
| Phase $G_2$ | 0,8±0,07 | 1,0±0,06 | >0,05 |
| Phae M | 1,4±0,1 | 1,4±0,08 | >0,05 |
| Prolymphocytes | | | |
| Growth fraction, percent | 63,3±3,7 | 64,7±3,5 | >0,05 |
| Mitotic cycle, h | | | |
| Generation time T | 15,2±0,5 | 12,9±0,8 | <0,05 |
| Phase $G_1$ | 6,0±0,3 | 2,9±0,07 | <0,001 |
| Phase S | 7,8±1,0 | 7,4±0,05 | >0,05 |
| Phase $G_2$ | 1,4±0,2 | 1,4±0,2 | >0,05 |
| Phase M | 1,4±0,1 | 1,3±0,07 | 0,05 |

once, in a dose of 40 MBq/kg. At least eight animals were used at each point of the investigation. The mice were killed 1 h after the last injection of the isotope. Blood films were prepared from the thymus and lymph nodes of the ileocecal complex, and used as squash preparations for autoradiographic study. The growth fraction was determined separately for lymphoblasts and prolymphocytes by calculating the labeling index of these cells 25 h after the beginning of the seven successive injections of $^3$H-thymidine into the animals. The generation time (T) and the durations of the S-phase (tS) and mitosis (tM) were found by Quastler's equations [2, 5]. The mean duration of the premitotic phase ($tG_2$) was estimated from the time of appearance of 50% of labeled mitoses. The duration of the presynthetic phase ($tG_1$) was determined by calculation [5]. The counting error during determination of labeling indices and mitotic indices did not exceed 14%. The results were subjected to statistical analysis by Student's test on the SM-3 computer.

## EXPERIMENTAL RESULTS

The time of disappearance of half the labeled cells from the blood of healthy and diabetic mice was taken as the characteristic of the life span of the lymphocytes [6]. Its average value in the former was 14 daqys and in the latter 9.9 days ($p < 0.01$). The percentage of labeled lymphocytes in the blood of healthy mice 25 h after the beginning of the series of seven injections of $^3$H-thymidine averaged 6.3, compared with 11.1 for the diabetic mice ($p < 0.01$). These results indicate that besides shortening of the life span of the lymphocytes in mice with alloxan diabetes, migration of these cells from the lymphopoietic organs into the blood stream was accelerated.

A marked degree of inhibition of thymic lymphopoiesis was found previously in mice with alloxan diabetes [3, 4]. Estimation of the kinetic parameters of the young proliferating cells in the thymus of animals with diabetes (Table 1) not only confirmed the previous conclusions, but also filled in some of the details: in the thymus of the diabetic mice the number of lymphoblasts and prolymphocytes in the mitotic cycle was significantly reduced, and the generation time of the proliferating lymphoblasts was significantly lengthened, mainly on account of lengthening of the presynthetic phase (by 3.1 times, $p < 0.001$). The premitotic phase (by 17%, $p < 0.01$) and the duration of mitosis (by 27%, $p < 0.001$) also were lengthened to a certain degree. Significant lengthening of the $G_1$- and $G_2$-phases was found in the dia-

betic mice in the thymic prolymphocytes also (on average by 1.5 times). Meanwhile the duration of the S-phase in the young thymic cells was not significantly changed in the diabetic mice. These results may indicate that the passage of the thymic lymphoic cells through the known bottlenecks of the cell cycle on the boundaries between the $G_1$/S- and $G_2$/M-phases is disturbed in diabetes [1]. The possible cause of this disturbance may be an absolute deficiency of insulin, which is a well-known serum factor of progression, which stimulates in particular the passage of cells through the W bottleneck on the boundary of the $G_1$/S-phases of the cell cycle [1].

The study of the kinetics of lymphocytes of the ileocecal lymph nodes in mice with alloxan diabetes showed (Table 1) that the growth fraction of lymphoblasts and prolymphocytes in these glands was not significantly changed compared with the healthy animals. There was likewise no significant change in any of the parameters of the mitotic cycle of nodular lymphoblasts studied. Meanwhile the cell cycle of the pyrolymphocytes from the lymph nodes of diabetic mice showed some reduction of the generation time, mainly due to shortening of the $G_1$-phase (by half, $p < 0.001$). These results indicate that the nodular lymphopoiesis in mice with alloxan diabetes not only is not inhibited, as was pointed out previously [3, 4], but is even accelerated to some degree on account of the fact noted above. It will be evident that proliferation of lymphoblasts from the ileocecal lymph nodes is independent of insulin, whereas proliferation of nodular prolymphocytes may perhaps even be facilitated by a deficiency of this hormone. At the same time, another possibility cannot be ruled out: it was shown in [7] that depopulation of the thymus-dependent zones takes place in the lymph nodes of rats with alloxan diabetes and, consquently, there is a change in the ratio of T- and B-lymphocytes in the general cell population; the changes which we found in the temporal parameters of the cell cycle of nodular prolymphocytes may probably also be connected with this fact.

On the basis of these results we have a better understanding of the mechanisms of disturbance of lymphopoiesis in diabetes and, in particular, of the mechanism of development of lymphocytosis B [4].

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