Leukocyte Kinetics in the Blood of Mice with Alloxan-Induced Diabetes

Yu. A. Kozlov, V. V. Novitskii, and A. N. Baikov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 120, № 7, pp. 33-35, July, 1995
Original article submitted August 11, 1994

An autoradiographic kinetic study of neutrophilic leukocytes, monocytes, and lymphocytes from the peripheral blood of male BALB/c mice with subacute diabetes induced by alloxan showed that the diabetes was accompanied by moderate neutrophilic leukocytosis, monocytopenia, depressed monocytopoiesis, prolonged circulation times of neutrophils and monocytes, and a shortened circulation time of lymphocytes.

Key Words: *alloxan-induced diabetes; kinetics of circulating neutrophilic leukocytes, monocytes, and lymphocytes*

Hematological disorders arising in diabetes mellitus play a substantial role in the pathogenesis of many of its complications [1,3,5,7,9]. Nevertheless, the kinetics of formed elements of the blood in diabetes mellitus (in particular, their daily production levels and their circulation times) remains virtually unexplored. The data reported in the literature [2,3,8,10,11] are fragmentary and shed little light on the mechanisms responsible for alterations in the quantitative and qualitative composition of leukocytes in this endocrinopathy [4,6].

In the present autoradiographic study we examined the kinetics of peripheral blood leukocytes (neutrophils, monocytes, and lymphocytes) in mice with alloxan-induced diabetes.

MATERIALS AND METHODS

The study was conduced on 22 male BALB/c mice (from the *Rassvet* Nursery, Tomsk) weighing 18-20 g. Two weeks before the tests, diabetes was induced with alloxan in 11 of the mice using the conventional procedure [2]. The diabetic animals had blood sugar concentrations of at least 14 mmol/liter, as measured by the orthotoluidine test. Thymidine-(5-methyl-^{3}H) (925 GBq/mmol; Izotop, Russia) was injected into all rats intraperitoneally once daily in a dose of 40 MBq/kg for a total of 14 days, after which the rats received, from day 15 to day 30, unlabeled thymidine (Fluka) in their drinking water in a concentration of 100 mg/liter. Throughout the 30-day period, blood smears were prepared at 1- to 3-day intervals for autoradiography and for calculating the labeling indexes of peripheral blood leukocytes (neutrophils, monocytes, and lymphocytes) [2]. In addition, blood leukocytes were counted and leukograms were examined during the indicated period. The circulation half-times ($t_{1/2}$) of labeled leukocytes in the blood of test and control rats were determined by analyzing the ascending and/or descending portions of the ^{3}H-thymidine labeling curves plotted for the leukocytes [6]. The mean circulation times (t) of these cells were determined from the formula $t_T=t_{1/2}/\ln 2$, based on the exponential law governing the elimination of blood cells from the circulation [4]. The turnover rates (V) of circulating leukocytes were calculated using the relation $V=0.693\ M/t_{1/2}$, where M is the absolute number of leukocytes of a particular variety (10^9 cells/liter [4].

Leukocyte labeling indexes in the autoradiographs were computed with an error of not more than 14% (criterion: 50 labeled cells). The significance of differences between the control and test mice was estimated by Student's t test.

Central Research Laboratory, Siberian Medical University, Tomsk (Presented by M. A. Medvedev, Member of the Russian Academy of Medical Sciences)

0007-4888/95/0007-0678$12.50

RESULTS

The results are summarized in Table 1 and Fig. 1. Neutrophil counts in the blood of diabetic mice were significantly higher than in the healthy controls, which agrees with the reported occurrence of neutrophilic leukocytosis in diabetes mellitus [1]. On the other hand, no significant difference was found between the two groups in the turnover rates of these cells. The results of our study indicate that neutrophilic leukocytosis in diabetes mellitus may be primarily due to substantially prolonged circulation times of neutrophilic leukocytes, probably as a result of their impaired migration to peripheral tissues. These findings are in agreement with those of Filyushina [8] in patients with diabetes mellitus.

The present study also enabled us to evaluate the productivity of monocytopoiesis and other kinetic parameters of monocytes in mice with experimental diabetes. The monocyte turnover rate in these mice was decreased by a factor of 2.2 ($p<0.001$) - an indication of strongly depressed monocytopoiesis. It is possible that the formation of only monocytes, which are insulin-sensitive [1], but not that of other mononuclear phagocytes, is inhibited in diabetes. Monocyte circulation times were also significantly prolonged in the diabetic mice. Monocyte counts in their blood proved to be significantly reduced, but in the presence of abnormalities detected in monocyte kinetics, the counts of these cells could be normal, as they often are in patients with diabetes mellitus [1].

In our previous study of blood lymphocyte kinetics in mice with alloxan-induced diabetes and control (healthy) mice [2], the peripheral blood of diabetic mice was found to contain twice as many labeled lymphocytes as that of controls 24 h after fractional administration of ^{3}H-thymidine at 4-h intervals, although on day 15 of such ^{3}H-thymidine dosing the level of maximal labeling was always lower in the former than in the latter mice. In the study reported here, mice with alloxan-induced diabetes had considerably shortened lymphocyte circulation times and were therefore at a high risk of developing lymphocytopenia [1].

Taken together, our results suggest that the formation of long-lived lymphocyte subpopulations is greatly reduced in diabetes mellitus, whereas short-lived lymphocytes continue to be produced at the normal level. Diabetes mellitus is known to impair lymphopoiesis only in the central lymphoid organs (bone marrow and thymus), and the cells whose formation and function suffer most in this disease appear to be cytotoxic T lymphocytes [1,3]

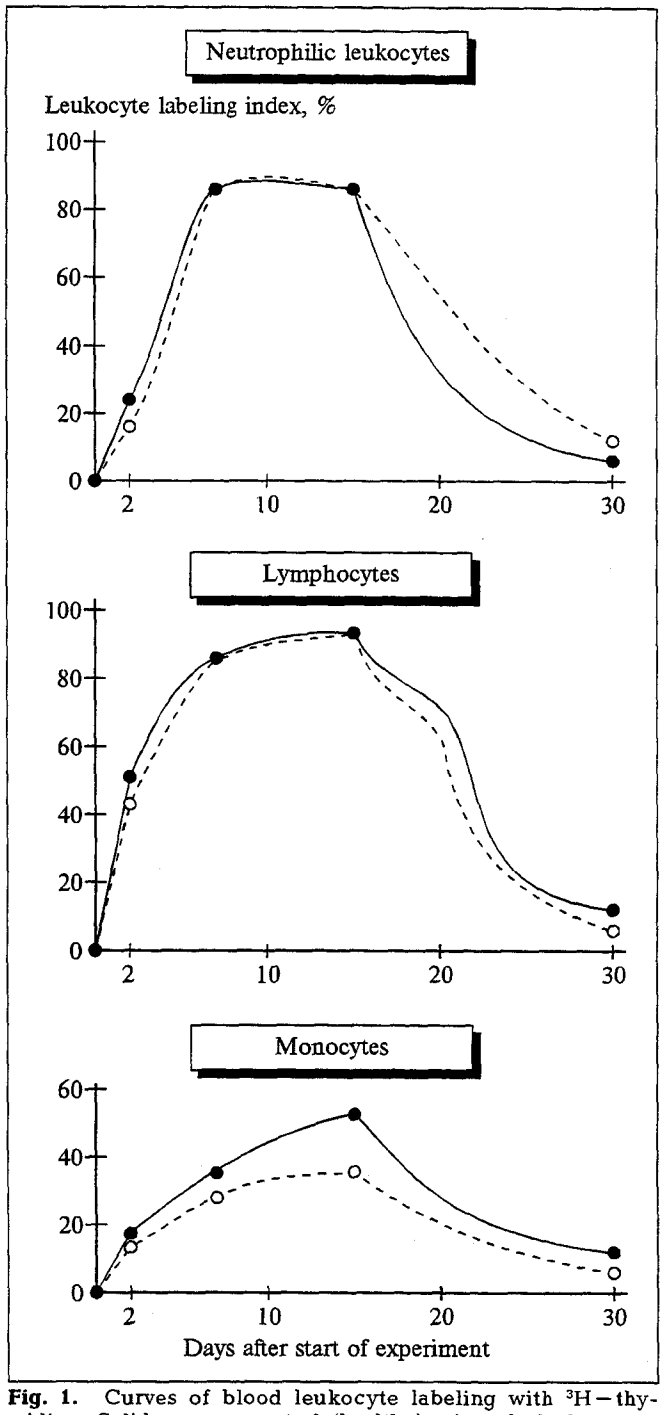

Fig. 1. Curves of blood leukocyte labeling with ^{3}H-thymidine. Solid curves: control (healthy) mice; dashed curves: test mice with alloxan-induced diabetes.

REFERENCES

1. Yu. A. Kozlov and V. S. Lavrova, *Usp. Sovrem. Biol.*, **105**, № 3, 505-520 (1988).
2. Yu. A. Kozlov, in: *Contributions of Basic Sciences to Medicine* [in Russian], № 2, Tomsk (1990), pp. 75-80.
3. Yu. A. Kozlov and T. A. Loktyushina, *Byull. Eksp. Biol. Med.*, **110**, № 10, 404-406 (1990).
4. V. M. Kotel'nikov, G. I. Kozinets, V. V. Kasatkina, and N. P. Kovalevskaya, in: *Kinetic Aspects of Hematopoiesis,*

TABLE 1. Major Kinetic Parameters of Blood Leukocytes in Mice with Alloxan–Induced Diabetes ($M \pm m$)

Parameter	Healthy mice (n=11)	Diabetic mice (n=11)
	Neutrophilic leukocytes	
Cells, $\times 10^9$/liter	3.09±0.33	4.38±0.42**
$t_{1/2}$, days	4.0±0.3	7.0±0.4***
t_T, days	5.8±0.4	10.1±0.6***
V, 10^9/liter×day	0.54±0.06	0.43±0.04
	Monocytes	
Cells, $\times 10^9$/liter	0.42±0.04	0.28±0.05*
$t_{1/2}$, days	1.33±0.08	1.93±0.13***
t_T, days	1.92±0.16	2.78±0.19***
V, 10^9/liter×day	0.22±0.02	0.10±0.02***
	Lymphocytes	
Cells, $\times 10^9$/liter	8.17±0.78	6.58±0.65
$t_{1/2}$, days	14.0±1.0	9.9±0.5**
t_T, days	20.2±1.4	14.3±0.7**
V, 10^9/liter×day	0.40±0.04	0.46±0.05

Note. *$p<0.05$, **$p<0.01$, ***$p<0.001$ in comparison with the healthy controls.

G. I. Kozinets and E. D. Gol'dberg (eds.) [in Russian], Tomsk (1982), pp. 149-211.

5. M. I. Martynova, V. V. Smirnov, and N. A. Mazurina, *Vopr. Okhr. Mat.*, № 5, 49-53 (1978).
6. E. N. Mosyagina, E. B. Vladimirskaya, N. A. Torubarova, and N. V. Myzina, *Kinetics of Formed Elements of the Blood* [in Russian], Moscow (1976).
7. V. S. Nemirovskii, M. N. Ostroumova, V. M. Dil'man, *et al.*, *Probl. Endokrinol.*, № 2, 8-11 (1980).
8. Z. G. Filyushina, *Byull. Eksp. Biol. Med.*, **68**, № 10, 48-50 (1969).
9. R. L. Jones and C. M. Peterson, *Amer. J. Med.*, **70**, № 2, 339-352 (1981).
10. Frank M. Ng, E. Babacan, and B. Dowsing, *Biochem. Int.*, **13**, № 2, 245-252 (1986).
11. P. D. Winocour, R. L. Kinlough-Rathbone, and J. F. Mustard, *J. Lab. Clin. Med.*, **109**, № 4, 464-468 (1987).