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BLOOD AND BONE MARROW CELL DISORDERS IN THE STAGES OF PROGRESSIVE DIABETES IN MICE

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Objective: to examine how the severity of tissue metabolic disorders affects the dynamics of the state of blood cells and bone marrow (BM) cells in patients with progressive diabetes mellitus (DM). **Materials and methods.** The genetic model of type 2 diabetes (T2DM) in db/db mutant mice (experimental group, n = 30) was used. Healthy mice of the same line – db/+m (n = 10) and line B10 (n = 5) served as control. The dynamics of laboratory and clinical parameters (blood glucose, glycosylated hemoglobin, body weight) and oxidative metabolism indicators in tissues were monitored FOR 6–6.5 months using Lasma-ST device. The state of blood cells (red blood cells, white blood cells, platelets) and BM cells were examined during the same period. Statistical processing of the results was done with preliminary use of the Shapiro–Wilk test; the significance of differences with the control was assessed using the parametric Student's t test, at $p < 0.05$. **Results.** In the development of T2DM, 3 stages of progressive metabolic disorders were identified: I – adaptation stage (1–2 months); II – progressive maladaptation stage (2.5–4.5 months); III – decompensation stage (from 5.0–6.5 months to death). It was found that in T2DM mice, blood content of red blood cells, Hb and leukocytes was reduced already in stages I–III; but in stage II and especially in stage III, there was increased platelet count and percentage of neutrophils, monocytes, eosinophils with a decrease in lymphocytes. A high percentage of live cells is preserved in the BM in stages I, II and early periods of stage III; in late periods of stage III, live cell percentages are frequently found to be low; in all periods of stage III, the total cell content in the BM is clearly reduced. **Conclusion.** Hematopoietic processes are inhibited in the BM as T2DM progresses. Individual assessment of the state of BM and its cells at the progressive stages of T2DM may be useful for prognostic purposes.

Keywords: diabetes mellitus, redox processes, blood cells, bone marrow cells.

INTRODUCTION

The prevalence of diabetes mellitus (DM) in the world is steadily increasing and has now become a pandemic [1]. Chronic course, severe vascular complications, early disability and high mortality among DM patients indicate the need to continue improving the therapy for this disease based on results from in-depth studies of pathogenetic mechanisms.

There are 2 most common types of DM – type 1 diabetes (T1DM) and type 2 diabetes (T2DM), which differ in terms of mechanisms of development and clinical manifestations at early stages of the disease.

Progressive hyperglycemia is a common clinical feature of T1DM and T2DM. It creates conditions for life-threatening complications in the body [2–4]. It has been shown that hyperglycemia has a damaging effect on various tissues of the body due to the toxicity of glycosylated proteins and lipoproteins accumulating in cells [5]. Among vital organs, the bone marrow (BM), which produces various types of blood cells (red blood cells, white blood cells, platelets) on a daily basis, turned out to be the most susceptible to the damaging effects of glycosylated proteins that accumulate in erythrocytes in the form of glycosylated hemoglobin [6]. As a result, red blood cells (RBC) change their functional properties (membrane potential decreases), and, along with white blood cells that produce proinflammatory cytokines [7], platelets play an active role in the development of life-threatening macro- and microvascular complications [8]. Other pathogenetic variables that emerge during the disease's progression promote disorders in the state of blood cells and BM in DM, particularly in T2DM. These include systemic inflammation against the background of developing immune dysfunction, oxidative stress and endoplasmic reticulum stress in cells, as well as disorders

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in the intestinal microbiome and barrier properties of the intestinal mucosa, etc., as a result of toxic damage to its cells [9–12]. Collectively, these factors aggravate metabolic disorders in the body by inhibiting redox processes in the cells of all organs and tissues, including blood and BM cells.

In recent years, as cell technologies have advanced, studies have been conducted on the feasibility of using bone marrow cells (BMCs) to treat metabolic disorders in T1DM and T2DM. These studies were based on the modern ideas that BM is not only the central organ of immunogenesis, but also the main regulator of the body's reparative regeneration processes [13]. However, it was discovered that autologous BM obtained from DM patients for induction therapy, even at early stages of the disease, have lower regulatory activity [14, 15] and are not always suitable for regenerative therapy [16] compared to BM from healthy allogeneic donors. We have not found any data on the state of BMCs at later, more severe stages of diabetes, when the patient undergoes not only drug therapy but also tissue (pancreatic islet cell transplantation) or organ (pancreas) transplantation. Meanwhile, it can be assumed that the outcomes of tissue and organ transplantation in patients with severe DM will be determined, among other things, by the state of their BMCs, their ability to adapt the body to the graft and support the graft viability in the recipient's body.

The aim of the present work is to study the dynamics of changes in the state of blood cells and BMCs depending on the severity of metabolic disorders in body tissues during the progressive course of diabetes on a genetic model of T2DM in mice.

MATERIALS AND METHODS

The dynamics of metabolic disorders, as well as changes in the state of blood cells and BMCs in T2DM were studied on mutant (homozygous) C57BL/KsJYLeprdb/+(B/Ks-Leprdb/+) – (db/db) mice, which carry a recessive gene – leptin receptor – Leprdb – (db) (linkage group 8, chromosome 4). The db gene in the homozygous state causes progressive DM, which is caused by a decrease in receptor-mediated sensitivity of body cells to endogenous insulin. The developing diabetes is similar to T2DM in humans and is characterized by cell degradation in pancreatic islets, but without deficiency of insulin production at early stages. There were a total of 30 mutant B/Ks-Leprdb/Leprdb (db/db) diabetic mice of both sexes used in the experiment (n = 30). Phenotypically healthy heterozygous mice of the same line – B/Ks-Leprdb/+ – (db/+m) (n = 10) and mice of non-diabetic line C57BL/10 – (B10) (n = 5) served as controls. So, the number of mice used in the experiment was 45 mice of the same initial age.

In these mice, changes in several functional indices developing in T2DM, which reflect the severity of the animal's clinical state, were studied dynamically over 6.0–6.5 months. Glucose and glycosylated hemoglobin (HbA1c) levels in the blood and body weight were measured, and the state of redox processes in the body tissues was assessed. Glucose levels were determined in fresh venous blood by photometric method on an Accu-Chek device (Switzerland), and the percentage of HbA1c was measured on an NycoCard READER device (Norway), which is designed for rapid *in vitro* determination of HbA1c by borate affinity analysis. The body weight of animals was determined using Mettler BD202 scale (Switzerland). Dynamic assessment of the state of redox processes was done noninvasively using a laser Doppler flowmeter, Lasma-ST [17]. This device allows measuring blood and lymph microcirculation in rodent tail tissues, determining in these tissues the activity level of mitochondrial coenzymes – NADN and FAD – and automatically calculating the oxidative metabolism index (OMI) based on the obtained results [17]. Determination of tissue microcirculation level, activity of mitochondrial coenzymes, oxidative metabolism index, as well as blood glucose levels in the course of animal life allowed us to identify 3 stages of metabolic disorders in T2DM mice (see the "Results" section of this paper). It was at these stages that we studied the dynamics of changes in the state of blood cells and bone marrow cells in T2DM mice.

In studying blood cells in T2DM, mixed (arterial-venous) blood was taken from cervical arteries and veins by decapitation of mice, preliminarily anesthetizing them with injection of Zoletil solution in saline at a dose of 40 mg/kg. Blood was collected in tubes with KZEDTA (tricalic salt of ethylenediaminetetraacetic acid). Hematologic parameters were evaluated – red blood cells (RBC, 10^{12}/L), hemoglobin (HGB, g/L), hematocrit (HCT, %), mean corpuscular volume (MCV, fl), mean corpuscular hemoglobin (MCH, pg), mean corpuscular hemoglobin concentration (MCHC, g/L), red cell distribution width (RDW-CV, %), red cell distribution width (RDW-SD, standard deviation, %), platelets (PLT, 10^9/L), relative platelet distribution width (PDW, %), plateletcrit (PCT, %), mean platelet volume (MPV, fl), white blood cells (WBC, 10^9/L), neutrophils (Neu, %), lymphocytes (Lymph, %), monocytes (Mono, %), Eosinophils (Eosi, %), basophils (Baso, %) were performed on an automatic hematology analyzer DYMIND VET DF50 (China) according to the manufacturer's guidelines. The data were presented as the average of three measurements.

BMCs were isolated from the femurs and tibias of mice using a standard protocol [18]. The isolated whole bones were cleared of muscles and ligaments, then the epiphyses were cut off and the bones were placed in 0.5 ml standard plastic centrifuge tubes with the bottom pre-pierced with a needle (G18–21). These tubes were placed inside 1.5 ml plastic centrifuge tubes and centrifuged for 10 seconds at 10,000g. The bone-purified BMCs sample was in the lower 1.5 mL tube after centrifugation.

Phosphatidylserine detection on the outer membrane of cells using labeled annexin V was used to assess the severity of BMCs apoptosis. The number of annexin-positive cells was assessed using the Annexin V-AF 488 Apoptosis Detection Kit (Lumiprobe, Russia) according to the standard flow cytometry protocol [19].

The obtained BMCs were suspended by pipetting in 100 μl of buffer at room temperature. From this suspension, so many cells were selected that their concentration in the reaction volume (100 μL) was $1 \times 10^5 \ldots 1 \times 10^6$ cells/mL. Annexin V-AF 488 was added to a concentration of 3 μg/mL and incubated for 15 minutes at room temperature. After that, 400 μL of chilled binding buffer was added.

Propidium iodide (PI) was used to assess cell membrane integrity. It was added to the samples before measurement on a flow cytometer to a concentration of 0.5–1 μg/mL.

Freshly prepared samples were analyzed on a BD FACSCalibur flow cytometer (Becton Dickson, USA) equipped with an argon laser (488 nm). Fluorescence emission (AF488, FITS) was recorded in a FL1 channel (515–545 nm) and in a propidium iodide FL2 range (620 nm). Between 15,000 and 25,000 events were accumulated for each sample. Data were collected using the CELLQuest program (Becton Dickson, USA). The data obtained in the pilot study were processed in the FlowJo program. The results were analyzed taking into account the guidelines outlined by Crowley et al. [19], without setting the target gate.

The numerical values of oxidative metabolism, glucose, HbA1c and body weight were statistically processed with preliminary use of the Shapiro–Wilk test on a small number of samples ($n < 5$) to demonstrate the normal distribution of data characterizing metabolism in individual periods. The significance of difference between the compared indicators was assessed using Student's t test (standard software package Microsoft Excel 2019) at $p < 0.05$.

RESULTS

The results of dynamic study of the clinical state of the animals during progressive development of T2DM and oxidative metabolism in their body tissues at the same age (time) periods are presented in Table 1 and Fig. 1. Table 1 shows that already after 1 month of life, the glucose and HbA1c levels of T2DM mice significantly increase, as does the body weight, compared to the controls. Continued examination of glucose and HbA1c levels in T2DM mice at 2, 4, and 6 months established further progressive increase. The body weight of T2DM mice at 2 and 4 months of life also continued to increase compared to controls, indicating obesity. However, starting at 5–6 months of age, the body weight of these mice became significantly lower than that of controls, and the animals acquired an emaciated appearance.

The characteristic clinical signs of T2DM, such as polydipsia, polyphagia and polyuria, became distinctly pronounced from 2 months after birth. On average, these mice drank 25.74 ± 1.18 ml of water per day, compared to 4.69 ± 0.35 ml in the control, $p < 0.05$; they ate 8.9 ± 0.29 g of feed compared to 3.74 ± 0.096 g in the control, $p < 0.05$ (control was briquette feed).

We identified the stages of increasing changes in the bodies of T2DM mice by dynamically measuring microcirculatory tissue indicators that characterize the state of redox processes in body tissues (Fig. 1) and comparing them to indicators of laboratory and clinical animal state (Table 1). It was found (Fig. 1) that at 1.0–1.5 months against the background of increasing hyperglycemia, the amplitude of the activity of coenzymes NADH and FAD increased, while OMI values (an oxidative metabolism indicator) decreased; however, when compared to the controls, the revealed changes in redox processes were not significant. This T2DM development period was recognized as stage I of T2DM and named the adaptation stage. Clinical signs of maladaptation appear at 2.0–2.5 months (significant increase in body weight, glycemia, HbA1c (glucose toxicity), polyuria, polyphagia) and last until 4.0–4.5 months. During this time span, the severity of impairment of redox process indicators progresses considerably (Fig. 1), although no visible consequences emerge yet. We defined stage II of T2DM as a period ranging from 2.0 to 4.5 months and dubbed it the progressive maladaptation stage. At the age

Table 1

Age dynamics of glucose content, HbA1c% and body weight in db/db, db/+m and B10 mice

Indicators of carbohydrate metabolism and body weight	Mouse lines		
	db/db (T2DM) Group 1 (n = 30)	db/+m (control) Group 2 (n = 10)	B10 (control) Group 3 (n = 5)
Age 1 month			
Glucose, mmol/L	10.3 ± 2.4*	5.4 ± 0.5	5.6 ± 0.3
HbAlc, %	4.9 ± 1.0*	3.5 ± 0.07	3.0 ± 0.08
Body weight, g	21 ± 2.5*	13 ± 1.2	15 ± 1.8
Age 2 months			
Glucose, mmol/L	18.7 ± 3.83*	5.8 ± 0.42	5.9 ± 0.03
HbAlc, %	7.9 ± 1.11*	3.6 ± 0.1	3.2 ± 0.13
Body weight, g	39 ± 2.37*	15 ± 2.69	18 ± 2.49
Age 4 months			
Glucose, mmol/L	25.5 ± 3.49*	4.6 ± 0.39	4.9 ± 0.69
HbAlc, %	8.6 ± 1.16*	3.7 ± 0.25	3.7 ± 0.22
Body weight, g	48 ± 2.68*	19 ± 2.26	21 ± 2.27
Age 6 months			
Glucose, mmol/L	27.4 ± 2.09*	5.7 ± 0.65	5.4 ± 0.38
HbAlc, %	8.9 ± 1.25*	3.9 ± 0.57	3.8 ± 0.49
Body weight, g	20 ± 2.35*	24 ± 1.80	27 ± 1.64

Note: *, $p < 0.05$ compared to control groups.

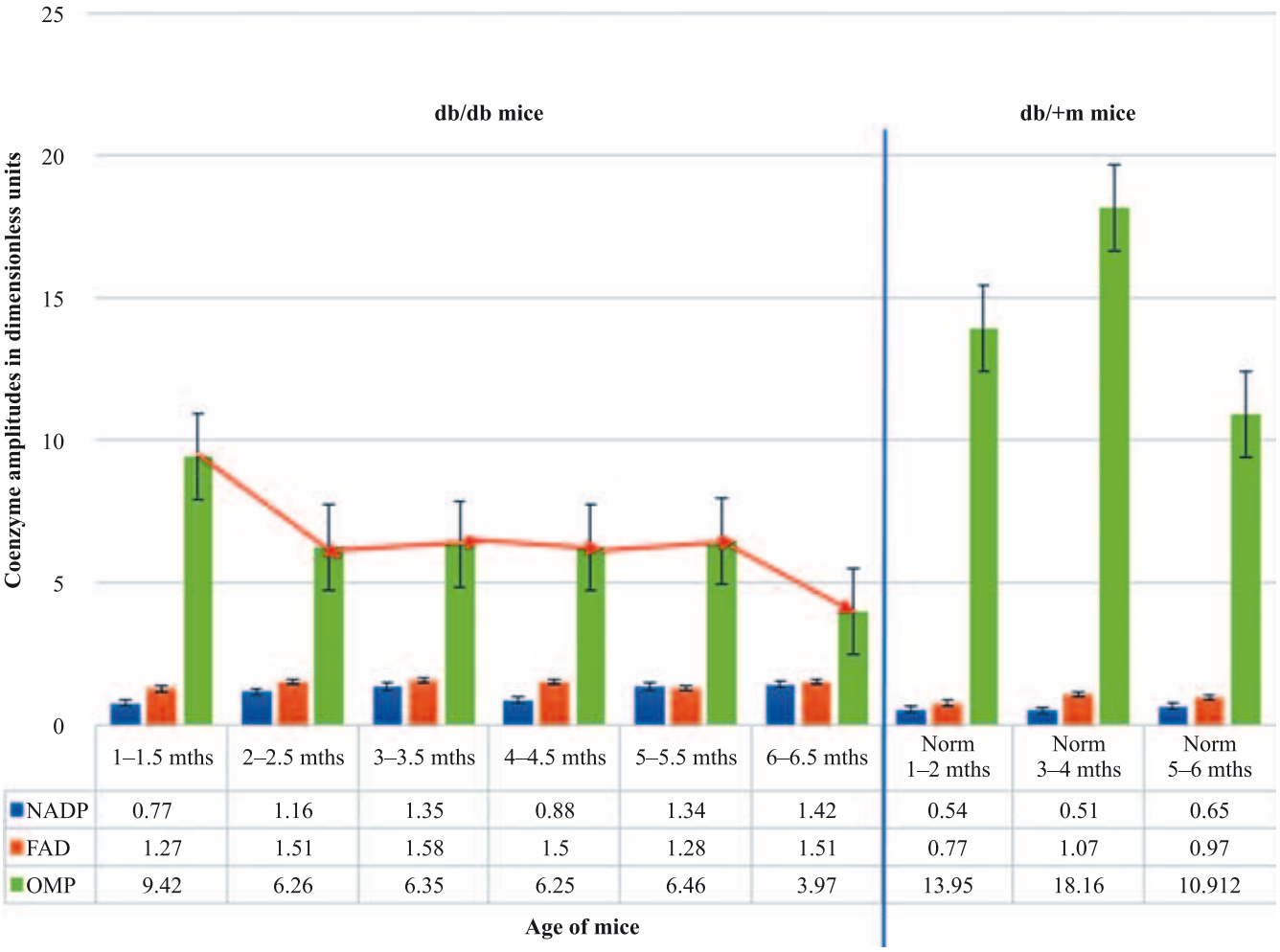
of 5.0–6.5 months, T2DM mice, on the background of worsening impairment of clinical parameters and indicators characterizing the state of redox processes in the body (NADN, FAD and OMI), with OMI reaching extremely low values – 3.97 ± 1.39 against 10.91 ± 2.04 in the control – at the same life stage (see Fig. 1), 30% of the animals developed late complications (skin maceration most often in the withers area), which persisted in the form of extensive wounds until the animals died (by 7–10 months). We defined this period (from 5.0–6.5 months until the animals died) as stage III of T2DM – the stage of decompensation of adaptation mechanisms with the development of deep tissue hypoxia, cell apoptosis and necrosis [8].

Having identified 3 clinical stages in the progression of metabolic disorders in T2DM, we proceeded to study the state of blood cells (RBCs, platelets and different types of WBCs) and BMCs, which produce them, since it is the state and functional properties of these cells that largely predetermine the adequacy of the course of redox processes in body tissues. Table 2 presents the results of a pilot study of the state of RBCs and platelets in healthy db/+m mice (control) and in db/db mice (T2DM model) at different stages of T2DM (abbreviations of the studied parameters are given in the "Materials and Methods" section).

Table 2 shows that already in the early life of T2DM mice (1.5–2.0 months, adaptation stage), their blood has a lower RBC content and reduced HGB level in RBCs compared to the control (db/+m mice). The increase in RBC at the decompensation stage is apparently a consequence of blood thickening on the background of polyuria.

In addition, T2DM mice showed a tendency towards increased MCV, RDW-SD and PLT, as well as decreased MCHC already at an early age. At the progressive maladaptation stage and decompensation stage, the same tendency towards an increase or decrease in some RBC characteristics was observed, which, apparently, indicates that structural alterations are emerging in these cells.

The PLT study clearly revealed a sharp increase in the number of these cells in blood at the stage of decompensation of redox processes and carbohydrate metabolism in the T2DM mice. In the study of WBC level (Table 3),



	1–1.5 mths	2–2.5 mths	3–3.5 mths	4–4.5 mths	5–5.5 mths	6–6.5 mths	Norm 1–2 mths	Norm 3–4 mths	Norm 5–6 mths
NADP	0.77	1.16	1.35	0.88	1.34	1.42	0.54	0.51	0.65
FAD	1.27	1.51	1.58	1.5	1.28	1.51	0.77	1.07	0.97
OMP	9.42	6.26	6.35	6.25	6.46	3.97	13.95	18.16	10.912

Fig. 1. Dynamics of microcirculatory and tissue parameters in db/db mice with DM and in db/+m mice without DM (normal) at different ages (the age of mice is indicated under the amplitudes of measured parameters: nicotinamide adenine dinucleotide phosphate (NADP), flavin adenine dinucleotide (FAD) and oxidative metabolism parameters (OMP) [17]

we also found a decrease in the total number of WBCs already at the early stages of life of the T2DM mice (1.5–2.0 months) compared to the control – 5.22×10^9/l *vs.* 9.92×10^9/l.

As the lifespan of the T2DM mice increased, the decrease in WBCs progressed, as seen by changes in the ratio of their individual populations. Early in the lives of the T2DM mice, the percentage of neutrophils (Neu), monocytes (Mono), and eosinophils (Eosi) increased, but the percentage of lymphocytes (Lymph) decreased. These changes intensified and became distinctly pronounced at the progressive maladaptation stages, especially during decompensation in the state of redox processes (Table 3).

The steady increase in platelet, neutrophil, monocyte and eosinophil count against a progressive decrease in lymphocyte count, as well as a sharp rise in the neutrophil-to-lymphocyte ratio suggest that systemic inflammatory response in T2DM is becoming more activated and that reparative processes are being inhibited, creating conditions for the development of micro- and macrovascular complications [25–27].

The detected decrease in the quantitative content of RBCs and WBCs already at early stages of life of T2DM mice in comparison with the control indicate that hematopoiesis processes were inhibited and that the state of BM responsible for hematopoiesis processes and regulation of homeostasis in the body of these mice needs to be assessed (Fig. 2).

Fig. 2 shows that during the development of T2DM, the percentage of large and medium-sized (proliferating) cells at stages I, II, and at early stage III decreases significantly compared to the control. In late (terminal) stage III of T2DM, the percentage of destroyed and small (nondividing) cells increases dramatically. Examination of the percentage of live and damaged cells in BM showed

Table 2

Results of dynamic study of the state of blood cells (red blood cells and platelets) in db/+m (control) and db/db (T2DM model) mice

Indicators studied	db/+m		db/db (T2DM)		
	1.5–2 mths.	3–4 mths.	1.5–2 mths. (adaptation period)	2.5–4.5 mths. (progressive maladaptation period)	5.0–6.0–6.5 mths. (decompensation period)
RBC, 10^{12}/L	8.68	8.36	7.5	7.73	8.12
HGB, g/L	157.5	154.5	132.25	147.3	155.7
HCT, %	40.75	40.35	39.15	40.8	43.88
MCV, fl	46.95	48.25	52.15	52.8	54.18
MCH, pg	18.1	18.45	17.6	19.07	19.18
MCHC, g/L	386	383	337.25	361	354.42
RDW-CV, %	15.35	13.65	18.42	17.53	17.4
RDW-SD, %	27.9	25.8	38.42	36.8	37.22
PLT, 10^9/L	881	732	898	753	1044.37
MPV, fl	6.7	6.7	6.85	6.33	6.7
PDW, %	6.5	6.75	6.22	7.2	8.07
PCT, %	0.59	0.49	0.61	0.48	0.69

Note: RBC, red blood cells; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW-CV, red cell distribution width; RDW-SD, red cell distribution width standard deviation; PLT, platelets; MPV; mean platelet volume; PDW, relative platelet distribution width; PCT, plateletcrit; fl, in femtoliters; pg, in picograms.

Table 3

Results of dynamic study of blood cells (white blood cells) in db/+m (control) and db/db (T2DM model) mice

Indicators studied	db/+m	db/db (T2DM)		
	2–4 mths.	1.5–2 mths. (adaptation stage)	2.5–4.5 mths. (progressive maladaptation stage)	5.0–6.5 mths. (decompensation stage)
WBC 10^9/л	9.92	5.22	4.37	3.32
Neu%	11.2	15.95	39.83	81.9
Lymph%	87.75	81.17	56.1	9.54
Mono%	0.65	1.22	2.3	6.14
Eosi%	0.3	1.57	1.67	2.33
Baso%	0.1	0.075	0.1	0.11
Neu/Lymph	0.13	0.20	0.71	8.58

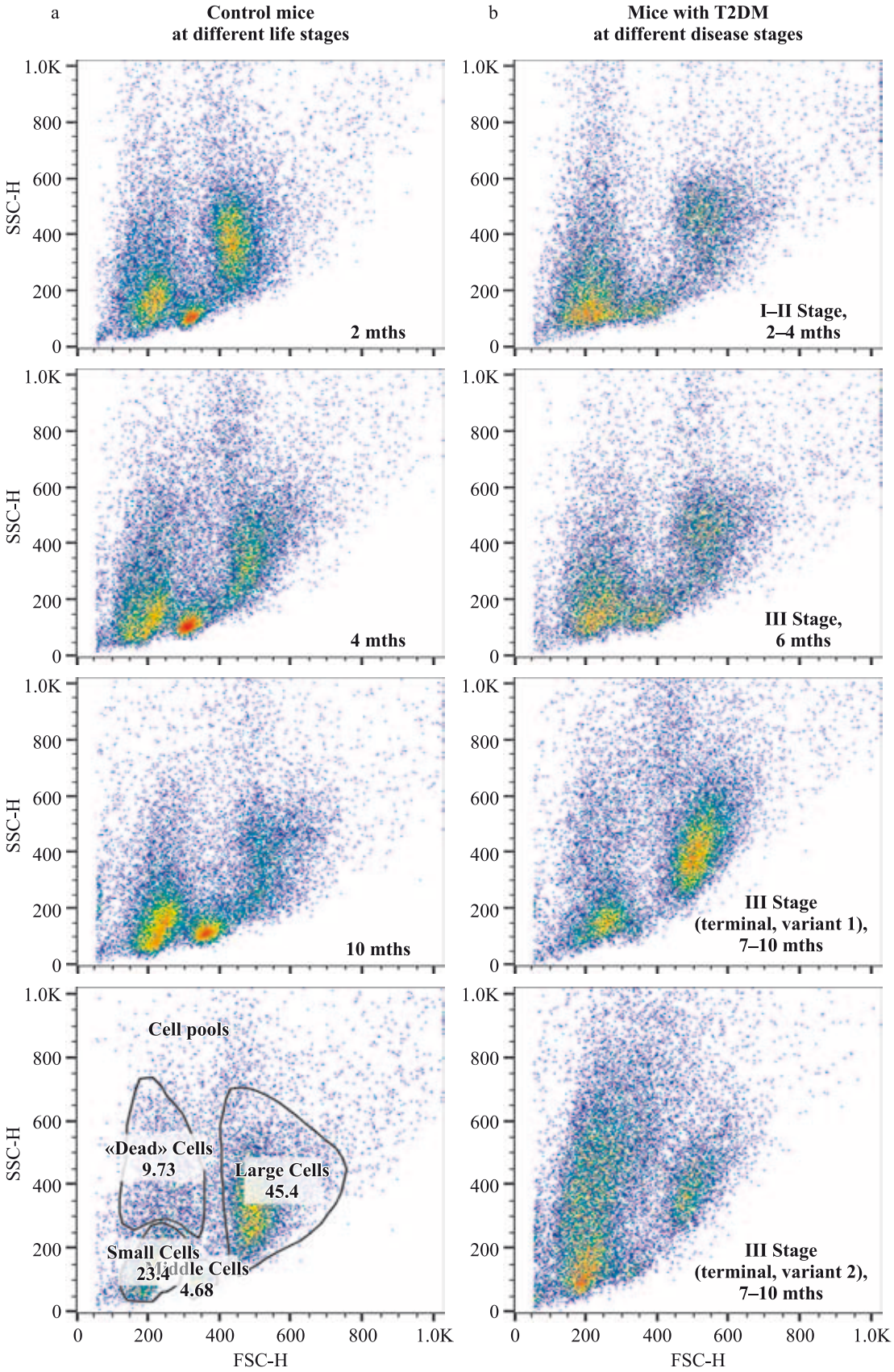


Fig. 2. Distribution of BM cells by size and texture in transmitted (FSC) and reflected (SSC) light in control mice (a) and mice with T2DM (b) at different life stages (disease stages). Bottom left shows the distribution of FSMs by population in control mice: small cells, middle cells, large cells, dead cells

(Fig. 3) that in healthy db/+m mice (control) at 2 months of age, the percentage of live cells averaged 68.95%, while cells in a state of necrosis, aponecrosis, and apoptosis averaged 31.05%. In db/db mice with T2DM at the same life span (adaptation period), the proportion of live cells was 71.35%, and cells in the state of necrosis, aponecrosis and apoptosis accounted for a total of 28.65%. At month 4 of life (period of developing maladaptation), as T2DM progressed, the percentage of living cells remained essentially unchanged, amounting to 70.5%; of the damaged cells, which made up 29.5%, the largest percentage (21%) were cells in the state of apoptosis.

In the early period of decompensation, which lasted for 5–6 months, however, we did not detect any deviations in the percentage of living and damaged cells in BM in contrast to the progressive maladaptation period (up to 4–4.5 months). Meanwhile, we noted a significant drop in the total count of BMCs during this period, as well as at later stages of clinical decompensation: BM gets depleted and it was necessary to collect BM from 2–3 tubular bones in order to study its cells.

At late stages of the decompensation period (7.0–9.5 months), the proportion of living cells in the BM of T2DM mice decreased significantly, reaching 30.4%; 69.6% of the cells were in necrosis, aponecrosis, or apoptosis, with aponecrosis accounting for the largest proportion of injured cells (44.7%). The BM of individual animals with T2DM showed a high percentage of living cells, even at late stages of decompensation, despite the fact that the total cell count was reduced when the cells were isolated from tubular bones (i.e., the bones contained a minimal amount of cellular material). Thus, in T2DM, as metabolic disorders worsen and redox processes become less efficient, the suppression of hematopoiesis processes also steadily increases in the BM, and cell necrosis, aponecrosis, and apoptosis intensify. These processes weaken the regulatory role and set the stage for the body to develop complications (skin maceration) and irreversible conditions. However, it is important to emphasize that even at the stage of clinical decompensation, at its early stage, and in some animals at the end stage, the BM cell pool is depleted, but not irreversibly damaged, indicating, in our opinion, the preservation of the regulatory and regenerative potential of BM in the body.

DISCUSSION

The issue of how clinical manifestations of diabetes and severe tissue metabolism disorders affect the state of blood cells and BMCs, particularly in the later stages of the disease, is still not well understood. Meanwhile, maintaining the mechanisms that keep the body in a state of homeostasis is crucial to the success of treatment and, most importantly, transplantation techniques (islet cell transplantation or pancreas transplantation) employed in the later stages of the disease. These primarily consist of BM, which is recognized as the central organ of immunogenesis, but also the main regulator of reparative regeneration in the body [13].

Regarding this, we set out to investigate in an experiment how the dynamics of changes in the condition of blood cells and BMCs are affected by the increasing disruption of carbohydrate and tissue metabolism in DM. We used a genetic T2DM model in 30 mutant db/db mice to investigate this issue. Healthy mice of the same line (db/+m) (n = 10) and mice of the B10 line (n = 5) were used as controls. In all these mice, laboratory and clinical parameters (blood glucose, HbA1c level, body weight, etc.), as well as the state of redox processes (by the level of microcirculation in tissues, amplitudes of coenzyme – NADH, FAD activity – and OMI, an indicator of oxidative metabolism) were monitored dynamically for 6.0–6.5 months from birth using the Lazma-ST apparatus [17].

T2DM was characterized by a progressive increase in hyperglycemia and glucotoxicity (increased HbA1c) until the end of the metabolic study period (Table 1). In the dynamics of body weight changes, two phases were identified: excessive weight gain over the course

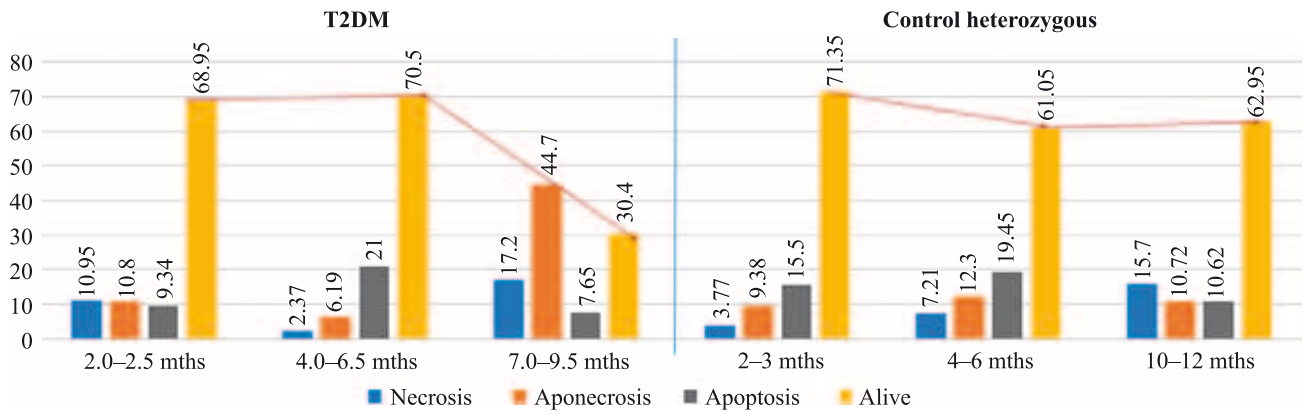


Fig. 3. Dynamics of changes in the state of bone marrow cells during the development of type 2 diabetes (in %)

of four months, followed by a fast decline over the next 5.0–6.5 months, all against the backdrop of persistent hyperglycemia and elevated HbA1c levels. This fact indicated profound metabolic disorders and the need for dynamic metabolic management.

In controlling the state of redox processes in the tissues of db/db mice, three stages of progressive T2DM were revealed (Fig. 1), which were characterized by a gradual increase in the severity of disorders in all parameters under study: I – adaptation stage (up to 2 months); II – progressive maladaptation stage (2.5–4.5 months); III – clinical decompensation stage (from 5–6.5 months to death of animals), at which vascular complications already appeared (skin maceration in 30% of mice). Additionally, we conducted a pilot study of the state of blood cells and BMCs in order to solve the task at the three stages. Against the background of hyperglycemia and elevated HbA1c, it was found that at stage I, the RBC count, HGB level, and WBC count all significantly decreased and stayed that way until the end of the observation (Table 2).

This fact can be explained by the fact that glycation of protein membranes of RBCs and other blood cells decreases their negative membrane potential, which accelerates aging and shortens the life span of blood cells [15]. In addition, a decrease in blood cell membrane potential in hyperglycemia promotes increased microviscosity, aggregation or adhesion of these cells, which first reduces their exit from the BM and then their production in the BM [16].

Patients with T2DM who experience prolonged episodes of hyperglycemia [17] and who already have microvascular complications [18] are observed to have decreased red blood cell count in the clinic. A decrease in erythrocyte count is also thought to be a result of erythropoietin production deficiencies in diabetic nephropathy patients, or of resistance to this hormone, as well as erythrocyte destruction that occurs in macro- and microangiopathies at advanced stages of T2DM [19].

However, we believe that in our experiments, the decrease in erythrocyte and leukocyte count already at stage I of T2DM in the model used, may be due to decreased membrane potential of cells as a result of early and accelerated glycation of the membrane proteins of these cells, caused by genetic features of the db/db mice. In stage II of T2DM, the persistent decrease in blood cell count may be due to all the above factors as well as to continuous rise in body weight.

Obesity is known to be accompanied by a state of chronic inflammation and a high level of circulating proinflammatory cytokines, which, having a long-term effect on the hematopoietic system and bone marrow niches, inhibit hematopoiesis processes in them [6]. The validity of this opinion is confirmed by our data on increased percentage of neutrophils, monocytes and eosinophils, and decreased lymphocytes in the blood (Table 3).

An increase in the neutrophil-to-lymphocyte ratio indicates activation of systemic inflammatory response in the body [20, 21] and even serves as a clinical predictor of worsening prognosis in the development of diabetic nephropathy [22, 23] and diabetic leg ulcers [24]. However, a cytometric analysis of BMC status, however, revealed that a significant portion (about 70%) of living cells remain in the BM at stages I, II, and even early stage III of T2DM (5–6 months) (Figs. 2 and 3). At the same time, the total cell count in the BM punctate always decreases significantly, even at early stage III. There is also always a significant drop in the total BMC count at the late progressive stage III of T2DM (7.0–9.5 months), a significant drop in the pool of living cells (up to 30.4%) and a high proportion of damaged cells (up to 69.6%); however, at late periods of stage III T2DM, a high percentage of living cells may also be retained in some animals. According to these findings, there are still T2DM animals, whose BMCs are resistant to the damaging effects of hyperglycemia and growing hypoxia, even at the decompensation stage of clinical and metabolic parameters (both at early and late stages). The ability of animals to contribute to the regulation and maintenance of homeostasis in the body is evident from the high percentage of living cells in their BM at the terminal stage of T2DM; their detection in the BM at the decompensation stage enables the prediction of a higher efficacy of therapeutic measures. A similar opinion is held by Gautier et al. (2015) [30], who consider it reasonable to predict the effectiveness of organ (liver) transplantation operations through preliminary measurement of the level of bone marrow CD34+ cells in peripheral blood, which characterizes the regenerative potential of cells in all body tissues [31].

CONCLUSION

1. In progressive T2DM in db/db mice, three stages are revealed, which differ in terms of degree of increase in the severity of redox processes and metabolic parameters: stage I (adaptation stage, at 1–2 months of life); stage II (progressive maladaptation stage, at 2.5–4.5 months of life); stage III (decompensation stage, from 5.0–6.5 months and until the animal dies).
2. Progressive T2DM occurs against the background of increasing hyperglycemia, elevated erythrocyte HbA1c levels, increased body weight at stages I and II and decreased body weight at stage III. These changes in clinical parameters occur against the background of a gradual decrease in the efficiency of redox processes (increased amplitudes of coenzymes NADH and FAD, and decreased OMI), especially pronounced at stage III.
3. In a pilot study of blood cell status in T2DM mice demonstrated a decrease in erythrocytes, Hb and leukocytes already at stage I. This persists at stages II and III. At stage II and especially at stage III, there is

a sharp increase in platelet count and percentage of neutrophils, monocytes and eosinophils, a decrease in lymphocyte count, as well as increased neutrophil-to-lymphocyte ratio, which indicate a systemic inflammatory response.

4. At stages I, II, and early stage III (5–6 months), the percentage of living and damaged cells in the BM of T2DM mice remains at the initial values; at late stage III (7.0–9.5 months), the percentage of living cells in the BM often decreases sharply and the percentage of damaged cells increases; at all periods of stage III, a decrease in the total cell count in the BM samples is diagnosed. Therapeutic interventions may be more successful in these animals if a significant proportion of living cells are preserved in the BM at both the early and late stages of the decompensation stage.
5. Individual assessment of blood cells and BMCs in progressive T2DM may be useful for prognostic purposes.

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