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Immune Alterations in Experimental Type 2 Diabetes Mellitus

Oralbek Ilderbayev
L.N. Gumilyov Eurasian National University

Kerim Mutig
I.M. Sechenov First Moscow State Medical University, RU

Darkhan Uzbekov
L.N. Gumilyov Eurasian National University

Gulzhan Ilderbayeva
L.N. Gumilyov Eurasian National University

Batyrlan Ayash
L.N. Gumilyov Eurasian National University

Zarina Amanova
L.N. Gumilyov Eurasian National University

Abstract: Type 2 diabetes mellitus is a multifactorial metabolic disorder characterized by insulin-independent hyperglycemia, impaired carbohydrate and lipid metabolism, and systemic complications. This study aimed to investigate metabolic and immune alterations in male laboratory rats with experimentally induced T2DM. Forty rats weighing 200 ± 20 g were divided into two groups: control and T2DM. Diabetes was induced via a single intraperitoneal injection of streptozotocin (30 mg/kg), and hyperglycemia was confirmed by fasting blood glucose levels exceeding 7.0 mmol/L. Metabolic analysis revealed significant hyperglycemia (20% increase, $p<0.05$), elevated immunoreactive insulin and C-peptide concentrations ($p<0.05$), and a trend toward increased glycated hemoglobin (HbA1c), indicating compensatory β-cell hyperactivity and early-stage metabolic dysregulation. Immunological assessment showed decreased total leukocyte and lymphocyte counts, with pronounced reductions in T-lymphocytes (CD3+) and T-helper cells (CD4+) ($p<0.05$), while T-suppressor cells (CD8+) remained unchanged, resulting in a reduced CD4/CD8 ratio. Functional tests demonstrated impaired lymphocyte mitogenic response, reduced antibody-forming cell activity, and suppressed natural killer cell activity ($p<0.01$), suggesting compromised innate and adaptive immunity. These findings indicate that experimental T2DM induces a combination of metabolic disturbances and systemic immune dysfunction. The observed interplay between hyperglycemia, β-cell stress, and immune suppression may contribute to increased susceptibility to infections and chronic inflammatory processes. Understanding these early alterations provides insight into the pathogenesis of T2DM and may inform the development of therapeutic strategies targeting both metabolic and immune pathways.

Keywords: Type 2 diabetes mellitus, Streptozotocin, Immunity

Introduction

Currently, the complex impact of chronic metabolic conditions, such as diabetes mellitus, on the human body is attracting significant attention from the scientific community. Type 2 diabetes mellitus (T2DM) is a

multifactorial disease characterized by insulin-independent hyperglycemia and disturbances in carbohydrate and lipid metabolism, developing at the intersection of metabolic, hormonal, and immunological dysfunctions. Two main clinical types of diabetes are distinguished: type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). T1DM is characterized by the autoimmune destruction of pancreatic β-cells, resulting in a complete cessation of insulin production. T2DM is more prevalent in adults and is associated with obesity, reduced physical activity, and genetic predisposition. In this form, insulin may be produced in sufficient quantities, but target cells exhibit decreased sensitivity, leading to the development of insulin resistance (Dedov et al., 2023, American Diabetes Association, 2024).

If diabetes remains uncontrolled over time, various systemic complications may develop, including angiopathies, nephropathy, retinopathy, and cardiovascular diseases, such as myocardial infarction and stroke (Tomic et al., 2022). Recent studies also indicate that immune system function is altered even in T2DM, playing a significant role in the pathogenesis of these complications. While disruptions in carbohydrate metabolism and insulin deficiency affect immune responses, the immune system itself actively contributes to the progression of diabetes.

T1DM is an autoimmune disease in which the body's immune system recognizes insulin-producing β-cells of the pancreas as foreign and destroys them. T-lymphocytes play a key role in this autoimmune reaction, recognizing antigens on the β-cell surface and initiating cytotoxic responses (Chen et al., 2024; Esser et al., 2014). In T2DM, hyperglycemia and insulin resistance exert systemic effects on the immune system by reducing lymphocyte functional activity and altering cytokine balance, thereby weakening the body's defense against infectious agents.

As a result, individuals with diabetes show increased frequency and severity of infections affecting the skin, genitourinary system, and respiratory tract. Additionally, T-cell dysfunction is commonly observed in diabetic patients, further contributing to impaired immune responses and the development of chronic subclinical inflammation (Todd,2010; Tkachuk et al., 2014). Chronic hyperglycemia also induces oxidative stress and elevates lipid peroxidation, adversely affecting immune cell membrane structure and energy metabolism, which reduces the effectiveness of immune responses.

Thus, diabetes mellitus is not only a metabolic disorder but also a pathology that exerts complex effects on the immune system. This dual impact contributes to the increased incidence of infectious and inflammatory complications in patients, as well as reduced efficacy of therapeutic interventions, highlighting the importance of considering immune parameters in clinical management.

Study Objectives

The study of immunological parameters in experimental modeling of type 2 diabetes mellitus in animals.

Methods

To achieve the stated objective, an experiment consisting of four series was conducted on 40 male white laboratory rats weighing 200 ± 20 g, maintained under vivarium conditions. Group I served as the control, while Group II included animals in which type 2 diabetes mellitus (T2DM) was experimentally induced. The rats were euthanized under ether anesthesia using a partial decapitation method, in accordance with the requirements of the local ethics committee and the international principles of humane treatment of animals outlined in the Helsinki Declaration (World Medical Association, 2002).

Type 2 diabetes mellitus was induced using streptozotocin (MP Biomedicals, USA), a compound that selectively targets pancreatic β-endocrinocytes. The drug was administered intraperitoneally at a dose of 30 mg/kg. This model is considered the closest pathogenetically relevant representation of human type 2 diabetes. To confirm the development of T2DM, fasting blood glucose levels were measured two weeks after streptozotocin administration. The inclusion criterion for the T2DM group was a glucose concentration exceeding 7.0 mmol/L.

Assessment of Metabolic Parameters. Blood glucose levels were determined using an express method with a glucometer. Glycated hemoglobin (HbA1c) was measured as an indicator of persistent hyperglycemia. The

functional activity of pancreatic β-cells and the degree of insulin resistance were assessed by measuring C-peptide levels in serum and immunoreactive insulin concentrations in plasma using specific assay kits.

Assessment of Immunological Parameters. Total leukocyte and lymphocyte counts in peripheral blood were determined for all experimental animals. The numbers of B- and T-lymphocytes and their subpopulations were assessed using immunofluorescent staining with FITC-conjugated antibodies. Stained cells were examined under a fluorescence microscope. For quantitative analysis, the immunoregulatory index (IRI) was calculated. The leukocyte migration inhibition reaction (LMIR) was performed using phytohemagglutinin (PHA) (Clausen, 1975). The concentration of circulating immune complexes (CIC) was determined (Jin et al., 2024), and neutrophil activity was assessed using the nitroblue tetrazolium (NBT) test (Damle et al., 2022).

Statistical Analysis. Statistical analysis was performed using STATISTICA 8.0 software. Group data were presented as mean ± standard deviation (M ± SD). The significance of differences between groups was evaluated using Student's t-test.

Results and Discussion

Metabolic Alterations. In the diabetic group of rats, a significant 20% increase in blood glucose levels was observed ($p<0.05$), reflecting the development of persistent hyperglycemia characteristic of this pathology. Simultaneously, the concentrations of immunoreactive insulin and C-peptide were elevated ($p<0.05$), which may indicate compensatory hypersecretion of insulin by pancreatic β-cells in response to developing insulin resistance. The level of glycated hemoglobin showed a tendency to increase, confirming the chronic nature of carbohydrate metabolism disturbances. Together, these changes correspond to the metabolic profile of the early stages of type 2 diabetes mellitus, when hyperglycemia is accompanied by compensatory stress on the insulin system (Table1).

Table 1. Changes in metabolic parameters of the experimental animals

Indications	I- control group	II – Experimental group
Glucose concentration, mmol /L	6,02±0,32	7,25±0,52*
Glycated hemoglobin, %	2,85±0,22	3,35±0,23
Immunoreactive insulin, ng /mL	0,64±0,09	0,87±0,07*
C-peptide, ng /mL	2,01±0,2	2,95±0,19*

Note: The difference is significant compared to the control group * - $p<0,05$; ** - $p<0,01$.

The elevation of glucose and glycated hemoglobin not only reflects impaired glucose utilization by tissues but also the activation of non-enzymatic protein glycation processes, leading to the formation of advanced glycation end-products (AGEs). These compounds can cause vascular damage, enhance oxidative stress, and activate inflammatory signaling pathways. Even moderate hyperglycemia can initiate a cascade of secondary pathological reactions, subsequently exacerbating endothelial dysfunction and metabolic instability.

The increased concentrations of immunoreactive insulin and C-peptide in diabetic animals are likely associated with compensatory hyperfunction of pancreatic β-cells. At the early stages of type 2 diabetes, β-cells attempt to overcome reduced insulin sensitivity of peripheral tissues by increasing insulin secretion; however, prolonged secretory stress ultimately depletes the insulin pool and reduces the functional reserve of the pancreas. Elevated C-peptide levels confirm preserved proinsulin secretion and reflect an adaptive response to rising insulin resistance.

Thus, the metabolic alterations observed in animals with experimental type 2 diabetes are characterized by chronic hyperglycemia, hyperinsulinemia, and activation of protein glycation processes, collectively creating a predisposition to systemic metabolic and inflammatory disturbances. These findings are consistent with literature reports indicating that prolonged carbohydrate metabolism impairment in diabetic animals leads to a complex of biochemical and endocrine adaptations aimed at maintaining energy homeostasis but with pathological consequences at tissue and organ levels.

Immune System Changes. Immunological parameters demonstrated pronounced shifts in the cellular component of the immune system. In diabetic animals, total leukocyte and lymphocyte counts were decreased ($p<0.05$), indicating reduced overall immune reactivity. Notably, T-lymphocytes (CD3+) and T-helper cells (CD4+) were significantly reduced in both absolute and relative terms ($p<0.05$), reflecting suppression of the T-cell compartment and disruption of cooperation among immune-competent cells. Meanwhile, T-suppressor cell

(CD8+) levels remained largely unchanged, leading to a reduction in the immunoregulatory index (CD4/CD8) and a shift of immune balance toward suppressor influence (Table 2).

Table 2. Changes in immune system parameters of the experimental animals

Indications		I- control group	II – Experimental group
WBC, $\times 10^9$/l	Abs. number	6,26±0,36	5,20±0,37 *
Lymphocytes, $\times 10^9$/l	Abs. number	2,61±0,16	2,18±0,14 *
	%	35,78±2,17	33,64±2,05
T-lymphocytes (CD3+), $\times 10^9$/l	Abs. number	1,58±0,09	1,30±0,10 *
	%	29,55±1,47	24,63±1,83 *
T-helpers (CD4+), $\times 10^9$/l	Abs. number	0,75±0,05	0,61±0,04 *
	%	18,48±1,54	14,58±1,13*
T-suppressors (CD8+), $\times 10^9$/l	Abs. number	0,56±0,03	0,50±0,03
	%	11,43±0,63	10,11±0,64
B-lymphocytes (CD20+) $\times 10^9$/l	Abs. number	0,43±0,03	0,48±0,03
	%	6,64±0,48	7,76±0,53
IRI (CD4/CD8)	-	1,34±0,07	1,22±0,08
LMIR	index	0,88±0,05	1,04±0,06 *
Circulating immune complexes (CIC)	conditional unit	1,36±0,08	1,12±0,08 *
Nitro blue tetrazolium (NBT-test)	%	3,78±0,26	2,44±0,18 **

Note: The difference is significant compared to the control group * - $p<0,05$; ** - $p<0,01$.

Increases in the lymphocyte mitogenic response index (LMTR) and decreases in antibody-forming cell (AFC) responses, as well as a significant reduction in natural killer (NK) cell activity ($p<0.01$), indicate impaired phagocytic and cytotoxic functions of innate immune cells. These changes are likely associated with oxidative stress, hyperglycemia, and disrupted lymphocyte metabolism in chronic diabetes. The reduction in CD3+ and CD4+ cells aligns with literature describing immunodeficiency in prolonged type 2 diabetes, which is linked to thymic dysfunction and T-cell apoptosis.

Chronic hyperglycemia leads to the accumulation of glycated proteins and lipid peroxides, acting as autoantigens and causing dysfunction of T- and B-lymphocytes. In type 2 diabetes, the production of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) is activated, promoting chronic subclinical inflammation and secondary exhaustion of immune responses. The decrease in T-helper cells with preserved B-lymphocytes (CD19+) indicates disrupted coordination between cellular and humoral immunity, reducing lymphocyte responsiveness to antigenic stimulation and potentially increasing susceptibility to infections.

Weakened NK-cell activity and reduced phagocytic capacity further indicate impaired innate immune defense. These alterations are likely related to membrane damage and impaired energy metabolism of immune cells under chronic oxidative stress. Hyperglycemia and elevated reactive oxygen species reduce the expression of pattern recognition receptors (PRRs) on monocytes and macrophages, impairing their phagocytic and antigen-presenting capabilities.

The results confirm the presence of systemic immune dysfunction in experimental type 2 diabetes. Metabolic disturbances, including hyperglycemia and hyperinsulinemia, are closely associated with immunodeficiency, forming a vicious cycle that exacerbates pathogenic processes. The observed combination of chronic metabolic stress and impaired immune competence may contribute to the development of diabetic complications, including increased susceptibility to infections, delayed wound healing, and chronic inflammatory foci. These findings highlight the interplay between metabolic and immune dysregulation, emphasizing the importance of targeting both pathways for therapeutic interventions.

Conclusion

Based on the data obtained, the following conclusions can be drawn regarding the complex alterations in animals with experimental type 2 diabetes mellitus. Experimental hyperglycemia was accompanied by a significant increase in blood glucose, immunoreactive insulin, and C-peptide levels, reflecting a state of compensated insulin resistance and β-cell activation in the pancreas. Concurrently, there was a trend toward increased glycated hemoglobin, indicating the chronic nature of carbohydrate metabolism disturbances and the

activation of non-enzymatic protein glycation processes, which contribute to oxidative stress and subclinical inflammation.

Metabolic disturbances were directly associated with immunodeficient changes. A decrease in the number of leukocytes, lymphocytes, T-lymphocytes (CD3+), and T-helper cells (CD4+) combined with a preserved level of T-suppressors (CD8+) resulted in immune imbalance and reduced immunoregulatory index (CD4/CD8). At the same time, reduced natural killer cell activity and phagocytic capacity indicate impaired innate immunity. These alterations can be explained by the effects of hyperglycemia and glycated products on immune cell membranes, as well as the activation of pro-inflammatory cytokines, which create a state of chronic low-grade inflammation.

Thus, experimental type 2 diabetes mellitus in rats is accompanied by the formation of a complex metabolic and immune homeostasis imbalance. Metabolic disturbances promote the development of immunodeficiency, while impaired immune defense further exacerbates the pathogenic processes of diabetes. These findings highlight the interrelationship between hyperglycemia, insulin resistance, and cellular immune dysfunction, which is crucial for understanding the mechanisms of diabetic complications and developing therapeutic strategies aimed at correcting both metabolic and immune disturbances.

Scientific Ethics Declaration

* The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

Conflicts of Interest

* The authors declare no conflict of interest.

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Author(s) Information

Oralbek Ilderbayev
L.N. Gumilyov Eurasian National University
010008 Astana, Satpayev 2, Kazakhstan.
Contact e-mail: *oiz5@yandex.ru*

Kerim Mutig
I.M. Sechenov First Moscow State Medical University, Russia

Darkhan Uzbekov
L.N. Gumilyov Eurasian National University
010008, Kazakhstan, Astana, Satpayev 2 Kazakhstan

Gulzhan Ilderbayeva
L.N. Gumilyov Eurasian National University
010008, Kazakhstan, Astana, Satpayev 2, Kazakhstan

Batyrlan Ayash
L.N. Gumilyov Eurasian National University
010008, Astana, Satpayev 2 Kazakhstan

Zarina Amanova
L.N. Gumilyov Eurasian National University
010008, Astana, Satpayev 2 Kazakhstan

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