Acta Scientific Gastrointestinal Disorders (ISSN: 2582-1091)

Volume 5 Issue 6 June 2022

Research Article

Study of the Role of Fas-Mediated Apoptosis of Peripheral Blood T-Lymphocytes in the Pathogenesis of Type 1 Diabetes Mellitus

Lugovaya AV[1]*, Mitreikin VPH[2] and Kalinina NM[3]

[1]*Pavlov State Medical University, Saint Petersburg, Russian Federation*

[2]*Professor, Department of Pathological Physiology, Pavlov State Medical University, Saint Petersburg, Russian Federation*

[3]*Professor, Department of Immunology, Pavlov First Saint Petersburg State Medical University, Leading Research Associate, Nikiforov Russian Center of Emergency and Radiation Medicine, Saint Petersburg, Russian Federation*

***Corresponding Author:** Lugovaya AV, Pavlov State Medical University, Saint Petersburg, Russian Federation.

DOI: 10.31080/ASGIS.2022.05.0437

Received: April 29, 2022

Published: May 30, 2022

Abstract

Introduction: It is known that impaired activation of Fas-mediated apoptosis in certain subpopulations of T cells plays an important role in the pathogenesis of type 1 diabetes mellitus (T1DM). The key point in the initiation of CD-1 is the resistance to apoptosis of activated autoreactive T-lymphocytes, which migrate from the bloodstream to the pancreas and are actively involved in the destruction of b-cells. It has been established that the Fas/FasL system plays a central role in maintaining peripheral autotolerance and tissue homeostasis of the body. Fas-mediated apoptosis is induced by binding of the Fas (CD95/APO-l/TNFRSF6)-receptor to the Fas(CD95L/CD178/TNFSF6)-ligand on the respective cells. Triggering the expression of cell surface Fas receptors (Fas) regulates the elimination of autoreactive T and B lymphocytes by apoptosis. To date, most of the results on the study of Fas-mediated apoptosis in T1DM have been obtained in experiments *in vitro*. There is no doubt that autoimmune changes *in vivo* are more profound, and the extrapolation of effects detected in the *in vitro* system to the body is not always valid.

The Aim: of the study was to evaluate the effectiveness of Fas-mediated apoptosis of T-lymphocytes in the blood of patients with T1DM depending on the phase of compensation and the duration of the course of the disease, as well as in individuals with a high risk of developing T1DM.

Material and Methods: We examined 63 patients with a reliably established diagnosis of T1DM and 15 people with a high risk of developing T1DM. The control group consisted of 30 healthy individuals, comparable in sex and age to patients with T1DM. Biomarkers of Fas-mediated apoptosis of peripheral blood lymphocytes in patients with T1DM and those at high risk of developing T1DM were studied. The surface expression of the Fas receptor in individual subpopulations of T-lymphocytes was assessed by flow cytometry. The concentration of soluble forms of the Fas receptor (sFas) and Fas ligand (sFasL) in the blood serum of the examined patients was studied by indirect enzyme-linked immunosorbent assay (ELISA).

Results: It has been established that in patients in the decompensation phase of T1DM, inhibition of Fas-mediated apoptosis of autoreactive CD95+ cells occurs with the participation of the soluble form of the Fas receptor (sFas-soluble Fas). When carbohydrate metabolism is compensated, apoptosis of lymphocytes is observed along the Fas pathway with the help of a soluble form of the Fas

ligand (sFasL-soluble FasL). In the compensation phase of T1DM and in individuals at risk, an increase in the content of sFasL was revealed. This probably has a protective value, since, according to the literature, sFasL is involved in the removal of autoreactive CD95+ cells in the peripheral blood.

Conclusions: The results obtained indicate a pronounced dysregulation in the Fas/FasL system, which is observed at all stages of the development of T1DM. The study found that the regulation of the FasL/Fas system affects the tropism of autoreactive T cells to islet antigens in patients with T1DM, and that Fas-induced death of β-cells mediated by T lymphocytes plays a significant role in the development and progression of T1DM, both in the latent stage and at the stage of advanced clinical manifestations of the disease.

Key words: Apoptosis; Type 1 Diabetes Mellitus; Death Receptors; Fas-Mediated Apoptosis; Fas Receptor; Fas Ligand; T Lymphocytes; B -Cells of the Pancreas.

Introduction

Type 1 diabetes mellitus (T1DM) is an organ-specific autoimmune disease that develops as a result of selective destruction of β-cells of the pancreatic islet apparatus by cytotoxic T lymphocytes (CTLs), type 1 T-helpers (Th1), and autoantibodies [1]. The onset of the disease is preceded by a long asymptomatic period - the pre-diabetic stage, characterized by the production of autoantibodies specific to islet autoantigens, including insulin. The inflammatory reaction in the islet microenvironment contributes to the induction and enhancement of the immune attack against pancreatic β-cells, and, at later stages, to the stabilization and maintenance of inflammatory infiltrates - insulitis [1,2]. During the pre-diabetic stage, autoreactive T cells begin to destroy β-cells, leading to a progressive loss of their secretory function. Clinical manifestation of T1DM occurs when 80-90% of β-cells are destroyed [2].

Despite the active study of the immunopathogenesis of T1DM, many key points in the development and progression of this disease remain unclear. The problems of early diagnosis of T1DM, ensuring a stable course of the disease and combating its secondary complications are still relevant [3,4]. In connection with the trend towards "rejuvenation" of the age of patients with T1DM, leading to early disability, an increase in the incidence rate, clarification of the mechanisms of immunopathogenesis and the development of new methods for the timely diagnosis of T1DM are of particular relevance [4,5].

In the immune system, Fas receptor (Fas) and Fas ligand (FasL) are involved in the regulation of immune responses and in T lymphocyte-mediated cytotoxicity. Fas-mediated apoptosis is the initiating phase of apoptotic signal transduction and refers to an external (receptor) pathway for triggering cell death [6].

Depending on the triggering mechanism of the apoptotic cascade, there are two main signaling pathways leading to the induction of apoptosis in mammalian cells: extrinsic (receptor) and intrinsic (mitochondrial or Bcl-2-regulated) [7,8]. The extrinsic pathway is mediated by DR (Death Receptor) cell receptors, which include the Fas receptor (Fas). Fas contains the domain DD (Death Domain), the central physiological regulator of apoptosis, in its cytoplasmic part [4,9]. Binding of Fas to the Fas ligand (FasL) activates the Fas-associated death domain adapter protein FADD (Fas-Associated Death Domain protein), which leads to the formation of the DISC (Death Initiating Signaling Complex) effector complex, a death initiating signaling complex [7,8,10]. DISC activates procaspase-8, which undergoes autocatalytic cleavage and becomes active. Thus, the interaction of Fas with FasL leads to the formation of active caspase-8, which belongs to the group of initiating caspases [4,11].

The Fas (CD95/APO-1/TNFRSF6) antigen is a membrane protein that is a member of the tumor necrosis factor receptor superfamily (TNFRSF-Tumor Necrosis Factor Receptor Super Family) and belongs to the DR (Death Receptor) group of cell receptors [12]. The latest literature reports that Fas is normally ubiquitously expressed in human tissues at the basal level, and its activation threshold must be strictly regulated to avoid excessive cell death [12,13]. Recently, negative regulators of FasR have been identified, the main of which is the FLIP (Flice-like inhibitory protein), which

is an inhibitor of caspase-8. A change in the cell balance between caspase-8 and its regulator FLIP can switch Fas-mediated signaling from apoptosis to proliferation and vice versa [4,11,12]. There is a functionally active soluble form of Fas, which is the result of proteolytic cleavage of membrane-bound receptors or is formed during alternative splicing [14].

Fas/APO-l-ligand (FasL/CD178/TNFSF6) is a membrane protein and is a member of the tumor necrosis factor (TNF) superfamily of ligands, which belong to cytokines [6,24]. Like the TNF ligand, FasL can be released from the cell surface and be physiologically active in a soluble form [14-16].

Fas-mediated apoptosis ensures the elimination of cells of the immune system that are undesirable for the body, and is also involved in the regulatory suppression of immune responses and cytotoxicity of T-lymphocytes [9]. If there is a defect in the Fas/FasL system, activated lymphocytes can accumulate, which leads to the development of autoimmune lymphoproliferative syndrome (ALPS), a group of genetic diseases characterized by mutations in the Fas and FasL genes, autoimmune disorders, and an increased tendency to develop malignant lymphoid formations [17]. The pathogenesis of ALPS is based on a genetically determined impairment of Fas-mediated apoptosis of activated T cells, leading to the appearance of double negative T lymphocytes (CD3+ CD4-CD8-) in the peripheral blood [6].

In modern literature, the role of death receptors in the destruction of β-cells in type 1 diabetes mellitus (T1DM) is widely discussed [2,4,9]. Studies using isolated human pancreatic islets have shown that exposure to stress factors (hyperglycemia, excess free radicals, reactive oxygen species, production of IL-1β by microenvironment cells) enhances Fas expression on b-cells, which leads to their death by mechanism of the Fas-mediated apoptosis [9]. Immunocompetent cells infiltrating pancreatic islet tissue produce pro-inflammatory cytokines: IL-1 β, TNF-a, and IFN-γ, which are known for their pro-apoptogenic properties [18,19,20]. IL-1β induces an increase in Fas expression on b-cells, which increases their readiness for Fas-mediated apoptosis, which is realized by autoreactive T cells expressing FasL. Activated cytotoxic T lymphocytes (CD8+CTL), which are part of the inflammatory infiltrates of the pancreas (insulitis), can also destroy β -cells in a Fas-dependent receptor way [2,9,21].

An increase in the expression of the Fas receptor in certain subpopulations of T lymphocytes in the peripheral blood of T1DM patients indicates their increased readiness for apoptosis. At the same time, this cannot guarantee the successful implementation of Fas-mediated apoptosis of these cells, since the soluble form of the Fas receptor competes with the membrane Fas receptor for binding of the Fas ligand and can block apoptosis of Fas-«positive» T cells [14]. It is necessary to consider the pathogenetic significance of soluble forms of Fas and FasL in the development of autoimmunity in T1DM, since the diagnostic and prognostic significance of these indicators of Fas-mediated apoptosis is actively studied in systemic and organ-specific autoimmune diseases, in sepsis, acute renal failure, and oncological diseases [15,16,22]. A comprehensive assessment of the effectiveness of Fas-mediated apoptosis with the determination of all markers involved in this variant of the receptor pathway for triggering the apoptotic program can provide a more accurate understanding of the mechanisms and significance of Fas/FasL system dysregulation in the pathogenesis of T1DM.

According to experts of the World Health Organization, T1DM will reach pandemic proportions over the next few decades [23]. Therefore, early diagnosis and prevention of the disease in people with a high risk of developing T1DM is of particular importance. In this regard, it seems relevant to assess the markers of Fas-mediated apoptosis of lymphocytes not only in patients with T1DM, but also in individuals with a high risk of developing autoimmune diabetes.

The Aim

To study the features of Fas-mediated apoptosis of peripheral blood lymphocytes in patients with DM-1 depending on the phase of compensation and the duration of the course of the disease, as well as in individuals with a high risk of developing T1DM.

Materials and Methods

Characteristics of the examined groups of patients. We examined 63 patients with a reliably established diagnosis of T1DM and 15 individuals with a high risk of developing T1DM. The control group (Group I) consisted of 30 healthy individuals, comparable in sex and age to patients with T1DM. The distribution of patients into groups was carried out depending on the phase of compensation and the duration of the course of the disease. Group II (decompensated T1DM) consisted of 17 patients with newly diagnosed T1DM (group IIa) and 19 patients with an average duration of T1DM of

15.3±5.1 years (group IIb). Group III (the state of compensation for T1DM) included 13 patients with a disease duration of up to 1 year (group IIIa) and 14 patients with an average duration of T1DM of 15.1±5.4 years (group IIIb). Group IV consisted of 15 persons with a high risk of developing T1DM, who are immediate family members of the examined patients with T1DM. Additional selection criteria for the risk group were an increased titer (> 1/20) of autoantibodies to cytoplasmic antigens of islet cells (Islet Cell Autoantibodies) in serum and impaired glucose tolerance. ICA can serve as a predictor of the disease in relatives of patients with type 1 diabetes, since they appear in the blood 1-8 years before the clinical manifestation of diabetes mellitus [2,24]. The characteristics of the groups of examined patients with T1DM are presented in table 1.

Main characteristics of the patient's groups		**The state of decompensation, newly diagnosed T1DM decompensation (IIa group)**	**The state of decompensation, the average duration of the T1DM is 15.3 ± 5.1 years (IIb группа)**	**The state of compensation, the average duration of the T1DM is 0,6 ± 0,2 years (IIIa группа)**	**The state of compensation, the average duration of the T1DM is 15,1 ± 5,4 years (IIIb группа)**
Number of patients in the group (n)		17	19	13	14
Age (years)		22,8 ± 3,9	44,6 ± 5,4	24,1 ± 4,2	42,9 ± 4,5
Sex	Men	10	11	5	6
	Females	7	8	8	8

Table 1: Characteristics of the examined groups of patients with T1DM.

Immunophenotyping of peripheral blood mononuclear cells was performed by flow cytometry using the following monoclonal antibodies manufactured by «Immunotech» (Beckman Coulter Corporation, USA): anti-CD3 conjugated with FITC (Fluorescein Isothiocyanate), anti-CD4-FITC, anti-CD8-FITC, anti- CD16-FITC, anti-CD20-FITC, anti-CD25-FITC, anti-HLA-DR-FITC, anti-CD95-FITC and their isotype controls.

To assess apoptotic processes in individual subpopulations of T lymphocytes, the level of surface expression of the Fas receptor was determined using a double fluorescent label - FITC (Fluorescein Isothiocyanate) and PE (Phycoerythrin). The study was performed using the following combinations of antibodies: anti-CD3-FITC/anti-CD95-PE, anti-CD4-FITC/anti-CD95-PE and anti-CD8-FITC/anti-CD95-PE. Cytometric analysis of lymphocytes was performed on an EPICS XL flow cytometer (Beckman Coulter Corporation, USA).

Determination of soluble forms of the Fas receptor (sFas) and Fas ligand (sFasL) in blood serum was carried out by indirect enzyme-linked immunosorbent assay (ELISA) using the «Human sFas Ligand ELISA» test systems (Bender MedSystems, Austria) and «Human Fas ELISA» test systems (BD Biosciences, USA).

For statistical processing of the obtained data, the nonparametric Wilcoxon-Mann-Whitney test was used to compare the means. The material was processed using the Statistica 10.0 software package (StatSoft, USA, Windows 10). The critical confidence level of the null hypothesis (the absence of significant differences) was taken equal to 0.05.

Research Results

Immunophenotyping of peripheral blood lymphocytes showed a statistically significant increase in the relative and absolute amount of CD95+ - lymphocytes in all groups of T1DM patients compared with the control group (group I). The data obtained indicate an increased activation and readiness of immunocompetent cells for apoptosis in T1DM (Table 2).

The maximum increase in the relative and absolute amount of CD95+ cells and the number of T-lymphocytes expressing the Fas

Groups	CD95+ - CELLS		CD3+CD95+ - lymphocytes	CD4+CD95+ - lymphocytes	CD8+CD95+ - lymphocytes
	The relative amount, %	The absolute amount, mm3	The relative amount, %		
I	4,2	80	3,0	2,3	0,9
IIa	12,4**	260**	10,8**	7,3**	6,0***
IIb	13,2**	222**	11,1**	8,1**	6,7***
IIIa	8,6*	212*	7,3*	5,1*	3,9**
IIIb	8,2*	152*	6,9*	4,8*	3,7**
IV	4,9	90	3,5	3,0	2,1*

Table 2: The relative and absolute amount of CD95+ - cells and T-lymphocytes expressing the Fas receptor in the peripheral blood of patients with DM-1 and persons at risk.

Notes: 1) *Differences in the studied indicator with the control group (I) are statistically significant ($p < 0,05$); 2) **differences in the studied indicator with the control group (I) are statistically significant ($p < 0,01$); 3) *** differences in the studied indicator with the control group (I) are statistically significant ($p < 0,001$).

receptor (Fas) was found in T1DM decompensation, regardless of the duration of the disease (groups IIa and IIb). These data indicate that in T1DM, an increase in the readiness of immunocompetent cells for apoptosis does not depend on the duration of the disease but is clearly associated with the decompensation of carbohydrate metabolism and the level of glycemia.

Among the subpopulations of T-lymphocytes, a significant increase in the percentage of $CD8^+CD95^+$-cells from the total amount of cytotoxic T-lymphocytes ($CD8^+$CTLs) in all groups of examined patients should be noted compared to the control group (Figure 1). The increase in the relative amount of Fas-expressing $CD8^+$CTLs is most pronounced with disease decompensation (groups IIa and IIb). An increase in the number of cells with the $CD8^+CD95^+$ phenotype in T1DM is probably a compensatory mechanism aimed at the elimination of autoreactive cytotoxic T-lymphocytes by apoptosis. According to the literature, it is the effector $CD8^+$CTLs that play the dominant role in the destruction of pancreatic β-cells [2,9].

At the same time, a significant increase in the number of cells with the $CD8^+CD95^+$ phenotype in the blood of individuals with a high risk of developing T1DM (group IV) compared with the control group (group I) is an unfavorable prognostic factor. These data indicate that already in the latent stage of T1DM there is an expansion of autoreactive clones of cytotoxic T-lymphocytes in peripher-

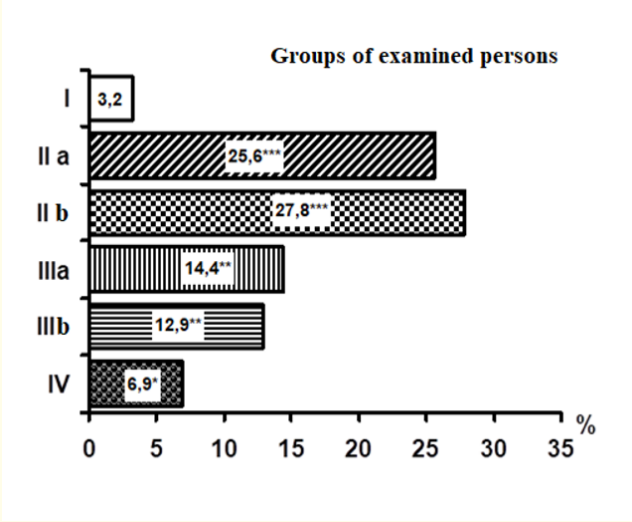

Figure 1: Percentage of CD8+CD95+ cells from the total number of cytotoxic T-lymphocytes.

Notes: 1) *Differences in the studied indicator with the control group (I) are statistically significant ($p < 0,05$); 2) **differences in the studied indicator with the control group (I) are statistically significant ($p < 0,01$); 3) *** differences in the studied indicator with the control group (I) are statistically significant ($p < 0,001$).

al blood, followed by their migration to the target organ (pancreas), which leads to the progression of the autoimmune process [1,2,22].

The study found that the concentration of soluble forms of the Fas receptor (sFas-soluble Fas) and Fas-ligand (sFasL-soluble FasL) in the blood serum of T1DM patients did not depend on the duration of the disease but changed depending on the state of carbohydrate metabolism compensation (table 3). In patients with T1DM in the phase of decompensation of the disease (group II), a significant increase in the concentration of sFas was observed in comparison with the control group (group I) and other examined groups of patients. The results obtained are consistent with literature data on the relationship between an increase in the concentration of sFas in the serum of patients and worsening of the course of the disease.

[14-16]. It has been established that a high level of serum sFas correlates with the severity of the septic process [14]. It has been shown that the content of sFas in the blood increased with the progression of renal dysfunction in patients with acute kidney injury [15,16]. According to the literature, the soluble form of Fas competes with the membrane Fas receptor for ligand binding, which prevents the "physiological" apoptosis of cells to be eliminated. With an increase in the concentration of sFas in circulation, not all “defective” cells can implement their apoptotic program. As a result, they accumulate in the peripheral blood, which leads to an aggravation of the pathological process.

Indicator	Control group (n = 8)	Patients with T1DM		Individuals with a high risk of developing T1DM (n = 15)
		The state of decompensation (n = 26)	The state of compensation (n = 24)	
	I	II	III	IV
sFas (pg/ml)	778	1501*	789	864
sFasL (ng/ml)	0,102	0,118	0,243*	0,403**

Table 3: The concentration of the soluble form of the Fas receptor and Fas ligand.

in the blood serum of patients with T1DM and in individuals with a high risk of developing T1DM

Notes: 1) *differences in the studied indicator with the control group (I) are statistically significant ($p < 0,05$); 2) **differences in the studied indicator with the control group (I) are statistically significant ($p < 0,01$).

The concentration of sFasL in the T1DM compensation group was significantly higher than in the decompensation group and significantly lower than in the risk group (Table 3). It should be noted a significant increase in the level of sFasL in the risk group, which is consistent with the literature data [22]. According to the authors, an increase in the level of sFasL in the latent stage of T1DM has a protective value and is aimed at eliminating autoaggressive lymphocyte clones by Fas-mediated apoptosis.

Thus, the results obtained indicate a pronounced dysregulation in the Fas/FasL system, which is observed at all stages of the development of T1DM.

Discussion

Dysfunction of the Fas receptor (Fas) and Fas ligand (FasL), as key inducers of receptor-dependent apoptosis, are actively studied in the pathogenesis of diseases associated with both inhibition and enhancement of apoptosis in cells of shock organs [6,25]. It has recently been established that Fas-mediated caspase-8 activation plays an important role in the regulation of pathogenetic mechanisms in bacterial infections [11]. A pronounced increase in the expression of Fas and FasL membrane forms on peripheral blood lymphocytes was found in patients with coronary heart disease, with acute cerebrovascular accident, in patients with viral and bacterial infections, with sepsis, with human immunodeficiency virus

[4,11,14,26]. It has been shown that an increase in the soluble form of FasL in the blood is one of the early markers of heart failure progression [26]. In our previous studies, we showed a significant increase in the content of $CD95^+$-mononuclear cells in the blood of patients with acute coronary syndrome (ACS) [27]. We found that oxidized low-density lipoproteins induce Fas-mediated apoptosis of both peripheral blood mononuclear cells and coronary endotheliocytes, which indicates the systemic nature of apoptosis in ACS [27,28].

In the pathogenesis of type 1 diabetes mellitus (T1DM), disturbances in Fas-mediated apoptosis are of a bivalent nature. So, if in relation to β-cells the development of apoptosis is associated with the progression of the disease, then from the point of view of the elimination of activated autoreactive lymphocytes, apoptosis is desirable and can slow down the destruction of pancreatic b-cells. The dual role of the receptor (external) pathway for triggering apoptosis in the pathogenesis of T1DM is due to the expression of its mediating molecules (Fas and FasL) both by effector cells (autoreactive T cells) and target cells (pancreatic β-cells) [9]. Identification of the relationship between the features of Fas-mediated apoptosis of peripheral blood lymphocytes and the state of carbohydrate metabolism compensation, the duration of autoimmune destruction of the pancreatic insular apparatus in patients with type 1 diabetes can contribute to a deeper understanding of the pathogenesis of the disease, which determined the purpose of this study. To assess the role of impaired apoptosis of peripheral blood mononuclear cells in the progression of the autoimmune process at the preclinical stage of diabetes, a group of individuals with a high risk of developing T1DM was examined.

The study found that in T1DM there is an increased readiness of immunocompetent cells for apoptosis, as evidenced by a significant increase in the number of $CD95^+$-cells in all examined groups of T1DM patients compared with the control group. The maximum increase in the relative and absolute content of $CD95^+$-cells and T-lymphocytes expressing the Fas receptor was found in T1DM decompensation, regardless of the duration of the disease. This is explained by the influence of hyperglycemia, which increases the sensitivity of peripheral blood lymphocytes to Fas-mediated apoptosis due to increased expression of the Fas receptor on their surface, and also induces p53-mediated apoptosis of target cells with the participation of effector caspase-3 [3,29-31].

Figure 2 shows a comparative assessment of the level of surface expression of the Fas receptor (CD95+) in individual subpopulations of T-lymphocytes in DM-1 patients in a state of carbohydrate metabolism decompensation and in the control group.

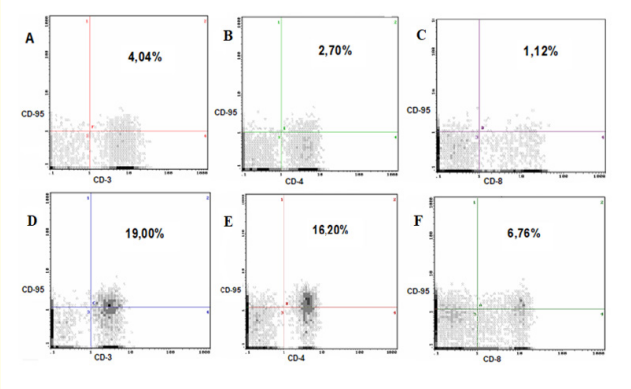

Figure 2: The histogram shows the number of $CD3^+CD95^+$-, $CD4^+CD95^+$- and $CD8^+CD95^+$-lymphocytes in the control group (A, B, C) and in the group of T1DM patients in the state of carbohydrate metabolism decompensation (D, E, F).

In all groups of examined patients, an increase in the percentage of $CD8^+CD95^+$-cells from the total number of cytotoxic T lymphocytes was observed compared with the control group, more pronounced with decompensation of the disease. This indicates the desire of the immune system to limit the activity of autoreactive cytotoxic T lymphocytes, which play a leading role in the destruction of the insular apparatus of the pancreas. For effective elimination of $CD95^+$-cells in the peripheral blood, it is necessary to maintain a balance between cells expressing Fas and FasL. It is important to consider the possible role of the soluble Fas receptor (sFas) in the inhibition of apoptosis via the Fas pathway [14,32].

Our results on an increase in the concentration of sFas during decompensation of T1DM are in accordance with the literature data reporting an increase in the concentration of the soluble form of Fas in some systemic and organ-specific autoimmune diseases [14-16]. The pronounced increase in the sFas level observed in our study against the background of a sharp increase in the number of $CD95^+$-cells in the blood of T1DM patients in a state of carbo-

hydrate metabolism decompensation indicates the inefficiency of Fas-mediated apoptosis due to inhibition of the elimination of $CD95^+$-cells along the Fas pathway. It has been shown that both membrane and soluble forms of the Fas ligand (sFasL) are involved in the removal of autoreactive human cells [12,14]. In the experiment, preliminary cultivation of diabetogenic T lymphocyte clones with sFasL completely inhibited the development of autoimmune diabetes in mice, which were then transplanted with diabetogenic T cells [9]. This means that the increase in in the concentration of sFasL in T1DM has a protective value and is aimed at establishing peripheral tolerance, which is necessary for protection against autoimmune aggression against β-cells. According to the literature, in individuals at high risk of developing T1DM, an increase in the concentration of sFasL was found against the background of a decrease in the number of autoreactive $CD4^+CD95^+$- and $CD8^+CD95^+$-lymphocytes in the blood, in connection with which the authors suggest the involvement of sFasL in the removal of pathogenic T cells on preclinical stage of the disease [22]. Therefore, the increase in in the concentration of sFasL that we have identified in patients with T1DM in the state of carbohydrate metabolism compensation and in the risk group, is a compensatory mechanism and plays a protective role.

Conclusion

These results suggest that the regulation of the FasL/Fas system affects the affinity of autoreactive T cells to islet antigens in T1DM patients, and that Fas-induced β-cell death mediated by T-lymphocytes plays a significant role in the development and progression of T1DM, both in the latent stage and at the stage of advanced clinical manifestations of the disease.

Thus, apoptosis is a highly regulated multi-step process that should be considered as a possible target for medical interventions. Changing the regular course of this process, its speed and outcome by blocking inducing and regulatory factors can make it possible to influence the dynamics of the development of T1DM, the frequency and duration of clinical remission. Treatment aimed at modulating apoptosis processes is promising [32]. In particular, as the study shows, Fas receptor and Fas ligand can act as potential therapeutic targets.

Conflict of Interest

The authors declare no conflict of interest.

Bibliography

1. Ryan A., *et al*. "Diabetes mellitus and apoptosis: inflammatory cells". *Apoptosis* 14.12 (2009): 1435-1450.
2. Vives-Pi M., *et al*. "How apoptotic β-cells direct immune response to tolerance or to autoimmune diabetes: a review". *Apoptosis* 20.3 (2015): 263-272.
3. Hammes HP. "Diabetic retinopathy: hyperglycaemia, oxidative stress and beyond". *Diabetologia* 61.1 (2018): 29-38.
4. Shukla V., *et al*. "Cerebral ischemic damage in diabetes: an inflammatory perspective". *Journal of Neuroinflammation* 14.21 (2017): 1-22.
5. Dedov II., *et al*. "National leadership". Moscow: GEOTAR-Media (2018): 596.
6. Khaitov RM. "Immunology: textbook". Moscow: GEOTAR-Media (2018): 496.
7. Green DR. "Cell death and the immune system: getting to how and why". *Immunological Reviews* 277.1 (2017): 4-8.
8. Sachet M., *et al*. "The immune response to secondary necrotic cells". *Apoptosis* 22.10 (2017): 1189-1204.
9. Joglekar MV., *et al*. "Human islet cells are killed by BID-independent mechanisms in response to FAS ligand". *Apoptosis* 21.4 (2016): 379-389.
10. Garg AD., *et al*. "Immunogenic versus tolerogenic phagocytosis during anticancer therapy mechanisms and clinical translation". *Cell Death and Differentiation* 23 (2016): 938-951.
11. Uchiyama R and Tsutsui H. "Caspases as the Key Effectors of Inflammatory Responses Against Bacterial Infection". *Immunologiae et Therapiae Experimentalis* 63.1 (2015): 1-13.
12. Rossin A., *et al*. "The Btk-dependent PIP5K1γ lipid kinase activation by Fas counteracts FasL-induced cell death". *Apoptosis* 22.11 (2017): 1344-1352.
13. Esposti MD., *et al*. "Changes in membrane lipids drive increased endocytosis following Fas ligation". *Apoptosis* 22.5 (2017): 681-695.

14. Bhatraju PK., *et al*. "Circulating levels of soluble Fas (sCD95) are associated with risk for development of a nonresolving acute kidney injury subphenotype". *Critical Care* 21.217 (2017): 1-9.

15. Liphaus BL., *et al*. "Increased serum sFas, sTRAIL, and reduced sFasL in juvenile-onset systemic lupus erythematosus". *Clinical Rheumatology* 36.12 (2017): 2847-2852.

16. Nabipour I., *et al*. "Influence of levothyroxine treatment on serum levels of soluble Fas (CD95) and Fas Ligand (CD95L) in chronic autoimmune hypothyroidism". *Endocrinology* 38 (3 (2010): 406-411.

17. Rieux-Laucat F., *et al*. "The Autoimmune Lymphoproliferative Syndrome with Defective FAS or FAS-Ligand Functions". *Journal of Clinical Immunology* 38.5 (2018): 558-568.

18. Hakonen E., *et al*. "MANF protects human pancreatic beta cells against stress-induced cell death". *Diabetologia* 61.10 (2018): 2202-2214.

19. Li X., *et al*. "IFNγ and TNFα synergistically induce apoptosis of mesenchymal stem/stromal cells via the induction of nitric oxide". *Stem Cell Research and Therapy* 10.18 (2019): 1-11.

20. Liang L., *et al*. "The suppressive effect of co-inhibiting PD-1 and CTLA-4 expression on H22 hepatomas in mice". *Cellular and Molecular Biology Letters*23.58 (2018): 1-11.

21. Blander JM. "The many ways tissue phagocytes respond to dying cells". *Immunological Reviews* 277.1 (2017): 158-173.

22. Tchorzewski H., *et al*. "Activated T lymphocytes from patients with high risk of type I diabetes mellitus have different ability to produce interferon-γ, interleukin-6 and interleukin-10 and undergo anti-CD95 induced apoptosis after insulin stimulation". *Immunology Letters* 75 (2001): 225-234.

23. Zhang M., *et al*. "MST1 coordinately regulates autophagy and apoptosis in diabetic cardiomyopathy in mice". *Diabetologia* 59.11 (2016): 2435-2447.

24. Abstracts of 52nd EASD Annual Meeting". *Diabetologia* 59.1 (2016): 581.

25. Zurochka AV., *et al*. "Flow Cytometry in biomedical research". *Yekaterinburg: RIO Uro RAN* 720 (2018).

26. Fefelova EV., *et al*. "Induction of lymphocyte apoptosis influenced by homocysteine contained in IHD patients' peripheral blood culture cell". *Online Scientific Educational Bulletin "Health and Education Millennium"* 18.11 (2016): 36-39. 2016.

27. Petrishchev NN., *et al*. "Content of soluble markers of apoptosis and circulating V annexin-connected apoptotic cells in the blood of patients with acute coronary syndrome". *VestnikSpbu (Russia)* 1 (2008): 14-24.

28. Vasina LV., *et al*. "Pathogenic significance of relative alteration in V-binding mononuclears and CD 59+-lymphocytes of peripheral blood in patients with acute coronary syndrome". *Medico-Biological and Socio-Psychological Problems of Safety in Emergency Situation (Russia)* 1 (2008): 74-80.

29. Baidwan S., *et al*. "Glucotoxicity promotes aberrant activation and mislocalization of Ras-related C3 botulinum toxin substrate 1 [Rac1] and metabolic dysfunction in pancreatic islet β-cells: reversal of such metabolic defects by metformin". *Apoptosis* 22.11 (2017): 1380-1393.

30. Cantley J., *et al*. "Disruption of beta cell acetyl-CoA carboxylase-1 in mice impairs insulin secretion and beta cell mass". *Diabetologia* 62.1 (2019): 99-111.

31. Sidarala V and Kowluru A. "Exposure to chronic hyperglycemic conditions results in Ras- related C3 botulinum toxin substrate 1 (Rac1)-mediated activation of p53 and ATM kinase in pancreatic β-cells". *Apoptosis* 22.5 (2017): 597-607.

32. Green DR., *et al*. "The clearance of dying cells: table for two". *Cell Death and Differentiation* 23 (2016): 915-926.