Chapter

The Role of Apoptosis in Autoimmune Destruction of Pancreatic b-Cells

Anna Vladimirovna Lugovaya, Vladimir Phylippovich Mitreikin and Natalia Mikhailovna Kalinina

Abstract

The purpose of this section of the monograph is to familiarize readers with the role of programmed cell death type 1—apoptosis in autoimmune destruction of the pancreas in type 1 diabetes mellitus (T1DM-1). The task of focusing the reader's attention on the mechanisms of pancreatic b-cells apoptosis is explained by the fact that the interest of scientists in this problem continues to grow. Sections of the chapter are devoted to the modern concept of T1DM-1 immunopathogenesis, the role of insufficient apoptosis of circulating effector T cells, on the one hand, and enhanced apoptosis of b-cells, on the other hand. Special attention is paid to the prospects for the treatment and prevention of T1DM. The chapter presents the results of experimental studies on the role of apoptosis in the immunopathogenesis of T1DM. Separately, the results of the authors' own studies are considered. The chapter was based on sources from international data bases: Scopus, Springer, PubMed. The authors express the hope that the chapter will contribute not only to a deeper understanding of the pathogenesis of T1DM, but also to arouse interest in the prospects for the treatment and prevention of this disease. The chapter is intended for students of medical universities and a wide range of readers with higher medical and biological education.

Keywords: apoptosis, autoimmune mechanisms of pancreatic destruction, efferocytosis, autoreactive T cells, proinflammatory cytokines, perforin, granzyme, T helpers, cytotoxic T cells, type 1 diabetes mellitus, C-peptide, b-cells

1. Introduction

The authors of this chapter have been dealing with programmed cell death in T1DM for many years. The area of scientific interests is the assessment of apoptosis of peripheral blood lymphocytes in the closest relatives of DM1 patients with a high risk of developing DM1. The risk group for DM1 was included by the first author of this chapter in her thesis on "Apoptosis of peripheral blood mononuclear cells in patients with type 1 diabetes mellitus."

The study of the mechanisms and significance of genetically programmed cell death began in the 1960s. The authorship of the term "apoptosis" belongs to the

English scientists—J. Kerr, E. Wylie and A. Kerry, who first put forward and substantiated the concept of a fundamental difference between physiological cell death (apoptosis) and their pathological death (necrosis). To date, tens of thousands of scientific papers have been devoted to the theory of apoptosis, revealing the main mechanisms of its development at the physiological, genetic, and biochemical levels. There is an active search for its regulators. Of particular interest are studies that allow the practical use of apoptosis regulation in the treatment of oncological, autoimmune, and neurodystrophic diseases.

Apoptosis (from the Greek ἀπόπτωσις—fall), the process of self-destruction or "leaf fall". However, not everything is so clear. Recent studies convincingly show that the processes of apoptosis and regeneration can be strongly interrelated. The removal of non-functioning cells by apoptosis can simultaneously serve as a signal for regenerative processes through the transmission of information through apoptotic bodies. The need for a clear coordination of tissue regeneration processes raises the question of the coordinators of this regulation and the signaling pathways through which different types of cells perform their functions in this process.

And since there is only one step from cell death to regeneration, the authors of the chapter are working hard to unravel the mystery: what molecules and what mechanisms can coordinate these processes? The issues of pancreatic cell regeneration in T1DM are the subject of research that we are currently doing in our laboratory.

2. Modern ideas about the immunopathogenesis of type 1 diabetes mellitus

Type 1 diabetes mellitus (T1DM) is an organ-specific autoimmune disease that develops as a result of the selective destruction of b-cells of the pancreatic islet apparatus by cytotoxic T lymphocytes (CTL), T helper type 1 (Th1) and autoantibodies [1, 2]. Together with interleukin 1b (IL-1b) and tumor necrosis factor α (TNF-α), interferon-g (IFN-g) is able to increase the expression of molecules of the major histocompatibility complex of the second class on pancreatic b-cells. This leads to recognizing them as alien [3, 4]. As a result of the gradual destruction of b-cells, insulin deficiency occurs, leading to a breakdown in glucose homeostasis and the manifestation T1DM. Clinically classic symptoms of T1DM—hyperglycemia and ketosis, appear only when 80–90% of the islets of Langerhans are destroyed [5].

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 [6]. The manifestation of T1DM is preceded by a long asymptomatic period—the prediabetic stage. It should be emphasized that during the asymptomatic stage T1DM there is an active production of autoantibodies to pancreatic b-cells and the formation of inflammatory infiltrates, the so-called "insulitis" [2]. 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 relevant.

The key point in the initiation of T1DM is the resistance of autoreactive T lymphocytes to apoptosis. Escape of autoreactive cells from immunological surveillance leads to active destruction of b-cells and subsequent manifestation of the disease [7, 8]. It has been established that in mice of the NOD (nonobese diabetic) line, which are a

model of spontaneous autoimmune diabetes similar to human T1DM, Th1-cells and cytotoxic T lymphocytes, even when the influence of IL-2, a factor that promotes the proliferation of T lymphocytes, is blocked on them exhibit increased resistance to apoptosis. At the same time, T-helpers are more resistant to apoptosis than cytotoxic T lymphocytes, which disrupts the normal balance of Th1-cells/cytotoxic T cells and contributes to the maintenance of autoimmune Th1-inflammation. Resistance of T lymphocytes of NOD mice to apoptosis is explained by increased expression of the apoptosis inhibitor Bcl-xL protein by T cells [9], as well as disturbances in the Fas/FasL system, which provides the receptor-mediated pathway for apoptosis [10].

Autoreactive lymphocytes resistant to apoptosis migrate from the bloodstream to the target organ—the pancreas and form insulitis, consisting of T and B cells, as well as macrophages, dendritic cells (DC), natural killer cells (NK cells) and natural killer T cells (T-NK cells) [2]. Immunocompetent cells infiltrating the islet tissue produce pro-inflammatory cytokines TNF-α, IFN-g, and IL-1b, nitric oxide, cytotoxic enzymes (perforin and granzyme B), excess free radicals, and other compounds that cause b-cell death by apoptosis [4, 5]. IL-1b alone or in combination with TNF-α and IFN-g enhances Fas-receptor expression on b-cells, which leads to their apoptosis as a result of interaction with autoreactive lymphocytes expressing Fas-ligand [1]. Some authors consider Fas-mediated apoptosis as the leading mechanism of b-cell destruction [11, 12].

In the pathogenesis of T1DM, disturbances in Fas-mediated apoptosis of lymphocytes, which play a major role in maintaining peripheral autotolerance, are important. The Fas/FasL system is involved in the clonal deletion of autoreactive T cells in peripheral lymphatic organs, in the elimination of activated T cells, and thus is central to the regulation of the peripheral immune respons. In the immune system, Fas receptor (FasR) and Fas ligand (FasL) are involved in the regulation of immune responses and in T-lymphocyte-mediated cytotoxicity. FasL is mainly expressed by activated CD4+ and CD8+ T cells [13]. FasR is constitutively expressed by mature T lymphocytes, but its expression is increased after antigen activation, making T cells more susceptible to apoptosis. If there is a defect in the Fas/FasL system, activated lymphocytes can accumulate [14–16]. Thus, if these cells are not eliminated by Fas-mediated apoptosis, the probability of developing an autoimmune disease increases [17].

Thus, from the modern point of view, T1DM is considered as a polygenic, multifactorial disease, in which a genetic predisposition in combination with environmental triggers triggers the activation of specific autoimmune processes leading to the death of b-cells. The leading links in the pathogenesis of autoimmune damage to pancreatic b-cells are immune dysregulation and programmed cell death. Disturbances in the processes of initiation and implementation of apoptosis become fundamental in the development of the disease [18]. Meanwhile, pathogenetic factors and markers of programmed cell death in autoimmune lesions have not yet been sufficiently studied, which is of interest for further research.

3. Role of apoptosis in immune-mediated death of pancreatic b-cells in type 1 diabetes mellitus

3.1 The role of Fas-mediated apoptosis in the pathogenesis of T1DM

It is known that the Fas/FasL system plays a central role in maintaining peripheral autotolerance and tissue homeostasis of the organism [19, 20]. Fas-mediated apoptosis is induced by binding of the Fas(CD95/APO-1/TNFRSF6) receptor to the

Fas(CD95L/CD178/TNFSF6) ligand on the corresponding cells [21]. Triggering the expression of cell surface Fas receptors (Fas) regulates the elimination of autoreactive T- and B-lymphocytes by apoptosis.

Fas-mediated apoptosis is the initiating phase of apoptotic signal transduction and refers to an extrinsic (receptor) pathway for triggering cell death.

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) [19, 22]. 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. 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 effector complex (Death Initiating Signaling Complex) [23–25]. DISC activates procaspase-8, which undergoes autocatalytic cleavage and transforms into its active form, caspase-8, which initiates apoptosis of the target cell [26, 27].

The Fas (CD95/APO-1/TNFRSF6) receptor 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 receptors [20]. 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 [18, 20]. 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 [28, 29].

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 [20, 28]. Like the TNF ligand, FasL can be released from the cell surface and be physiologically active in a soluble form [29–31].

Fas-mediated apoptosis ensures the elimination of cells of the immune system that are undesirable for the organism, and is also involved in the regulatory suppression of immune responses and cytotoxicity of T-lymphocytes [20].

According to literature, the role of death receptors in the destruction of b-cells in type 1 diabetes mellitus (T1DM) is widely discussed [1, 5, 20]. Studies using isolated human pancreatic islets have shown that exposure to stress factors (hyperglycemia, excess free radicals, reactive oxygen species, production of IL-1b by microenvironment cells) enhances Fas expression on b-cells, which leads to their death by mechanism of the Fas-mediated apoptosis [17]. Immunocompetent cells infiltrating pancreatic islet tissue produce pro-inflammatory cytokines: IL-1b, TNF-a, and IFN-g, which are known for their pro-apoptogenic properties [20, 32, 33]. IL-1b 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 b-cells in a Fas-dependent receptor way [18, 30].

There are conflicting data in the literature on the surface expression of the Fas receptor on peripheral blood T lymphocytes in T1DM. In experimental studies in NOD mice, a decrease in the expression of the proapoptotic Fas antigen on the surface of T lymphocytes was revealed [8, 9]. The results of our own studies revealed a statistically significant increase in Fas receptor expression in certain subpopulations of peripheral blood T lymphocytes in T1DM patients, regardless of the duration and state of disease compensation, compared with the control group [34].

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.

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).

The data we obtained are presented in **Table 1**. The maximum increase in the relative and absolute content of T-lymphocytes expressing the Fas receptor was found during decompensation of T1DM, which is explained by the influence of hyperglycemia, a secondary immune response to the so-called "late apoptotic" b-cells due to their inefficient phagocytic clearance and is consistent with the data literature [2]. It has been established that hyperglycemia increases the sensitivity of T cells 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 [35, 36].

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

Figure 1 shows a comparative assessment of the level of surface expression of the Fas receptor (CD95+) in individual subpopulations of T-lymphocytes in T1DM patients in a state of carbohydrate metabolism decompensation and in the control group.

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
Ia	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*

*Notes: (1) I—control group (healthy persons); Ia group—the state of decompensation, newly diagnosed T1DM; IIb group—he state of decompensation, the average duration of the T1DM is 15.3 ± 5.1 years; IIIa group—the state of compensation, the average duration of the T1DM is 0.6 ± 0.2 years; IIIb group—the state of compensation, the average duration of the T1DM is 15.1 ± 5.4 years; IV group—persons with a high risk of developing T1DM. (2) *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 ($p < 0,001$).*

Table 1.
The relative and absolute amount of CD95+-cells and T-lymphocytes expressing the Fas receptor in the peripheral blood of patients with T1DM and persons with a high risk of developing T1DM.

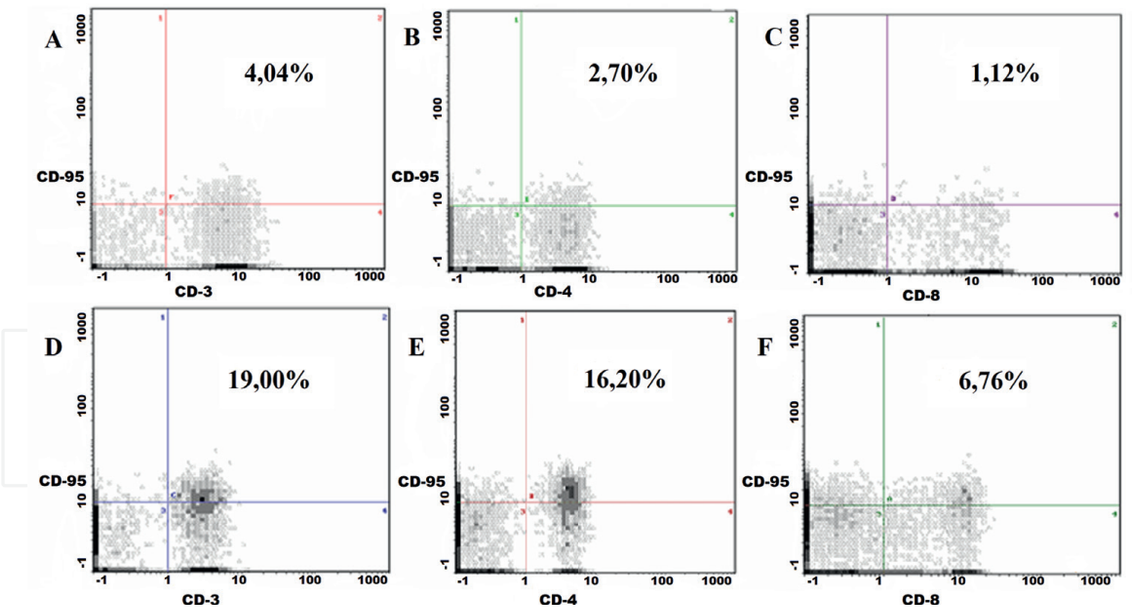

Figure 1.
The histogram shows the number of CD3+CD95+-, CD4+CD95+- and CD8+CD95+ -lymphocytes in the control group (A–C) and in the group of T1DM patients in the state of carbohydrate metabolism decompensation (D–F).

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 2**). 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

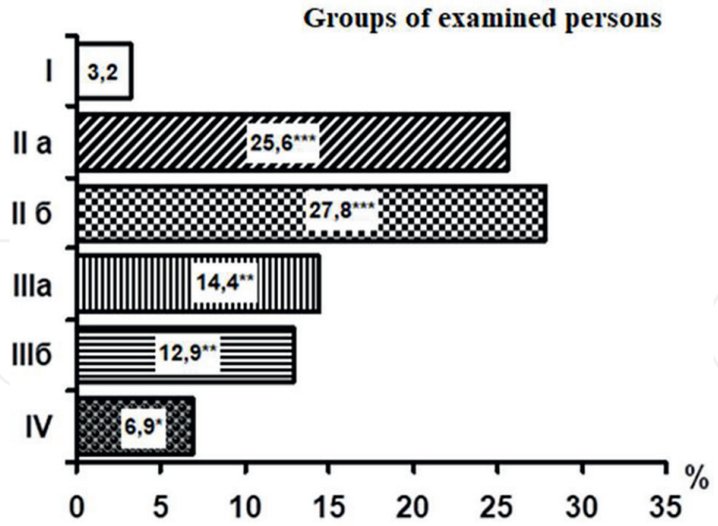

Figure 2.
*Percentage of CD8+CD95+ cells from the total number of cytotoxic T-lymphocytes. Notes: (1) *Differences in the studied indicator with the control group (I) are statistically significant ($p < 0.05$); (2) **differences in the studied indicator with the control group (I) are statistically significant ($p < 0.01$); (3) *** differences in the studied indicator with the control group (I) are statistically significant ($p < 0.001$).*

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, 20].

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 peripheral blood, followed by their migration to the target organ (pancreas), which leads to the progression of the autoimmune process.

In addition to assessing the surface expression of the Fas receptor and Fas ligand, 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, oncological diseases [29, 32, 37]. A comprehensive assessment of the effectiveness of Fas-mediated apoptosis with the determination of all biomarkers (surface and soluble) 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.

We found that the content of soluble forms of Fas (sFas _ soluble Fas) and FasL (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. In patients with T1DM in the phase of decompensation of the disease, a significant increase in the content of sFas was observed compared with the control group and other examined groups of patients (patients in the phase of T1DM compensation, persons with a high risk of developing T1DM) [34]. Our results 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. It

has been reported that a high level of serum sFas correlates with the severity of the septic process [28]. It has been shown that the content of sFas in the blood increased with increasing renal dysfunction in patients with acute kidney injury [29, 31].

According to the literature data, the soluble form of Fas competes with the membrane Fas receptor for the binding of the Fas ligand, which prevents the "physiological" apoptosis of the cells to be eliminated. With an increase in the sFas content in the 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.

We also studied the content of the soluble form of Fas-ligand in the serum of T1DM patients and those at risk for developing T1DM.

The content of sFasL with compensation for T1DM was significantly higher than in the decompensation group and significantly lower than in the risk group (**Table 2**). It should be noted a significant increase in the content of sFasL in the risk group, which is consistent with the literature data. According to the authors, an increase in sFasL in the latent stage of T1DM has a protective value and is aimed at eliminating autoaggressive lymphocyte clones by Fas-mediated apoptosis [8].

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

Disturbances in the functioning 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 [30]. It has recently been found that Fas-mediated caspase-8 activation plays an important role in the regulation of pathogenic mechanisms in bacterial infections [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 [29].

In the pathogenesis of T1DM, disturbances in Fas-mediated apoptosis are of a bivalent nature. So, if in relation to b-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) by both effector cells (autoreactive T cells) and target cells (pancreatic b-cells) [20].

Indicator	Control group (n = 28)	Patients with T1DM		Persons 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 (ng/ml)	778	1501*	789	864
sFasL (pg/ml)	0.102	0.118	0.243*	0.403**

*Notes: (1) n—the number of examined persons; (2) *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 ($p < 0.01$).*

Table 2.
The concentration of the soluble form of the Fas receptor and Fas ligand in the blood serum of patients with T1DM and in persons with a high risk of developing T1DM.

Efficient elimination of autoreactive T lymphocytes from the peripheral blood requires maintaining 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 [30].

Our results on an increase in serum sFas in the state of decompensation of T1DM are consistent with the results of a number of authors who reported an increase in the content of the soluble form of Fas in some systemic and organ-specific autoimmune diseases [31, 37].

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 2**). It should be noted a significant increase in the level of sFasL in the risk group, which is consistent with the literature data [8].

Several experimental studies have shown that both membrane and soluble forms of the Fas ligand (sFasL) are involved in the removal of autoreactive human cells [20, 21]. According to the literature, in an experimental study, 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 [20]. This means that the increase in sFasL in T1DM has a protective value and is aimed at establishing peripheral tolerance, which is necessary for protection against autoimmune aggression against b-cells. An increase in sFasL was reported in individuals at high risk of developing T1DM 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 at the preclinical stage of the disease [8]. According to the literature, an increase in the soluble form of the Fas ligand (sFasL) is a compensatory mechanism aimed at the elimination of activated autoreactive peripheral blood lymphocytes and plays a protective role.

3.2 Apoptosis in the regulation of immune mechanisms involved in the pathogenesis of T1DM

Both humoral and cellular immunity factors are involved in the development of T1DM. Islet cell autoantigens are recognized by autoantibodies and autoreactive effector T lymphocytes resistant to apoptosis, which are involved in the destruction of b-cells through the release of a triad of pro-inflammatory cytokines TNF-α, IL-1b, IFN-g, cytotoxic enzymes (perforin, granzyme B) and other compounds, including free radicals [7]. Experimental studies on mice of the NOD (nonobese diabetic) line on the creation of autoimmune diabetes, close to human T1DM, showed that the central role in the pathogenesis of this disease belongs to autoreactive T-lymphocytes, specific for pancreatic islet b-cells [1, 5].

Autoreactive clones of T cells that react with the islets of Langerhans have been described both in animals and in humans [5, 7]. From the peripheral blood of children with newly diagnosed T1DM, a clone of T-lymphocytes (CD4Th1 + cells) was isolated, which recognizes glutamate decarboxylase, an epitope of the B-chain of the insulin molecule, and other autoantigens of the islets of Langerhans. It has been shown that by transplanting autoreactive T lymphocytes of a sick animal, it is possible to induce T1DM in a healthy syngeneic animal. Strong evidence for the involvement of T cells in the immunopathogenic mechanisms of T1DM is that monoclonal antibodies to the

CD3 antigen can interrupt early T1DM in NOD mice and restore their tolerance to b-cell autoantigens [38, 39]. Most often, diabetogenic clones of T-lymphocytes consist of CD4+ cells, but there are clones formed by CD8+ cells. Some authors believe that CD8+-lymphocytes without the presence of CD4+ cells are not able to lead to the destruction of b-cells [38–40].

In recent years, the cellular mechanisms of death of b-cells of the islets of Langerhans are considered leading [5, 7]. The direct effect of immunocompetent cells on target cells (including b-cells), leading to the destruction of the latter, is referred to as cellular cytotoxicity. Two main pathways for the realization of cellular cytotoxicity have been described, involving perforin- and Fas-dependent mechanisms. It has recently been established that in some cases such destruction is accompanied by the development of apoptosis [1, 7]. A specific feature of the development of apoptosis in this case is the primary damage to the membrane of target cells, which is atypical for this process, with the penetration of granzyme proteins into them. It is believed that it is the latter that include the mechanism of programmed cell death [7]. If CD8+CTLs realize their cytotoxicity by activating both mechanisms, then natural killer cells use exclusively the perforin-dependent pathway, while CD4+ lymphocytes activate Fas-dependent mechanisms and are restricted by the major histocompatibility complex of the second class [1, 2]. Here it is necessary to mention the role of cytokines in the immune-mediated destruction of b-cells. Together with IL-1 and TNF-a, IFN-g is able to increase the expression of molecules of the major histocompatibility complex of the second class on the cells of the pancreatic islets. This leads to recognizing them as alien. The death of pancreatic islet cells occurs as a result of apoptosis, direct cytotoxic action of TNF-a and cytotoxic T lymphocytes (CD8+CTL), as well as by antibody-dependent cytotoxicity [6]. According to a number of researchers [41, 42], the initial step in the development of T1DM is the presentation by macrophages or dendritic cells of specific autoantigens of b-cells to T helpers, which is carried out in association with molecules of the major histocompatibility complex of the second class.

In accordance with modern concepts, type 1 diabetes mellitus is considered as an autoimmune insulitis, in the pathogenesis of which, in addition to autoantibodies, the role of cellular immunity reactions is undeniable [2]. Autoreactive lymphocytes migrate from the bloodstream to the target organ (pancreas) and penetrate into the islets of Langerhans, forming inflammatory infiltrates—insulitis. This is evidenced by the results of histological studies that reveal lymphocytic infiltration of pancreatic islets, formed mainly by CD8+ and CD4+ T cells [1, 5, 7].

Activated macrophages secrete IL-12, which stimulates CD4+ cells that secrete IFN-g and IL-2. IFN-g activates "resting" macrophages, which in turn produce IL-1b and TN-Fa, which is accompanied by a sharp rise in the level of free radicals in b-cells [7]. IL-2 causes the migration of peripheral CD8+ lymphocytes to the islets of Langerhans, probably due to the induction of the expression of specific homing receptors. Naive cytotoxic T cells that carry specific receptors for b-cell autoantigens differentiate into effector cytotoxic CD8+ lymphocytes after recognizing a specific b-cell peptide associated with molecules of the major histocompatibility complex of the first class, which occurs in the presence of CD4+ lymphocytes. Then CD8+ lymphocytes start the process of destruction of b-cells due to the secretion of perforin and granzyme B. CD4+ lymphocytes expressing the Fas-ligand destroy b-cells by the mechanism of Fas-mediated apoptosis, as well as indirectly—due to the secretion of cytokines IFN-g and TNF-a. In this way, macrophages, CD4+ and CD8+ cells are thought to act synergistically to destroy b-cells, leading to the onset of autoimmune diabetes [41, 42].

3.3 Apoptosis as the final mechanism of immune-mediated destruction of pancreatic b-cells

The most important stage in the pathogenesis of T1DM is the dysregulation of apoptosis processes associated with the preservation of autoreactive clones of lymphocytes that are tropic for b-cells and are able to "escape" from apoptosis [11].

To date, most studies in the field of studying the role of apoptosis of immune-mediated destruction of pancreatic b-cells in T1DM have been performed on experimental models of type 1 diabetes mellitus in vivo and in vitro. However, it should be emphasized that the induction and regulation of apoptosis in animal models of T1DM may differ significantly in T1DM in humans [2]. In particular, nicotinamide, which effectively protects rat b-cells from apoptosis [43], can protect human b-cells from necrosis caused by free radicals, but not from cytokine-induced apoptosis [44]. Data obtained in experiments in vitro show that the sensitivity of human b-cells is definitely lower than that of animal b-cells [45, 46], which must be considered when approximating the results from model systems to the human body.

The detection of apoptosis in vivo and the study of its role in the destruction of b-cells in T1DM encounters significant methodological difficulties. However, in some models of T1DM in experimental animals, it is possible to detect apoptosis in pancreatic b-cells and show a correlation between the degree of insulitis and apoptosis of b-cells. In particular, it has been shown that apoptosis is the predominant mechanism of b-cell death in NOD mice [11].

3.3.1 Signal proteins involved in the implementation of apoptosis of pancreatic b-cells

When apoptosis is induced by cytokines produced by T-helpers and macrophages, the death of b-cells can be mediated by signaling systems associated with ceramides or with mitogen-activated protein kinases (MAPK). The combination of the cytokines TNF-a, IL-1b, and IFN-γ has been shown to induce beta cell death in vitro [5]. Cell death is induced by activation of the transcription factors JNK (c-Jun N-terminal kinase), NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), and STAT-1 (Signal Transducers and Activators of Transcription), which induce iNOS transcription. and subsequent production of the free radical NO. Inactivation of the JNK pathway makes b-cells less susceptible to IL-1-mediated death through a NO-independent process [47, 48]. Other components of the MAPK signaling pathway, p38 and ERK1/2 (extracellular signal-regulated kinase1/2), also play a possible role in cytokine-induced b-cell death [49, 50].

3.3.2 Bcl-2 family members in b-cell apoptosis

In addition to the signaling proteins listed above, members of the Bcl-2 family play a significant role in the regulation of b-cell apoptosis in humans. The Bcl-2 family is known to include both the anti-apoptotic Bcl-2 and Bcl-xL proteins and the pro-apoptotic Bax, Bim, Bik, and Bak proteins. These proteins are regulators of the mitochondrial apoptosis triggering pathway [51], which plays an important role in b-cell death in T1DM [5, 52]. The Bax/Bcl-2 ratio regulates the balance between the processes of induction and inhibition of apoptosis [34, 53]. It has been established that overexpression of antiapoptotic proteins of the Bcl-2 family protects b-cells from apoptosis in T1DM [53–55]. Bcl-2 inhibits apoptosis by blocking the transport

of cytochrome C protein from mitochondria, which plays an important role in the realization of the death signal.

It is believed that therapeutic methods aimed at enhancing the expression of anti-apoptotic members of the Bcl-2 family can protect b-cells from apoptosis and reduce the area and degree of damage in T1DM. To date, convincing results in this area have been obtained only in experimental models using NOD mice [56]. Nevertheless, research into the therapeutic potential of Bcl-2 is ongoing.

3.3.3 The role of nitric oxide and inducible NO-synthase in the destruction of b-cells

The study of the biological significance of nitric oxide (NO) was a significant breakthrough in understanding the mechanisms of destruction of pancreatic b-cells. Nitric oxide is a relatively unstable free radical with a half-life of several seconds [57]. Takamura et al. found that in b-cells of transgenic mice that develop type 1 diabetes mellitus, an increased expression of induced NO synthase is determined, which confirms the role of excessive nitric oxide formation and the development of T1DM in these animals without signs of insulitis [46]. Nitric oxide produced by activated macrophages functions as a specific effector molecule, but can also be involved in the destruction of b-cells [5]. Corbett and Daniel showed that the expression of inducible NO-synthase in macrophages and the formation of nitric oxide by them practically does not affect the function of the pancreatic islets. At the same time, the expression of inducible NO synthase directly in b-cells and the formation of NO here completely inhibit insulin secretion [58]. Thus, the main damaging value is attached to nitric oxide, which is formed directly in the b-cell. Eizirik and Mandrup-Poulsen showed that b-cells of human pancreatic islets are more resistant to the damaging action of various alkylating compounds (alloxan, streptozotocin), cytokines, oxygen free radicals and nitric oxide compared to rat pancreatic islets [59]. Induction of NO synthase in human pancreatic islets is observed several days after simultaneous exposure to IL-1b, TNF-a and IFN-g. Darville and Eizirik showed that in human pancreatic islets the expression of induced NO synthase occurs with the obligatory participation of IL-1b and IFN-g [60]. The transcription factors c-fos (protooncogene), JNK, and the nuclear factor NF-kB are involved in the regulation of induced NO-synthase mRNA expression. In vitro experiments have shown that the expression of NO synthase, which is activated by cytokines and various endotoxins, is inhibited by dexamethasone and insulin, which may be important in the prevention of type 1 diabetes [57].

The synthesis of nitric oxide in b-cells induced by pro-inflammatory cytokines can lead to their death by apoptosis, which is preceded by the appearance of many biological signs of the apoptotic process, including internucleosomal DNA fragmentation [11].

3.3.4 Role of pro-inflammatory cytokines in apoptosis-mediated b-cell death

It should be emphasized that TNF-α is a pleiotropic cytokine that plays a key role in many physiological and pathological cellular processes, including the role of an inducer of activation apoptosis of target cells [61]. It was believed that TNF-α could directly destroy b-cells, since it contains a death domain in its receptor. However, experimental studies using b-cell culture have shown that this is not the case. It turned out that in the presence of TNF-α, NF-kB (Nuclear Factor of κ-chain B-lymphocytes) which is known for its antiapoptotic properties, is activated [56, 62]. This is supported by in vivo experimental studies using NOD mice indicating that

inhibition of NF-kB in b-cells increases their susceptibility to TNF-α-mediated apoptosis [62, 63].

Recent studies show that the priority of the latest therapeutic developments in the field of type 1 diabetes is the suppression of inflammation in the target organ. Resolvin D1 has been shown to reduce the severity of streptozotocin-induced T1DM by reducing oxidative stress and suppressing inflammation. The action of the drug is largely aimed at suppressing the production of pro-inflammatory cytokines TNF-α and IL-6. As a result of the action of Resolvin D1 in the peripheral blood of experimental animals, a statistically significant decrease in the concentration of TNF-α and IL-6 was recorded compared to the initial values ($p < 0.001$) [5]. In another study, the use of immunomodulatory therapy in individuals with a high risk of developing type 1 diabetes contributed to the suppression of the production of TNF-α and IFN-g, in combination with an increase in the concentration of C-peptide compared to pre-treatment levels. Teplizumab treatment improved b-cell function, as evidenced by a quantitative and qualitative improvement in insulin secretion [64]. Thus, monitoring of the cytokine profile and timely therapy aimed at suppressing anti-inflammatory cytokines is of critical importance in the pre-diabetic stage. New approaches to preventing the progression of clinical T1DM. to irreversible destruction of b-cells and insulin deficiency are a promising direction in modern diabetology.

IL-1b, produced by macrophages, also plays a significant role in the destruction of b-cells. It has been established that after stimulation of pancreatic b-cells in vitro with IL-1b, the expression of Fas receptors increases on their surface, as a result of which b-cells become targets for T-lymphocytes carrying FasL [1]. Expression of Fas receptors by b-cells has been confirmed not only by in vitro studies, but also in vivo in experiments using laboratory animals [1, 6]. Thus, IL-1b is involved in the destruction of b-cells by stimulating the expression of the Fas receptor on the membranes of b-cells. In b-cells after their interaction with IL-1b, characteristic signs of apoptosis are revealed: DNA fragmentation, nuclear condensation and the formation of apoptotic bodies [65].

IFN-g usually acts in combination with other pro-inflammatory cytokines, such as TNF-a or IL-1b, and sometimes both. IFN-g stimulates the production of IL-1b APC [66]. When IFN-g and TNF-a bind to cognate receptors on b-cells, either caspase-dependent b-cell apoptosis is triggered or b-cell apoptotic death is activated by inducible NO synthase (iNOS) [62]. The triad of pro-inflammatory cytokines TNF-a, IFN-g, and IL-1b is detrimental to b-cells, since it promotes the triggering of various mechanisms of apoptotic death of the islets of Langerhans. Maintaining high concentrations of all three cytokines in the microenvironment of b-cells enhances their apoptotic death [67].

Thus, many signaling proteins are involved in the apoptotic death of b-cells in T1DM, each of which can become a potential therapeutic target in the development of new methods of therapy for this disease.

A general scheme of the mechanisms of apoptotic death of b-cells in T1DM is shown in **Figure 3**.

The B-cell antigen is captured by antigen-presenting cells (APCs). Antigen processing results in the formation of antigenic fragments that form a complex with MHC II. The antigen-MHC II complex is recognized by T cell receptors (TCRs). T-cell activation occurs due to antigen recognition and co-stimulation by secondary signals of co-stimulatory molecules—CD28-B7. An activated cytotoxic T lymphocyte (CD8+CTL) produces IFN-g, which subsequently stimulates APC to produce additional cytokines

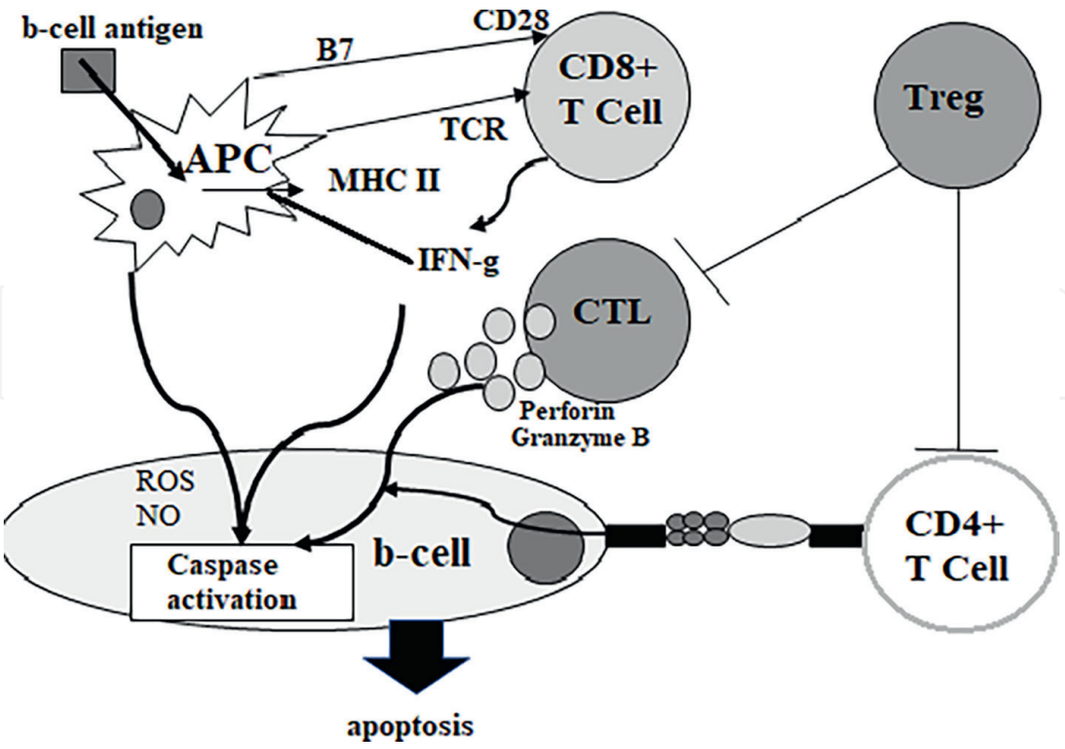

Figure 3.
The main mechanisms of pancreatic b-cells apoptosis in T1DM.

IL-1b and TNF-a. In addition, CD8+CTL produces proteins: granzyme B and perforin. IL-1b increases expression of the Fas receptor on b-cells, which increases their sensitivity to Fas-mediated apoptosis mediated by CD8+ and CD4+ T cells. Conversely, regulatory T cells (T-regs) suppress CD8+ and CD4+ T cells. [1].

4. Perspectives for the treatment and prevention of T1DM

It is clear from the above data that the process of apoptosis as the central mechanism of b-cell death in T1DM deserves further study. The field of research into the process of apoptosis is one of the most promising areas in cell biology. In the future, therapeutic intervention could be implemented at the level of immune cells and/or target cells.

The accumulation of knowledge about b-cell apoptosis is likely to progress rapidly in the near future. If apoptosis is the general mechanism by which b-cells die in T1DM in response to immune attacks by cytokines and/or T lymphocytes (cellular cytotoxicity), then new strategies may be developed to prevent the process of b-cell death, and hence the manifestation of the disease itself.

It has been established that protection against apoptosis can be implemented at four different levels: (1) "interception" of stimuli that induce apoptosis; (2) functional antagonism of apoptosis triggers; (3) intervention in the signal cascade; (4) blockade of catabolic enzymes involved in cell self-destruction. The study of all levels of intervention can serve as the basis for the development of a new strategy to prevent the death of b-cells [2, 7].

Treatment aimed at suppressing the initiation of the apoptosis process is promising. For example, some methods may include blockade of death ligand binding (TNF, FasL). In addition, the goals of therapy may be aimed at increasing resistance to apoptotic

stimuli by increasing the expression of anti-apoptotic members of the bcl-2 family. Insulin may have a potential positive effect in preventing disease at the preclinical stage, due to its potential immunomodulatory effect and its ability to induce b-cell "rest" [5].

Considering the numerous mechanisms by which apoptosis affects individual stages of the pathogenesis of T1DM, it is advisable to consider possible ways of its modulation in order to influence certain targets of the pathological process for therapeutic purposes.

5. Conclusion

Over the past 10 years, a real breakthrough has been made in immunology, both in the field of basic research and in clinical medicine. The rapid development of immunotherapy, the discovery and introduction into practice of new biomarkers, the improvement of immunological methods of laboratory diagnostics indicate that we live in an era of "every second" innovations. Using the example of T1DM, which is an autoimmune disease, we are seeing new advances in therapy and a significant improvement in the quality of life of patients. It should be emphasized that we owe all these achievements to experimental medicine, which is the first rung of the ladder leading to the success of clinical medicine.

The authors express the hope that this chapter will help readers to better understand the molecular mechanisms of T1DM immunopathogenesis, as well as to appreciate the significance of experimental studies in science and practical medicine. Perhaps the material presented in this section will inspire someone to their own scientific research.

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Conflict of interest

Authors declare no conflict of interest.

Author details

Anna Vladimirovna Lugovaya[1*], Vladimir Phylippovich Mitreikin[2] and Natalia Mikhailovna Kalinina[3,4]

1 First Pavlov State Medical University of Saint Petersburg, Russian Federation

2 Department of Pathological Physiology, First Pavlov State Medical University of Saint Petersburg, Russian Federation

3 Department of Immunology, First Pavlov State Medical University of Saint Petersburg, Russian Federation

4 Nikiforov Russian Center of Emergency and Radiation Medicine, Saint Petersburg, Russian Federation

*Address all correspondence to: g89213159748@gmail.com

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