



Review

Role of immune system in type 1 diabetes mellitus pathogenesis



Leszek Szablewski *

General Biology and Parasitology, Center of Biostructure Research, Medical University of Warsaw, 5 Chalubinskiego Str., 02-004 Warsaw, Poland

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ABSTRACT

The immune system is the body's natural defense system against invading pathogens. It protects the body from infection and works to communicate an individual's well-being through a complex network of interconnected cells and cytokines. This system is an associated host defense. An uncontrolled immune system has the potential to trigger negative complications in the host.

Type 1 diabetes results from the destruction of pancreatic β -cells by a β -cell-specific autoimmune process. Examples of β -cell autoantigens are insulin, glutamic acid decarboxylase, tyrosine phosphatase, and insulinoma antigen. There are many autoimmune diseases, but type 1 diabetes mellitus is one of the well-characterized autoimmune diseases. The mechanisms involved in the β -cell destruction are still not clear; it is generally believed that β -cell autoantigens, macrophages, dendritic cells, B lymphocytes, and T lymphocytes are involved in the β -cell-specific autoimmune process. It is necessary to determine what exact factors are causing the immune system to become unregulated in such a manner as to promote an autoimmune response.

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1. Introduction

Diabetes mellitus is a metabolic disorder that leads to the development of a number of complications including ketoacidosis, kidney failure, heart disease, stroke, and blindness. Type 1 diabetes usually starts

in people younger than 30 and is therefore also termed juvenile-onset diabetes, even though it can occur at any age. It was also termed insulin-dependent diabetes mellitus. Type 1 diabetes incidence has more than doubled in the past 20 years and is set to double again before 2020 [1]. It is a well-known autoimmune disease that is characterized by a specific adaptative immunity against β -cell antigens. This disease is one of the classical examples of organ-specific autoimmune disease characterized by lymphocytic infiltration or inflammation in pancreatic

* Tel.: +48 22 621 26 07; fax: +48 22 628 53 55.
E-mail address: leszek.szablewski@wum.edu.pl.

islets called “insulinitis.” Insulinitis ultimately leads to the destruction of the insulin producing β -cells, resulting in diabetes. This is usually associated with infiltration of innate immune cells. These cells produce cytokines which promote β -cell apoptosis and increase infiltration of islet-specific T cells. Islet-specific T cells attack and destroy β -cells.

T cells play an important role in the induction of type 1 diabetes mellitus. The inflammatory infiltrate in islets consists mostly of T lymphocytes, but the data on the predominance of CD4 + (Th4 or helper) [2] or CD8 + (Th8 or cytotoxic) [3]. CD4 + T cells have been subdivided into different subset based on their cytokine secretion profiles: Th1, Th2, Th17, and Treg (regulatory T cells) [4,5]. Cytokines produced by Th1 are mediators in cellular immunity, whereas cytokines produced by Th2 are stimulators of humoral immune responses and antibody production. Th17 T cells may play a role in the induction of autoimmune tissue injury. Th1 cells are responsible for aggressive disease, while Th2 T cells infiltrate more slowly and do not induce diabetes [6]. The inflammation generated by Th1 cells might attract T cells that would not normally accumulate in the islets. Treg cells are primary controllers of immune responsiveness and peripheral immunological tolerance [7]. These cells also regulate several organ-specific autoimmune diseases such as type 1 diabetes [8]. Treg cells control natural killer (NK) cells in an insulinitic lesion. They also control progression to type 1 diabetes in NOD mice by reining in NK cells, inhibiting both their proliferation and production of interferon- γ [9]. A variable number of B cells or macrophages have also been described in the inflammatory infiltrate [3]. Studies using human and murine models of diabetes have demonstrated that the autoimmune destructive process in type 1 diabetes requires both CD4 and CD8 T cells as well as macrophages [10,11]. These cells are nondestructive; however, they accumulate in an islet lesion [12]. A triggering event needs to occur, which promotes β -cell destruction. It is to note that clinical manifestations of diabetes occur after 90% of an individual's β -cell mass is destroyed [13].

2. Autoantibodies

Islet cell autoantibodies reacting with antigens located in the cytoplasm of all endocrine cells within the pancreatic islets were first described by Bottazzo et al. [14]. Islet reactive T cells exist in patients with type 1 diabetes. Studies identified autoantibodies to four islet gene groups: insulin or proinsulin (PI), glutamic acid decarboxylase (GAD65 or GAD67), insulinoma-associated antigen-2 (IA-2 or ICA512 or IA-2 β or PHOGGRIN), and zinc transporter 8 (ZnT 8) [15]. Autoantibodies to other islet antigens, such as heat shock protein (HSP), chromogranin A and their peptides [16], islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP) [17], and human islet amyloid polypeptide (IAPP) precursor protein [18] have been identified. In addition to mentioned autoantigens, still others have been identified, such as autoantibodies to glucose transporter 2 (GLUT2) [19], sulphatide [20], ganglioside GM2-1 [21], carboxypeptidase H [22], and three distinct-associated autoantigens of 38 kDa molecular mass (autoantigen to nuclear transcription factor jun-B) [23], Imogen 38 [24] and glima 38 (glycosylated islet cell membrane antigen of 38 kDa) [25]. Islet autoantibodies rarely appear prior to age 6 months [26]. Risk is incremental in relation to whether antibodies are against two, three, or four of the antigen group. On the other hand, type 1 diabetes risk can vary in relation to which of the islet autoantibodies are present [27]. For example, the presence of antibodies to IA-2 is associated with highest risk [15]. Autoantibodies alone do not appear to be sufficient to induce β -cell destruction. Transplacentally transferred antibodies related to type 1 diabetes are usually eliminated from the peripheral circulation of infants before 9 months of age [28]. No differences were found in autoantibody frequencies between the offsprings from mothers vs. fathers with type 1 diabetes up to the age of 5 years [29].

The development of the pathology involved several cell types of both the innate and adaptive systems. This disease is under the control of several genetic loci, but also is influenced by environmental factors.

3. Role of HLA genes in type 1 diabetes risk

In humans, the major histocompatibility complex (MHC) is known as the human leukocyte antigen (HLA) and contains over 200 genes [30]. It is located on chromosome p21.3 [31], encoding HLA class I and class II molecules [32]. Genes of HLA class I and II are highly polymorphic and consist of many different alleles [32]. These genes encode cell surface proteins that are required for interaction with cells of immune system, and are involved in immune recognition and killing. Distinct loci within HLA region determine risk [33], though the HLA class II region appears to be most influential [34]. The HLA is considered to contribute about half of the familial basis of type 1 diabetes [35]. Disease susceptibility is highly associated with inheritance of the HLA alleles DR3 and DR4 as well as the associated alleles DQ2 and DQ8. Several genes of HLA class II were found to determine a susceptibility hierarchy ranging from protection to strongly at-risk.

More than 90% of patients with type 1 diabetes express either DR3DQ2 or DR4DQ8. The DRB1*1501-DQA1*0102-DQB1*0602 haplotype, found in ~20% of the population but only 1% of patients, confers dominant protection against type 1 diabetes [36]. Heterozygous genotypes DR3/DR4 are the most in children diagnosed with type 1 diabetes prior to the age of 5 (50%) [37]. Individuals with the haplotype DRB1*Q302-DQA1*0301, especially when combined with DRB*0602-DQA1*0501, are highly susceptible to type 1 diabetes. The risk of type 1 diabetes in these individuals increases 10–20-fold. The HLA DRB1*03, *04; DQB1*0302 genotype, which confers with the highest risk of type 1 diabetes, is present in 39% of patients who develop type 1 diabetes before age 20 [38]. On the other hand, HLA class II haplotypes such as DR2DQ6 confer dominant protection [39]. Individuals with the haplotype DRB1*0602-DQA1*0102 rarely develop type 1 diabetes. The precise mechanisms through which HLA-DQ determines disease susceptibility is still not clear [40].

An association has been also found for class I alleles [41]. Most notably the presence of the HLA-B*39 allele was found to be a significant risk factor. HLA-A*02 increases the risk in individuals possessing the high-risk class II DR3/4-DQ8 haplotype [42]. HLA-A*0201 is one of the most prevalent class I alleles in patients with type 1 diabetes. The presence and functionality of HLA-A*02-restricted CD8 T cells reacting against β -cell antigens such as insulin, glutamate decarboxylase and islet amyloid polypeptide (IAPP) were found in patients with type 1 diabetes and islet transplant recipients [18,43]. Alleles at HLA DP class II loci and class I loci, such as HLA*24, B*38, and B*39, also contribute to type 1 diabetes risk, but they have not been incorporated into risk prediction models [15].

How to explain the influence of HLA genes on the development of type 1 diabetes? Several hypotheses have been introduced. A peptide competition model suggested by Nepom [44] proposed that a diabetogenic peptide is permissive for disease when it binds to a disease associated class II molecule. The competition of diabetogenic peptides between resistant and susceptibility HLA alleles might be a mechanism of disease protection. The competition effect is more profound when the source antigen is rare so that the abundance of peptide-DQ8 complex is reduced. The reduced abundance of this complex can lead to a diminished activity of DQ8-restricted autoreactive T cells. Another model focused on the failure of the immune system to maintain tolerance to β -cells in type 1 diabetes [45]. It was suggested that HLA DQ genes associated with type 1 diabetes bias the immunologic repertoire toward autoimmune specificities, creating an immune prone individual, followed by amplification and triggering events that promote subsequent immune activation [46]. MHC class II alleles conferring susceptibility and resistance to diabetes select completely different sets of immunogenic epitopes from the β -cell autoantigen GAD65. Results obtained by Chao et al. [47] showed that substitutions of two amino acids within the I-A β chain, that distinguish a diabetes-susceptibility from a diabetes-resistance allele are sufficient to alter peptide binding and MHC restriction. These substitutions may also influence antigen

presentation and the selection of the T cell repertoire. According to authors' suggestion, the molecular mechanisms for class II-mediated selection of immunodominant epitopes are complex and differ for each individual peptide epitope. Another studies showed that DQ8 and DR4 are able to bind the same human GAD65 peptides [48]. The expression of DR4 diminished DQ8-restricted T cell response. It was demonstrated that the diminished T cell response varied according to the specific DRB1*04 alleles. Authors suggest that DR4 is able to modulate DQ8-restricted T cell response. The decreased DQ8-restricted CD4 T cell activity due to peptide competition may be the mechanism explaining the modulation effect of DR4 to type 1 diabetes susceptibility [48].

4. Other genes and environmental factors in type 1 diabetes susceptibility

Candidate gene studies also identified the insulin gene as the second most important genetic susceptibility factor, contributing 10% of genetic susceptibility to type 1 diabetes [49]. Over the last decade, whole genome screen has indicated that there are at least 15 other loci associated with type 1 diabetes [50], such as cytostatic T-lymphocyte antigen (CTLA-4) [51], phosphotyrosine protein phosphatase, non-receptor 2 and 22 (PTPN2 and PTPN22) [52,53], interleukin 2 receptor alpha (IL2R2) [54], interferon induced with helicase domain 1 (IFIH1) [55], vitamin D receptor (VDR) [56], KIAA0350 (also known as CLEC 16A) [57], and other genes [58].

Environmental risk factors may trigger an autoimmune process leading to β -cell destruction and overt type 1 diabetes in genetically susceptible individuals [59]. These factors include, for example, dietary compounds (such as cow's milk, plant proteins, nitrates, wheat gluten) and viruses (Coxsackie B viruses, rubella viruses, cytomegaloviruses and retroviruses) [60]. The viral infection can lead to immune cell activation through various possible mechanisms. Viruses may alter a host cell and the immune system identifies it as an immunological target. These cells are lysed and release self peptides and fragments into the circulation. In circulation they may be processed and presented via antigen-presenting cells [61]. Another mechanism proposed by mentioned authors suggest that viruses can alter the immune system of the host by inducing polyclonal B cell activation, the release of lymphokines, the activation of immune cells, and the disruption the balance between Th1 and Th2 type responses. These events promote unwarranted immune activation [61]. It is to note that viruses produce proteins similar to those of the host. Certain viral components share a distinct homology with identified β -cell antigens targeted in an autoimmune response [61]. As the example, GAD is an example of an autoantigen in type 1 diabetes that shares homology with viral proteins, especially with the P2-C viral sequence of the Coxsackie B virus and the major outer capsid protein of rotavirus [62,63]. There are also several other hypotheses [64].

5. Innate immunity and type 1 diabetes mellitus

The innate immune system is the body's first line of defense against pathogens. It recognizes pathogens and foreign molecules without having been exposed to them and without generating long-term immunological memory. The innate system uses invariant pattern recognition molecules, which recognize conserved molecular patterns present in foreign particles that enter the host, as well as several cell products that can be associated with a breach in defenses. These pattern recognition molecules can be soluble, membrane-bound, or cytosolic [5].

Response of the innate immune involves a group of cellular receptors known as pattern recognition receptors (PRRs). These receptors are found in intracellular compartments, secreted through tissue fluids and blood, and on cellular surfaces [79]. Cells of the innate immune system use several types of PRRs to help initiate an immune response. These PRRs include Toll-like receptors (TLRs), nucleotide oligomerization domain-like receptors (NOD-like receptors, NLRs), and retinoic acid-inducible gene-I-like helicases [80]. These receptors recognize

pathogens through the interaction with pathogen-associated molecular patterns (PAMPs). Upon PAMP binding to its cognate PRR, a variety of defense mechanisms are induced, including the release of cytokines and chemokines responsible for inflammatory pathways, that protect the body against further tissue injury from foreign pathogens [81]. On the other hand, excessive or defective inflammatory signaling can have adverse consequences and increase the risk of developing an autoimmune disease [81].

TLRs are an evolutionary conserved class of PRRs, which when activated cause the activation of the immune system [82]. Upon recognition of a ligand, TLR activation brings about a cascade of proinflammatory cellular responses, such as up-regulated production of cytokines, chemokines, and costimulatory molecules [83]. It has been suggested that in type 1 diabetes, TLRs may be priming an unwarranted adaptive immune response because of autoreactive processes directed against self antigens [84]. Innate immune cells activated against self antigens may be responsible for a break in tolerance, and the body may promote autoreactive immune response [64]. Results obtained by Devaraj et al. [85] showed that the expression of TLR2 and TLR4 were both increased in monocytes obtained from type 1 diabetic patients as compared to healthy control individuals. The activity of investigated TLRs along with downstream targets of TLR signaling such as MyD88, nuclear factor- κ B (NF- κ B), and TIR-domain-containing adapter-inducing interferon- β (TRIF) were all significantly up-regulated in these patients. It is suggested that the inflammatory response initiated by TLR2 and TLR4 signaling may have significant consequences in type 1 diabetes development [80].

On the other hand, by using animal models (NOD mice), it was found that TLR3 is not required for autoimmune diabetes onset while TLR9-deficient mice had a significantly decreased incidence of diabetes as compared to TLR9 heterozygotes [86]. By using animal models, it was shown that the TLR3 expression and functionality on pancreatic β -cells and TLR3 ligand induce in these cells apoptotic pathways [139]. The above described results indicate that TLRs have different roles in the development of type 1 diabetes.

TLRs are not the only PRRs used by cells of the innate immune system. Other receptors, such as NLRs, triggering receptors expressed on myeloid cells (TREM), and C-type lectin receptors (CLRs) have not been directly linked to autoimmunity, but represent a class of receptors that may be responsible for triggering autoimmune responses or autoimmune initiation of the adaptive immune system [87]. The innate immune system uses multiple cell type, including macrophages, dendritic cells, NK cells, and neutrophils, each of which has their own specific function in an innate response.

5.1. Macrophages

Macrophages are responsible for sensing, integrating, and responding appropriately to myriad stimuli in their tissue milieu. The role of macrophages in the pathogenesis of type 1 diabetes has been suggested in early studies [88]. These cells are detected in the islet infiltrates of young NOD mice and inhibition of this macrophage influx into the pancreas, by inhibiting an adhesion-promoting-receptor on this cell, or their depletion, prevents the development of type 1 diabetes [89]. In animal studies, macrophages could play a pathogenic role on β -cells through their production of proinflammatory cytokines TNF- α and IL-1 β [90,91]. Macrophage activity is channeled through two distinct response patterns designated as classically and alternative activation [88]. Both macrophage populations are central in diabetes.

Classically activated macrophages trigger an inflammatory response which initiates insulinitis and pancreatic β -cell death during type 1 diabetes. These macrophages respond to intracellular pathogens by secreting proinflammatory cytokines (TNF- α) and interleukins (IL-1 β , IL-6, IL-8, IL-12, and IL-23), express costimulatory molecules important in T cell activation (MHC, CD40, CD86), and produce bactericidal mediators (toxic mediators) such as NO through the expression of iNOS and

reactive oxygen species (ROS) [88,92,93]. The β -cells are very sensitive to free radicals because β -cells exhibit very low free radical scavenging activity [94]. Reactive oxygen species can damage β -cells by causing DNA strand breaks, which induce the DNA repair enzyme poly(ADP) ribose polymerase (PARP). Enzyme uses and depletes nicotinamide adenine dinucleotide (NAD), leading to necrosis of β -cells [95]. According to Jun et al. [96], macrophages are required to the activation of cytotoxic T cells that can destroy pancreatic β -cells. Classically activated macrophages also have an enhanced antigen presenting ability [97]. These macrophages are induced by Th1-cell-derived IFN- γ and microbial products, such as bacterial lipopolysaccharide (LPS).

Alternatively activated macrophages decrease hyperglycemia, insulinitis and inflammation in the pancreas [92]. These cells are induced during Th2-type responses (during helminthic infection and allergic responses). Activation of alternatively activated macrophages is dependent upon stimulation with IL-4/IL-13 [98], through the IL-4R α receptor [99], signal transducer and activator of transcription factor 6 (STAT6) [100], and with helminth antigens [101]. Alternatively activated macrophages produce IL-10, TGF- β , and selected proinflammatory cytokines secreted by classically activated macrophages. They express high levels of PD-1 ligands (program-death 1) such as PDL-1 and PDL-2, and inhibit the proliferative response of activated T cells [102]. Helminth-induced alternatively activated macrophages decrease type 1 diabetes-triggering inflammation, as well as glucose tolerance during obesity [103].

In summary, macrophages play the role of antigen presenting cells (APCs). They can directly pick up and present β -cell antigens. This leads to the activation of β -cell toxic CD8 T cells and the generation of autoreactive CD4 effector T cells [12].

5.2. Dendritic cells

Dendritic cells (DCs) are a heterogeneous group of innate effectors that serve two general functions in controlling T cell immunity. The first function is the presentation of antigens to T cells, which is essential for T cell activation and expression. Second, these cells secrete cytokines that condition the extracellular milieu and determine the nature of T cell response [104]. DCs have opposing roles because they are capable to inducing immune responses against foreign antigens, as well as tolerance to self-antigens [105]. Dendritic cells are the prototype professional antigen presenting cells derived from hemopoietic cells and are essential link between innate and adaptive arms of the immune system. They are widely distributed in different types of tissues, serving as sentinels of the immune system [106]. As mentioned above, dendritic cells are heterogeneous and several subtypes with distinct features have been identified in lymphoid and non-lymphoid tissues in human and mouse (for details see Maddur et al. [107]). Phenotypically DCs are identified in “immature” and “mature” stage [108]. Immature dendritic cells have high capacity to capture and internalize antigens due to surface expression of receptors such as Toll-like receptors (TLRs), C-type lectin receptors (CLRs), and intracytoplasmic nucleotide oligomerization domain (NOD)-like receptor (NLRs). These cells also possess cytokine receptors that enable them to respond to the appropriate stimuli. Immature dendritic cells act as immunological sensors that receive stimuli from environment to alert the immune system. Of note, immature dendritic cells are poor stimulators of T cells and must undergo processes of maturation and activation [107]. Maturation is the phenotypic changes that enable DCs to initiate immunity as antigen presenting cells. Mature DCs secrete immunoregulatory cytokines and chemokines such as IL-1 β , IL-6, IL-10, IL-12, IL-23, TNF, CCL17, CCL12, and CCL22. Upregulation of costimulatory and adhesion molecules CD80, CD86, CD40, CD54, and CD58 critical in mediating clustering with and activation of T cells is also observed [107].

Summers et al. [109] have found that circulatory DC subsets exist in normal frequencies and phenotypic states in diabetic patients. In vivo, DCs were located around the pancreatic islets in type 1 diabetic patients, but were absent in pancreatic tissue of healthy controls. Naturally occurring DCs within the blood of patients with type 1 diabetes circulate

in normal numbers, exist in a normal resting state, and consist of similar proportions of myeloid DC and lymphoid DC subsets. Authors suggest that the presence of DCs within the pancreas of type 1 diabetic patients indicates that β -cell destruction may be facilitated either directly or indirectly by DCs that have migrated into the pancreas [109].

Dendritic cells are important APCs that are instrumental to the initiation and perpetuation of type 1 diabetes [109]. Observations in type 1 diabetic patients suggest dysregulation in the T cell response to antigen presentation by DCs. Dendritic cells would be expected to impact the functional balance between pathogenic T cells and Tregs in type 1 diabetes. Studies in NOD mice have shown that myeloid dendritic cells exhibit a hyperinflammatory phenotype. These cells have an elevated capacity to stimulate T cells and secrete proinflammatory cytokines such as IL-12 [110]. Studies in diabetic patients showed that monocyte-derived DCs from peripheral blood exhibit a limited T cell stimulatory capacity relative to at-risk or healthy individuals [111]. Of note, IFN- α and IFN- β are secreted in large amounts by plasmacytoid dendritic cells in autoimmune diseases. In type 1 diabetes, elevated expression of interferons was found in the pancreas of recently diagnosed patients, though the role of plasmacytoid dendritic cells is unclear [104].

In summary, dendritic cells are the most sought after cells in inciting autoimmunity and are sustenance to autoimmune diseases. In these diseases, dendritic cell priming of the immune response in lymphoid organs is a requisite first step that leads to the activation of T cells and production of pathogenic autoantibodies and the ensuing chronic inflammatory process. On the other hand, using mixed lymphocyte reaction (MLR), it was found that dendritic cells expressing HLA-G mediated the inhibition of autologous T cell activation. It is suggested that IFN- β can increase HLA-G in DCs from diabetic patients. Therefore it may prevent the immune regulatory pathway in the diabetic pathogenesis [112].

5.3. Natural killer cells (NK) cells

Data on NK cells in human type 1 diabetes are sparse and so far inconclusive. Initial studies reported conflicting results on the frequency and activity of NK cells in peripheral blood samples from type 1 diabetic patients. A limitation of the studies on NK cells in humans is the use of peripheral blood samples that might not reproduce the ongoing process in the infiltrated pancreatic islets [113]. Among the many cell types of the immune system, natural killer cells are one of the earliest cell types to arrive at target organs of inflammation. These cells can be swiftly mobilized by danger signal and are among the earliest arrivals at target organs of disease. These cells can recirculate between organs; therefore, the distribution of natural killer cells is not static [114].

In humans, NK cells are identified by the lack of the surface marker CD3 and the presence of CD56 with or without CD16. Most circulating natural killer cells are CD56lowCD16+, and can kill target cells, and produce cytokines following specific recognition of their targets [115]. The other subset of NK cells, CD56hiCD16-, is the predominant NK cell subset in peripheral lymphoid organs. These cells produce large amounts of cytokines such as IFN- γ , TNF- α , and granulocyte-macrophage-colony-stimulating factor (GM-CSF) following stimulation by pro-inflammatory cytokines and acquire cytotoxicity only after prolonged activation [115].

Evidence from animal models suggests that natural killer cells can be important players in the development of type 1 diabetes, although data in humans are still sparse. NK cells are potentially involved in onset of type 1 diabetes, given their ability to kill target cells and to interact with antigen-presenting cells and T cells. These cells act through the cytotoxic destruction of their target cells. NK cells recognize the target cells through specific molecules on the surface of these cells. Studies in animal models and in man have shown that NK cells are involved both in disease progression and in disease protection, thus suggesting a sort of dual role of these cells in type 1 diabetes pathogenesis, possibly reflecting a certain degree of heterogeneity in patients as well as in

experimental models. The contribution of NK cells to disease pathogenesis may differ in different stages during the disease's natural history [113].

When activated, NK cells induce apoptosis of the target cells mainly through the production and secretion of perforin and granzyme. As mentioned above, these cells also secrete pro-inflammatory cytokines such as IFN- γ , TNF- α [140], and granulocyte-macrophage colony-stimulating factor and macrophage inflammatory protein 1 α and 1 β [116]. Because NK cells are a major source of IFN- γ , a key pro-inflammatory cytokine, it is hypothesized that their pathophysiological impact on diabetes is to modulate the aggressiveness of the immune attack and the rate of progression from insulinitis to overt diabetes [117]. Of note, NK cells can kill autologous cells [118]. They might influence the downstream response and these cells can affect the proliferation and generation B and T autoreactive lymphocytes. These cells might prevent the presentation of self-antigens by dendritic cells; therefore, they also seem to be necessary for the initiation of autoreactive B and/or T cell response [119]. NK cells can also secrete cytokines that suppress B and T cell responses or even destroy them [120].

Two main categories of NK receptors were identified in humans: NKG2A, specific for HLA E molecules, and killer cell immunoglobulin-like (KIR) receptors, that recognize HLA A, B, and C molecules [121]. NKG2D is one of the main NK activating receptor. This receptor is expressed by the majority of human NK cells but also in other lymphocytes such as CD8 [121]. KIR receptors are not always inhibitory. These receptors belong to diverse family receptors, both stimulatory and inhibitory, expressed mainly by NK cells, but also by some groups of T lymphocytes [122].

Impaired NK cell function has been observed in the blood of diabetic patients and in lymphoid tissues of NOD mice. Some of these alterations are linked to type 1 diabetes onset while others seem to be a consequence of the disease. A slightly decreased expression of NKG2D in type 1 diabetic patients relative to control subjects was observed, independent of the duration of disease [117]. Qin et al. [123] have observed that NK cells from type 1 diabetic patients were present at reduced cell numbers compared to nondiabetic control subjects. These cells had diminished responses to IL-2 and IL-15. After IL-2 stimulation, NK cells from type 1 diabetic patients failed to downregulate the NKG2D ligands, MHC class I-related chain A and B, upon activation. These cells obtained from diabetic patients also exhibited decreased NKG2D-dependent cytotoxicity and IFN- γ secretion, and showed defects in NKG2D-mediated activation of the phosphoinositide 3-kinase-AKT pathway [123]. The NKG2D receptor is underexpressed in NOD NK cells and these cells exhibit decreased NKG2D-dependent functioning [124]. On the other hand, type 1 diabetic patients had an increased frequency of KIR gene haplotypes that include the activating KIR2DS3 gene, with a genetic interaction between the KIR and HLA complexes [117].

Animal studies showed that proliferating and activated NK cells were detected only in the early pre-diabetic infiltrates [125]. During the later stages of diabetes development, most pancreatic NK cells of NOD mice became hyporesponsive, as reflected by diminished cytokine secretion, and a lower capacity to degranulate in response to antibodies specific for activating receptors [126]. It is suggested that hyporesponsive phenotype of NK cells might be partly caused by interaction between NK cell receptors and their ligands expressed by β -cells. Ligands for NKG2D might downregulate the expression of NKG2D and "turn off" NK cells to avoid excessive autoimmunity [127].

NK cells can exhibit protective functions in β -cell autoimmunity. Several studies performed mainly in animal models, but also in man, have shown that NK cell can be involved in disease protection. Experiments performed in BDC2.5 transgenic mice on an NOD vs. C57BL/6H-2g7 genetic background showed that NK-cell infiltration was increased in islets in the aggressive C57BL/6H-2g7 lesions. NK cell participation was also enhanced in the aggressive lesions provoked by cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) blockade in BDC2.5/NOD mice [128]. In this context, depletion of NK cells significantly

inhibited diabetes development. Blockade of the NK cell activating receptor NKG2D prevents disease in NOD mice [129]. It was shown that NK cells mediate the protective effects possibly through the down-regulation of autoreactive cytotoxic T lymphocytes (CTL) and through the secretion of IFN- γ [130]. These cells have an important role in islet allograft tolerance in C57BL/6 mice induced by blocking interactions between CD40 and CD154 [131]. NK cells have an important role in mediating the capacity of complete Freund's adjuvant (CFA) to protect NOD mice from diabetes [132].

In summary, given prosperity of NK cells to produce IFN- γ and kill other cells, these cells may influence the development of autoimmune diseases through direct tissue destruction or indirectly via the regulation of adaptative immune responses or the modification of antigen presenting cells [133].

5.4. Neutrophils

Neutrophils are the essential component of the innate immune system. These cells, being the member of the non-specific immune system, play a critical role in the host defense mechanism against various bacterial infections. Neutrophils play an important role during the early host response to infection by a coordinated series of effector functions such as chemotaxis, phagocytosis, and the generation of reactive oxygen species.

In search for the roles of neutrophils in the pathogenesis of diabetic complications, many studies on the functions of these cells have been carried out and ended with many controversies [134]. Although several studies have been carried out to resolve the controversial non-specific immune responses in type 1 diabetes, inexplicable controversies with regard to possible functional defects of neutrophils still persist. Observed defects in chemotaxis, phagocytosis, killing of bacteria and release of superoxide in neutrophils of type 1 diabetic patients and animal models [134–136] are probably an effect of disease and not a cause of disease [137].

6. Adaptive immunity and type 1 diabetes mellitus

The adaptative immune system is body's second line of defense against pathogens and disease. It is an antigen-specific structure that discriminates non-self molecules through the recognition of antigens using receptor interactions between T cells and antigen presenting cells. The adaptative immune response generates long-term immunological memory. It triggers clonal expansion of T cells, which in turn cross-talk to B cells to produce antigen-specific antibodies. T cells and their specific T cell receptor (TCR) are a crucial part of the adaptative immune system. This system depends on the ability to assemble rearranged genes for both TCR and the immunoglobulin gene. Both the naïve T and B cell receptor repertoire are generated by interaction with self-ligands, such as the MHC, which in turn can signal to T and B cells to mature and survive. Once recognition of peptide-bound MHC complex occurs, a cascade of signaling events takes place, leading to either CD4 + T cell or CD8 + T cell response. It depends on which class of MHC was initially recognized by TCR.

It is currently accepted that type 1 diabetes is an autoimmune disease in which T cells play an important role. These cells are the key players in the autoimmune attack of β -cells [65]. Two animal models of type 1 diabetes have been extensively analyzed: the biobreeding (BB) rats and the nonobese diabetic (NOD) mice. Anti-islet T cells, both CD4 and CD8 T cells have been identified in type 1 diabetic patients as well as in the animal model. The autoimmune nature of type 1 is supported by experiments in NOD mice. Importantly, transfer of anti-islet specific CD4 or CD8 T cells induces diabetes in immuno-incompetent NOD mice. In contrast, antibodies do not transfer the disease [66,67]. Insulinitis and diabetes can be adoptively transferred with T cells from diabetic mice into non-diabetic recipients [67], whereas B cells are not needed [68]. It is widely-held theory that the insulinitis seen in patients

with type 1 diabetes is initiated by diabetogenic T cells that then recruit a heterogeneous mixture of cells. This mixture includes bystander T cells which are autoantigen-specific and/or do not contribute to the disease [69]. The ability of T cells to infiltrate or accumulate in the pancreatic islets is cell-autonomous, antigen-specific and tightly regulated [69].

CD8⁺ T cell-mediated β -cell killing is likely a major mechanism of β -cell destruction. CD8 T cells directly attack β -cells carrying the target autoantigens [70]. These T cells were found in insulinitic lesions in NOD mice and in human, and can destroy β -cells upon activation via MHC class I expressed on pancreatic β -cells [17]. According to observations of Hamilton-Williams et al. [71], deficiency in MHC class I due to lack of beta-2 microglobulin or beta-restricted MHC class I deficiency is sufficient to arrest diabetes development and prevent β -cell destruction in NOD mice. Destruction of β -cells can involve the release by CD8 T cells of cytolytic granules containing perforin and granzyme B. According to Van Belle et al. [17], CD4 T cells mostly provide help to both B cells and CD8 T cells by providing cytokines, such as interleukin-21 (IL-21), and a positive-feedback loop via CD40L–CD40 interactions to antigen-presenting cells, cumulating in a proficient autoreactive CD8 T cell response. Of note, IL-21 is required for the development of type 1 diabetes in NOD mice [72]. CD4 T cells are activated by β -cell antigen presenting cells (macrophages or dendritic cells). Macrophages secrete IL-12, stimulating CD4 T cells to secrete interferon- γ (IFN- γ) and IL-2. IFN- γ stimulates other resting macrophages to release in turn other cytokines, such as IL-1 β , tumor necrosis factor- α (TNF- α), and free radicals, which are toxic to β -cells. Secreted proinflammatory cytokines and free radicals are mediators of β -cells death. Cytokines produced by activated CD4 T cells attract T and B lymphocytes and encourage them to proliferate in the islet leading to insulinitis. Th17 cells are able to produce IL-17A, IL-17F, and IL-22. These cells provide protection in certain infections but have also linked to a number of autoimmune diseases, a function previously assigned to Th1 cells and IFN- γ ; however, it is not entirely clear if the Th17 pathway is influenced in type 1 diabetes [5]. B lymphocytes produce antibodies against released β -cells antigens. Chemokines that are subdivided into four families termed CXC (CXCL1–16), CX3C (CX3CL1), C (CCL1–2), and CC (CCL1–28) are chemotactic cytokines that attract leukocytes to tissues [59]. Pancreas infiltrating CD4 T cells produce CC (CCL2–5, CCL7, and CCL12), CXCL10, and XCL1. In a transgenic model of autoimmune diabetes, these cytokines produced by islet-specific Th1 cells promote induction of type 1 diabetes [60]. The presence of CD4 T cells in insulinitic lesions suggests a beacon role and direct inflammatory properties.

CD8 T cell epitopes are derived from preproinsulin signal peptide [73], IA-2, IAPP [18], IGRP, ZnT 8 [74], and GAD65 [43]. CD4 T cell epitopes are derived from GAD65, IA-2, and PI. Smaller contributions are obtained from HSP-60, HSP-70, and IGRP.

Lymphocytes can directly kill β -cells through a cytotoxic process. These cells can also kill β -cells through the release of proinflammatory cytokines. Proinflammatory cytokines, such as IL-1 β , IFN- γ , as well as free radicals, are mediators of pancreatic β -cell death. Cytokines influence the expression of inducible nitric oxide synthase (iNOS), leading to nitric oxide (NO) production. Nitric oxide synthesis decreases expression of ATPase 2b (SERCA2b), depletes Ca²⁺ stores and activates endoplasmic reticulum stress pathway, which is a mechanism to β -cell death [75]. Nitric oxide contributes to cytokine-induced apoptosis through activation of Jun NH2-terminal kinase (JNK) and suppression of members of the serine/threonine-specific protein kinase (Akt) family [76]. Free radicals can induce, in turn, apoptosis and necrosis of β -cells by activating the caspase pathway and inducing excessive cells stress, respectively [77]. T cells can also induce in β -cells pathways of apoptosis through FAS and FAS ligand-dependent interactions [5,17,75,78]. IL-1 β and TNF- α induce the nuclear factor κ B (NF- κ B) signaling pathway, which promotes apoptosis of β -cells by increasing of FAS. TNF- α and IFN- γ act synergistically to activate the transcription factor signal transducer and activator of transcription-1 (STAT-1) signaling,

iNOS overexpression, and secretion of NO, thereby promoting apoptosis of β -cells by the p53 pathway [77].

In phase 2 of type 1 diabetes disease progression, both T and B lymphocytes accumulate in an islet lesion. Here these cells can be activated against self antigens to trigger an immunological response that leads to the destruction of pancreatic β -cells. It is important, that both MHC class I and MHC class II restricted T cells are necessary for type 1 diabetes progression in both humans and NOD mice [2,138]. It is thought that interactions between antigens and MHC molecules can cause the development of an autoimmune T cell repertoire.

In summary, T cells secrete large amounts of cytokines in response to antigen-specific activation. Th1 cells, as mentioned above, produce IFN- γ , which enhances cellular immunity and is important for intracellular defense, autoimmunity, and anti-tumor response. Th2 cells produce IL-4, IL-5, and IL-13, which enhance humoral immunity and are important for extracellular defense. Th17 subset of T helper cells was first recognized during assessment of the involvement of IL-23 in autoimmune disease. Th17 cells have been linked to a number of autoimmune diseases, but it is not entirely clear if the Th17 pathway is influenced in type 1 diabetes [5]. It is necessary to determine what exact factors are causing the immune system to become unregulated in such a manner as to promote an autoimmune response [64].

7. Prevention trials and therapy for type 1 diabetes mellitus

Patients with type 1 diabetes can have a near-normal life relying on the administration of exogenous insulin by daily injections, continual pump therapy, or islet transplantations. Through these methods, diabetic patients can optimize the glycemic control and decrease the incidence of diabetes complications. In order to optimize the treatment for this disease, large investigations have been designed and conducted to evaluate prevention trials [141] and therapy for type 1 diabetes. Based on current concept of the disease, it seems to be possible to delay or prevent type 1 diabetes.

7.1. Primary prevention trials

Primary prevention is treatment in infants with increased genetic risk. These studies include several dietary manipulations, such as free of cow's milk, delayed exposure of gluten-containing foods, and vitamin D supplementation. To date, all primary prevention trials have been dietary interventions designed to interrupt putative environmental factors of type 1 diabetes. Because none of the specific dietary factors has been shown to be an unequivocal risk factor for type 1 diabetes, and their effects have still been contradictory, it is difficult to evaluate the efficiency of the primary interventions [141,142].

7.2. Secondary prevention trials

Secondary prevention is targeted at individuals with persistent islet autoantibodies. These prevention trials involve the use of nicotinamide or antigen-specific therapies. In animal studies nicotinamide has been shown to increase insulin synthesis and inhibit the development of diabetes if administered prior to the onset of disease [143]. The results obtained from participants either to nicotinamide or placebo groups showed that the rates of type 1 diabetes development were essentially the same [144].

Antigen-specific therapy is based on the concept that the appropriate administration of a diabetes autoantigens controls the autoimmune response. This kind of therapy, according to idea, changes immune system to a protective rather than destructive response [141]. Antigens used in this trial were parenteral insulin, oral and nasal insulin or intradermal administration of proinsulin peptides, and a vaccine with GAD. In the case of parenteral insulin, the intervention included low-dose subcutaneous ultravalente insulin twice every day with a total dose of 0.25 units/kg body weight per day [145]. The results failed to demonstrate

the delay or prevention in type 1 diabetes. As only one dose of insulin was tested, it was impossible to evaluate the effect of insulin in the protection of the β -cells. The study with subjects positive for anti-insulin autoantibodies that received oral insulin demonstrated that it was no delay in the clinical onset of type 1 diabetes as compared to participants treated with placebo [146]. The trial showed that the nasal insulin had no effect on the protection of the disease [147]. A pilot safety study with a single proinsulin peptide administration has been performed in patients with established type 1 diabetes [148] and recently, several trials with various proinsulin peptides are underway [141]. Whether a vaccine using glutamic acid decarboxylase can promote the prevention of type 1 diabetes is being assessed by the Diabetes Prevention-Immune Tolerance Study in Southern Sweden [144]. It is to note that a French pilot trial indicated that immunosuppression with low-dose cyclosporine may delay the development of type 1 diabetes [149].

7.3. Pancreatic β -cell regeneration

The key of treating type 1 diabetes is to replenish the lost β -cells. During the last two decades, much effort has been made into exploring β -cell regeneration. It is commonly accepted that the total number of pancreatic islets remains constant in the life time while the size of islet increases with age. Moreover, pancreatic β -cells which have longer life-span do not undergo proliferation frequently [150]. On the other hand, many preclinical studies revealed that given proper stimulation some β -cells may regain the potential to proliferate [151]; however, only a small group of specialized β -cells will regain the potential to proliferate under certain conditions [152].

Two categories of factors or hormones have been used to promote β -cell regeneration. The first category includes factors involved in the development and differentiation of pancreatic β -cells such as cyclin D1/D2, cyclin-dependent kinase 4 (CDK4) [154], glucagon-like peptide-1 (GLP-1), neurogenin 3 (Ngn3) [155] or pancreatic duodenal homeobox-1 (Pdx1) [153]. From mentioned factors, CDK4, GLP-1 and Ngn3 were demonstrated to be effective to promote β -cell regeneration [155,156]. As a second category there are mitogenic factors: vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF). These factors seemed to work through combination with the native mechanism of β -cell regeneration in a synergistic or additive manner [157]. A hormone called betatrophin, produced by liver and fat, was identified [158]. According to author's observations, this hormone worked specifically on pancreatic β -cells to promote replication. The injection of betatrophin led to a 17-fold increase in β -cell proliferation in comparison to the control [158].

7.4. Stem cell transplantation therapy

Stem cells with proliferation capacity and transdifferentiation potential are being explored as a promising new treatment for tissue regeneration, although their immunomodulatory properties are not yet completely understood. These cells were used to repair bone defect [159] and heart infarct [160] and can be used to promote β -cell regeneration [161]. Low immunogenic potential of mesenchymal stem cells (MSCs) together with their effects on immune response makes them a promising therapeutic tool for severe refractory immune diseases, such as type 1 diabetes [162]. Stem cells therapy, based on the generation of insulin-producing cells derived from mesenchymal stem cells, represents an attractive possibility. As shown by in vitro experiments, MSCs can modulate the immunological activity of different cells. The most important being their inhibitory effect on T cell proliferation and dendritic cell differentiation – key factors for activating autoimmune disorders. These cells inhibit proliferation of CD4, CD8, memory and naïve T cells [163], and can also stimulate the production CD8 + Treg which inhibits lymphocyte proliferation in allogeneic transplants [164].

It is important that MSCs can be differentiated into insulin-producing cells by using a specific culture medium. Insulin-producing cells, obtained

from MSCs, express gene related to pancreatic development and function, such as insulin I and II, GLUT 2, glucose kinase, islet amyloid polypeptide, and pancreatic duodenal homeobox. These cells synthesize C peptide and insulin [165]. As a source of insulin producing cells there are bone marrow MSCs (BM-MSCs), adipose-derived MSCs (ADSCs), umbilical cord blood MSCs (UCBhMSCs), pancreatic MSCs [162], and amniotic stem cells [166].

Several studies have reported on the treatment of newly diagnosed type 1 diabetes using stem cell transplantation. For example, newly diagnosed type 1 diabetic patients treated with autologous hematopoietic stem cell transplantations achieved good glycemic control [167]. The majority of children with Fanconi anemia had normal glucose tolerance and normal β -cell function after hematopoietic cell transplantation [168]. Patients with insulinopenic diabetes showed a 30–50% decrease in insulin requirements after human adipose-derived MSCs transplantation [169].

Stem cell therapy which is still under preclinical studies has faced more practical challenges. Stem cell therapy has demonstrated its potential in many reports, as a potent immunosuppressant and trophic mediator. These results suggest the use of MSCs in clinical practice as a new therapeutic option to treat type 1 diabetes.

7.5. Pancreatic or islet transplantation

β -cell replacement therapies represent a therapeutic alternative for insulin injections with careful blood glucose monitoring [170]. Human pancreatic transplantation has demonstrated partially successful results in reversing long-term renal and neural complications in selected type 1 diabetic patients. This therapy also offers glycemic control and sufficient prevention of hypoglycemia without the need for exogenous insulin administration [171].

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