Review paper

Ethiopathogenesis of autoimmune diabetes mellitus in humans. A review

DANIELA KANTÁROVÁ[1], MILAN BUC[2], MARTINA STUCHLÍKOVÁ[2], MARIÁN MOKÁŇ[1], MOJMÍR VRLÍK[3]

[1]I Internal Clinic, Comenius University, Jessenius School of Medicine and Martin Center of Immunology, Martin, Slovakia; [2]Department of Immunology, Comenius University School of Medicine, Bratislava, Slovakia; [3]Martin Center of Immunology, Martin, Slovakia

Abstract

Type 1 diabetes mellitus is an autoimmune disorder characterized by the destruction of insulin-producing β-cells of the pancreatic islets of Langerhans' and lack of endogenous insulin. Susceptibility to type 1 diabetes mellitus is influenced by both genetic and environmental factors. It is generally believed that the environmental agents, such as viral infections, dietary factors in early infancy or climatic influences, trigger disease development in genetically susceptible individuals. The HLA genes are the strongest determinants for type 1 diabetes – there are some susceptibility genes, especially HLA-DR3, -DR4, HLA-DQ2, -DQ8. β-cells destruction is caused by T cell mediated autoimmune reaction. Effector mechanisms probably are dominated by cell-mediated β-cell destruction. The phase prior to the onset of insulin deficiency is heralded by the development of circulating autoantibodies against multiple antigens (insulin, β-cell antigens).

Key words: *autoantibodies, autoreactive T cells, HLA alleles, IDDM, viruses.*

(Centr Eur J Immunol 2006; 31 (3-4): 102-110)

Diabetes mellitus is a heterogeneous group of metabolic disorders characterized by glucose intolerance and hyperglycemias. The clinical manifestations are the result of either absolute or relative deficiency of insulin secretion. Diabetes mellitus is a leading cause of blindness, amputations and end-stage renal disease, and is a major factor contributing to cardiovascular diseases and premature death. An expert committee of American Diabetes Association has proposed four categories of diabetes, based on aetiology of the disease. The categories are type 1 (formerly defined as insulin-dependent diabetes mellitus), type 2 (with a major component of insulin resistance, defined as non-insulin dependent diabetes mellitus), gestational diabetes a other types of diabetes (e.g. the maturity-onset diabetes of youth) [1].

Type 1 diabetes accounts for about 10% of all diabetes, affecting app. 20 million people worldwide [2, 3]. It can be further divided into type 1A (immune mediated) and 1B (not immune mediated but with profound loss of insulin secretion), respectively [4, 5].

Type 1A diabetes mellitus (DM 1A) is a condition caused by an autoimmune response against insulin-secreting beta cells of the pancreas resulting in their destruction. It is usually characterized by acute onset and dependence on exogenous insulin for survival.

Epidemiology of Type 1A diabetes mellitus

Current estimates indicate that autoimmune diabetes mellitus represents app. 5 to 10% of the diabetes developing in adults. A recent analysis of data on published incidence trends showed that the incidence of DM 1A had been generally increasing by 3.0% per year, and it would be app. about 40% higher in 2010 than in 1998 [6].

When an individual presents with DM 1A, it indicates that he/she and his/her relatives have an increased risk of suffering from or developing a series of autoimmune disorders, such as celiac disease (app. 5% patients with DM 1A), Grawes-Basedow´s disease, Addison´s disease, pernicious anaemia or other [7, 8].

A general description of the disease process begins with a genetic susceptibility to the development of DM 1A,

Correspondence: Daniela Kantárová, MD, Internal Clinic, Faculty Hospital, Kollárova 2, 036 59 Martin, Slovakia, Email: daniela.v@pobox.sk

a triggering event followed by autoimmune reactions that cause complete islet β cells destruction [9]. It means that the autoimmune response causing insulitis is generally triggered by an environmental stimulus, but occurs primarily in those who are genetically predisposed to the disease.

Type 1A diabetes tends to run in families and there is substantial evidence that genetics plays a significant role in causing the disease [10]. Identical twins of patients with DM 1A have an overall risk of developing the disease 50%. This risk varies dramatically with the diabetic twin's age of disease onset. If the identical twin develops diabetes after age 5, the risk for the other twin exceeds 50%. In contrast if the twin develops diabetes after age 25, the risk is less than 10%. The risk of developing diabetes is app. 5% for a sibling or an offspring of the DM 1A patient and that in common population is app. 1:300. The incidence decreases in the third decade of life to increase again in the fifth to seventh decade [11].

One of the most striking characteristics of DM 1A is large geographic variability in its incidence. Scandinavia and the Mediterranean island of Sardinia, respectively, have the highest incidence rates in the world contrary to the Oriental population with the lowest rate. A child in Finland is 400-times more likely to develop diabetes than one in China. The incidence rate of DM 1A in developed countries has been clearly rising and affecting younger children [12]. The geographical and ethnic variations in type 1A diabetes reflect the prevalence of susceptibility genes or causal environmental factors, or both [13-15].

DM 1A diagnosed in adulthood seems to be associated with male excess with a male: female ratio between 1.3 and 2.15 in most populations of European origin and there is also the pubertal peak of incidence in females [16, 17].

Genetics of type 1A diabetes mellitus

DM 1A is a genetically determined disease and many genes or genetic regions were found to be associated with its induction [for review see 18, 19]. Several genome-wide linkage studies have been conducted to identify candidate regions that may contain unidentified susceptibility genes. About 20 candidate regions for diabetes genes have been reported in linkage studies of affected sib pairs. Most of the known or suspected susceptibility loci have been designated IDDM, e.g. IDDM1 refers to genes mapping to the HLA region at 6p21, IDDM2 to the insulin region at 11pl5, etc. [20] (table 1).

The genetic region of the major histocompatibility complex in man, **HLA** (***h****uman* ***l****eukocyte* ***a****ntigens*), is located on the short arm of chromosome 6 (p21.3) and occupies a large segment of DNA, extending about 3,600 kb (3.6 cM). It is a region of highly polymorphic genes that form separate gene clusters, class I (telomeric) and class II (centromeric). These two regions are separated by another cluster of unrelated genes called class III [21-23] (figure 1). A major determinant of genetic susceptibility to autoimmune diseases resides in the HLA class II region. HLA class II molecules, particularly DR and DQ, account for app. 40% of the genetic risk for DM 1A development. As the HLA region displays a significant degree of linkage disequilibrium (i.e. specific DQ and DR alleles are non-randomly associated with each other), associations of HLA alleles with disease must be considered as haplotype specific and not allele specific.

Individuals with the highest risk for type 1A diabetes express both predisposing haplotypes: DQA1*0501-DQBl*0201 (DQ2), which is almost always inherited with DRBl*0301 (DR3) and DQA1*0301-DQBl*0302 (DQ8), inherited with DRBl*0401 or DRBl*0402 (DR4) [24-26]. These individuals have been referred to as DR3/DR4, DQ2/DQ8 heterozygotes. Individuals who carry this high risk haplotypic combination have ~5% absolute risk of DM 1A, however within affected families, this genotype shows ~20% risk; app. 40% of diabetic children possess this

Table 1. DM-1A susceptibility loci

Locus	Region	λ_s	LOD
IDDM1 *(HLA)*	6p21	1.7-4.2	65.8
IDDM2 *(INS)*	11p15	1.6	4.28
IDDM3	15q26		
IDDM4	11q13	1.0-1.5	2.7
IDDM5	6q25		4.5
IDDM6	18q12-q21	1.0-1.5	1.1
IDDM7	2q31	1.0-1.6	1.2
IDDM8	6q27	1.0-2.1	3.6
IDDM9	3q22-q25	1.0-1.7	3.4
IDDM10	10p11-q11	1.1-2.2	2.8
IDDM11	14q24-q31		4.0
IDDM12 *(CTLA-4)*	2q31-q33		3.57
IDDM13*	2q34-q35		
IDDM15	6q21		2.36
IDDM16 *(IGH)*	14q32		
IDDM17	10q25		2.38
IDDM18 *(IL-12p40)*	5q33		
	7q25		1.81
	16q22-q24	1.6	3.93
	1q42		2.2
	8q22-q24		2.4

** The symbol IDDM14 has been reserved, but not published.*
λ_s – lamda-s – these values reflect a sibling risk of a disease in relation to its population prevalence.
References to particular findings are availablein the publication Kantarova D et al. [19]

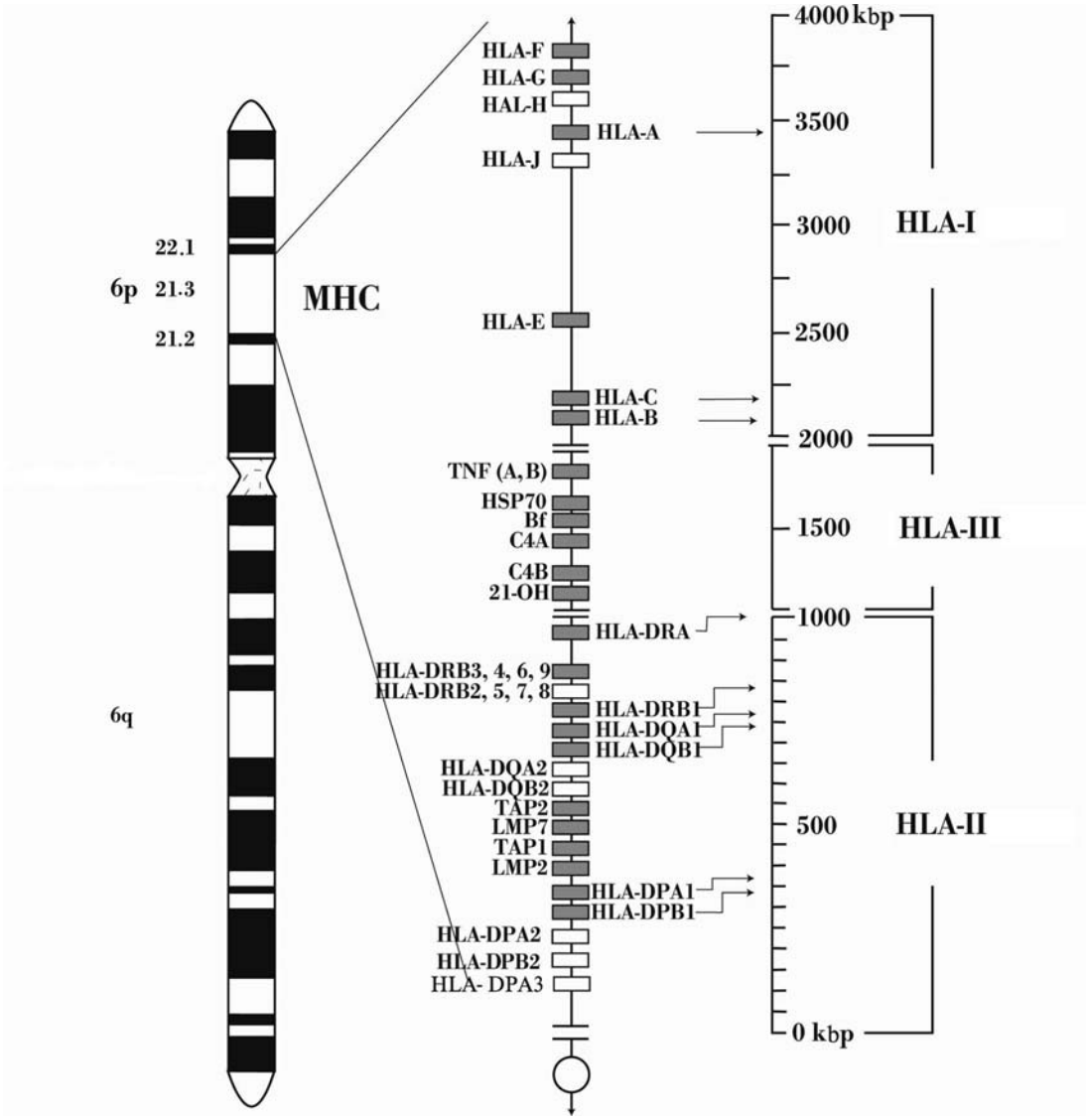

Fig. 1. Scheme of genetic region of the major histocompatibility complex in man
The genetic region of the major histocompatibility complex in man, HLA (**h**uman **l**eucocyte **a**ntigens), is located on the short arm of chromosome 6 (6p21.3) and occupies a large segment of DNA, extending about 3600 kb. It is a region of highly polymorphic genes that form separate gene clusters, class I (telomeric) and class II (centromeric). These two regions are separated by another cluster of unrelated genes, which are called class III genes. The classical class I HLA loci are HLA-A, -B, and -C, those of class II are HLA-DR (DRA, DRB), -DQ (DQA, DQB) and -DP (DPA, DPB). Class III HLA region comprises the complement genes C2, C4, B, heat shock genes HSP70 and others

genotype compared to 2% of children in the healthy population [20, 24, 25].

HLA-alleles have also been associated with protection from type 1A diabetes, esp. the haplotype DQA1*0102/DQB1*0602/DRB1*1501 (DQ6, DR15) confers strong protection. Evidence suggests that such protection may be mostly encoded by the DQB1*0602 allele; however, although this protective effect is extremely powerful, it is not absolute [24, 27, 28]. To summarize, as some HLA haplotypes are associated with high, moderate, low risk and even "protection", their identification is useful in disease prediction (table 2).

There are also some reports on associations between the last group of class II HLA alleles and DM 1A. HLA-DPB1*0101, -DPB1*0301, and -DPB1*0202, respectively, were reported to be positively and HLA-DPB1*0402 negatively associated with [29-31].

The influence of the HLA class II molecules on the risk of DM 1A is related to their central role in antigen presentation and the activation of a helper T cell mediated immune response [for a review see 22].

The second well established susceptibility locus to DM 1A is on chromosome 11p15.5, which is mapped to a region containing the variable number of tandem repeat (VNTR) polymorphism in the promoter region of the **insulin gene** (IDDM2). This VTNR region is categorised into classes I to III. VNTR I homozygous individuals develop DM 1A more likely than those with VNTR III; VNTRII is associated with resistance to the disease induction. VNTR I allele predispose to DM 1A by reducing tolerance to insulin and its precursors via lower insulin transcription in the thymic medullar epithelial cells [18, 32, 33].

The next DM A1 susceptibility genetic region, **IDDM12**, is located on the long arm of chromosome 2 (2q33) and contains the CTLA-4 (*cytotoxic T lymphocyte antigen-4*) gene [34, 35]. CTLA-4 plays an important role in regulation of the immune response, esp. in suppressive activities of CD^+CD25^+ T regulatory cells (T_{reg}) [36].

The fourth established human DM 1A susceptibility locus is **PTPN 22** (*protein tyrosine phosphatase non-receptor type 22*; chromosome 1p13). It encodes a lymphoid protein tyrosine kinase (LYP) that is important in negative control of T cell activation and in T cell development [37, 38].

It is well recognized that IL-2 has paradoxical functions in T cell homeostasis, acting as a potent T cell growth factor during the initiation of immune responses and having a crucial function in the termination of T cell responses and maintenance of self-tolerance. The latter function has been proposed to be due to a requirement for interleukin 2 (IL-2) signalling for the development and function of regulatory T cells. From this point of view is very interesting evidence that the region containing the gene IL2RA encoding the alpha chain of the IL-2 receptor (CD25) on chromosome 10p15-p14 could be the fifth susceptibility locus for human DM 1A [39].

IDDM4 is a region on chromosome 11q13 and one of its genes that might be involved in DM 1A genetic predisposition can be that coding for FADD, a molecule involved in the apoptosis process (40). **IDDM7** on chromosome 2q31-33 may identical with NeuroD gene that is involved in morphogenesis of β cells of Langerhans´ islets [41, 42]. NRAMP1 gene is probably identical with **IDDM13**, a region on the long arm of chromosome 2 (2q34-35) [41]. It encodes a protein responsible form macrophage resistance to intracellular parasitic bacteria. **IDDM18** (5q31.1-q33.1) is a genetic region harbouring p40 chain of interleukin 12 [43]. IL-12 drives the differentiation of T cells towards the T_H1 subset and autoreactive T cell response induced by IL-12 might predispose to self-destructive immunity [44]. There is also a report on association **CD4 SNP promoter polymorphism** (12p) and type 1 diabetes mellitus [45]. CD4 is a principal differentiation antigen of T helper cells involved in cell cooperation and signal transduction.

Table 2. Spectrum of diabetes risk HLA haplotypes

High risk haplotypes			
DR3	DRB1*0301	DQA1*0501	DQB1*0201
DR4	DRB1*0401	DQA1*0301	DQB1*0302
	DRB1*0402	DQA1*0301	DQB1*0302
	DRB1*0405	DQA1*0301	DQB1*0302
Moderate risk haplotypes			
DR1	DRB1*01	DQA1*0101	DQB1*0501
DR8	DRB1*0801	DQA1*0401	DQB1*0402
DR9	DRB1*0901	DQA1*0301	DQB1*0303
Protective haplotypes			
Strong protection			
DR2	DRB1*1501	DQA1*0102	DQB1*0602
DR6	DRB1*1401	DQA1*0101	DQB1*0503
DR7	DRB1*0701	DQA1*0201	DQB1*0303
Moderate protection			
DR5	DRB1*1101	DQA1-0501	DQB1*0301
Weak protection			
DR4	DRBl*0401	DQAl*0301	DQBl*0301
	DRBl*0403	DQAl*0301	DQB1*0302
DR7	DRB1*0701	DQAl*0201	DQBl*0201

There are reports on other associations and one can expect that genetic DMA-1A map will be completely filled up in the near future.

Environmental factors in the ethiopathogenesis of Type 1A diabetes mellitus

Although DM 1A susceptibility has an apparent genetic origin, what exactly is responsible for stimulating the autoimmune response remains unclear. Various studies have shown that the concordance rate for type 1A diabetes among monozygotic twins is about 50%, meaning that environmental factors must also play a significant role. There is also some seasonal variation in the incidence of DM 1A, with more patients presenting with the disease in the fall of summer and winter months. This variation has been ascribed to potential viral infections causing beta cell destruction [46, 47].

Congenitally acquired **rubella virus** was the first reported to be associated with DM 1A in 1969 [48]. Rubella virus can be grown from foetal pancreas and app. 1/5 of children infected with rubella *in utero* progressed to DM

1A [49]. These data suggested that direct infection could be a mechanism for DM 1A development. However, attenuated live rubella vaccination, largely eradicating congenital rubella in the developed world, has not been linked with islet autoimmunity [50]. The mechanism of rubella-associated DM 1A remains therefore unresolved. Early direct infection, perhaps resulting in a breakdown of β cells differentiation during foetal development and not autoimmunity may be responsible for DM 1A following congenital rubella.

Mumps epidemics have been associated with an increase in incidence of DM 1A [51], and the **parotitis virus** was shown to infect human β cells *in vitro* [52]. However, similarly as with rubella, the mumps vaccination has not been associated with development of islet antibodies. Mumps virus-associated DM 1A remains therefore unproven [53, 54].

The association of enteroviruses (including entero-, echo- and coxsackie viruses) with DM 1A has attracted most attention when reported that enterovirus infections had been diagnosed in 51% of cases and 28% of controls in the six months before the development of islet antibodies [55, 56]. It was suggested that these viruses could stimulate a β cell autoimmune response. The sequence of the region of GAD (amino acid residues 250-273) is significantly similar to the **Coxsackie virus** B4 strain p2C protein (CB4--virus) [57]. It was thought that these viruses induced activation and proliferation of T cells specific for a viral epitope that mimics a protein unique to β cells (phenomenon of *molecular mimicry*) causing therefore cytotoxic T cells to respond to the β cells autoantigens [46]. However, further studies have proved that enteroviruses could be potentially causal at DM 1A onset, but not at the initiation of islet autoimmunity [58, 59].

Given sparse evidence for the initiation of islet autoimmunity by enteroviruses, attention was paid to another group of RNA viruses, to **rotaviruses**. Rotaviruses are double-stranded RNA viruses of the reovirus family and are the predominant cause of gastroenteritis in infants. Strong sequence similarities between rotavirus VP7 and two T cell islet antigen epitopes, one in GAD65 and one in IA-2 (54) make rototaviruses very suspect from inducing autoimmune processes resulting in DM 1A; however it needs to be confirmed in further studies.

The innate immune system senses invading microorganisms by a phylogenetically conserved family of proteins – pattern recognition receptors of which **Toll-like receptors** (TLRs) are ones of the most important [for a review see 60]. TLR3 is important in the induction of antiviral defence mechanisms. The replicative cycle of viral infections in many cases involves double-stranded RNA (dsRNA) production where dsRNA is either a by-product generated by a symmetrical transcription of DNA virus genomes or is an essential intermediate in viral RNA synthesis. dsRNA is a very potent inducer of type I interferons and other cytokines, which exert potent antiviral and immunostimulatory activities. As TLR3 is not only important in innate immunity, but is also present on β cells [61], it may be involved in mechanisms leading to autoimmune diabetes [62].

Some dietary factors may contribute to the development of diabetes too. Bovine milk ingestion, particularly in the first months of life is associated with development of the disease. One theory suggests that exposure to cow's milk can induce an autoantibody response to proteins (e.g. p69) expressed by β cells [63]. A possible explanation of the findings can be that in genetically predisposed individuals no tolerance to cow milk proteins develops; however, the question is not resolved as antibodies to various cow milk proteins can be also detected in healthy first degree relatives.

An increase of allergy and autoimmune prevalence has occurred in "westernized" societies over the past few decades. This is therefore thought to be primarily due to changes that have taken place in the environment in developed countries as result of reducing infections during early childhood. A consequence may be a bias of the physiological development of the immune system to either to T_H2 (allergies) or T_H1 (autoimmune disorders) direction or an insufficient activity of regulatory T cells (a hygiene hypothesis) [for reviews see 22, 64].

Autoimmune mechanisms in destruction of beta cells of pancreas

Type 1 diabetes mellitus results from an organ-specific autoimmune-mediated loss of insulin-secreting β cells of pancreas. This chronic destructive process involves both cellular and humoral components of immunity. Markers of this process are detectable in the peripheral blood months or even years before the onset of clinical diabetes [57].

The two distinctive features of type 1A diabetes are the infiltration of pancreatic islets by macrophages and lymphocytes and the presence of autoantibodies in the plasma [65]. Islet inflammation or insulitis is the hallmark lesion of DM 1A. Mononuclear cells, initially macrophages and dendritic cells, accumulate first around the islets. Subsequently, B and T cells (both helper and cytotoxic) arrive and form a periinsulitis. Insulitis occurs when macrophages and activated T cells invade the islets of Langerhans´ in the pancreas and attack the insulin-secreting β cells. Insulitis occurs during the pre-diabetic phase ultimately leading to the complete depletion of β cells, which is the characteristic of type 1 diabetes [3].

Infiltration of autoreactive T cells into the islets is essential for the development of diabetes. Studies performed in NOD mice have revealed that the influx of T cells into the pancreas is associated *de novo* formation of lymphoid follicles what is a prototypic feature of a chronic progressive inflammation. The experimental results show that the interaction between membrane lymphotoxin ($LT\alpha1\beta2$) and its receptor ($LT\beta R$) is

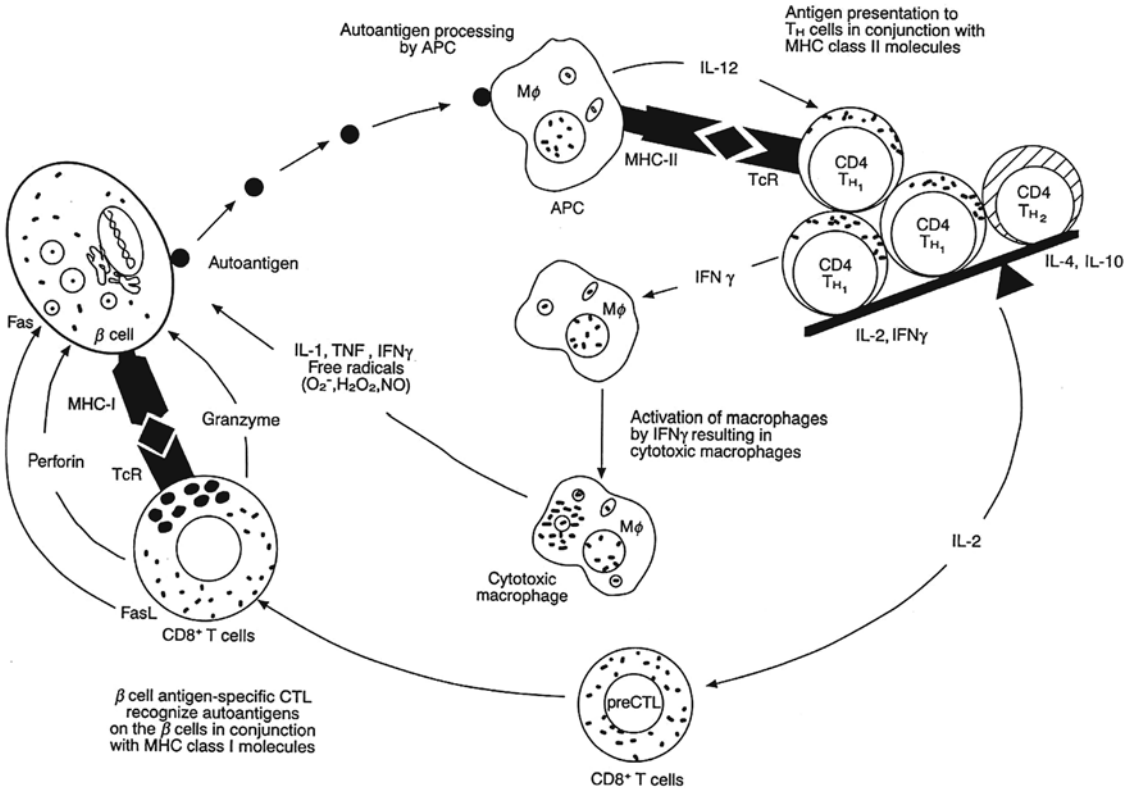

Fig. 2. A schematic description of the roles of various subsets of T cells and their cytokines in β-cell destruction, leading to diabetes (adapted from Muir A et al. [74]). Islet cell proteins are presented by antigen presenting cells (APC, i.e. dendritic cells or macrophages or B cells) to naive T_H0 type cells in association with HLA class II molecules and other co-stimulatory molecules. These APC secrete IL-12, promoting the differentiation of T_H0 cells to T_H1 type cells. T_H1 cells secrete IL-2 and IFN-γ that further stimulate macrophages or specific cytotoxic $CD8^+$ T cells leading to β-cell destruction and eventually diabetes. T cells on other hand can prevent β-cell destruction by secreting IL-4 early during the differentiation of T_H0 cells, favouring a benign T_H2 type response and down-regulating T_H1 cells. IL-4 and IL-10 secreted by $CD4^+$ T_H2 type cells and reduced IL-2 and IFN-γ secretion prevents β-cell destruction and diabetes

essential for the development and maintenance of this lymphoid microenvironment. Lymphotoxin knock out mice and wild-type mice treated with LTβ receptor-immunoglobulin fusion protein (LTβR-Ig) reverse insulitis and prevents the formation of lymphoid follicles, even when insulitis is well established [66]. This effect is likely caused by a failure to induce lymphoid tissue chemokines and adhesion molecules [67].

The autoimmune process is initiated by activation of autoreactive $CD4^+$ T_H1 cells. The primary antigenic stimulus to β-cell autoimmunity, however, remains unknown. One possibility is that T cell activation occurs within the islets, where autoantigens are presented either by β cells or by APCs in the vicinity. This seems, however, unlikely as naïve T cells circulate through peripheral lymphoid organs rather than through tissues. Another way could be an activation of T cells in the regional lymph nodes [68]. The third contingency is that potentially autoreactive T cells are first stimulated at a distant site, either in a tissue or in one of the peripheral lymphoid organs: T cells are activated by cross-reactive environmental antigens (*molecular mimicry* – see above) or microbial superantigens. Activated T_H1 cells start to produce IFN-γ and IL-2; they activate cytotoxic T cells that subsequently mediate the destructive processes of insulitis. There is also a class I HLA molecules hyperexpression that can increase cell-mediated β cell destruction. Macrophages by their production of proinflammatory cytokines, esp. TNF and IL-1, may induce apoptosis that contributes to β cell demise; reactive oxygen species and nitric oxide may have an additional destructive effect [1, 22, 69] (figure 2). The resolution of severe insulitis by LTβR-Ig treatment [66] suggests that migration of inflammatory cells into the islets is a dynamic process in which these cells may constantly move in and out of the target tissue what results in continuous

activation of autoreactive T cells by previously unrecognized autoantigens (epitope spreading) and tissue damage.

NKT cells are a population of T cells (T) that share some characteristics with natural killer (NK) cells. The key features characteristic of NKT cells include heavily biased T-cell receptor gene usage (Vα24JαQ and Vβ11, so called Vβ24*i*-NKT cells), CD1d restriction and high levels of cytokine production, particularly IL-4 and IFN-γ [70]. Because of this property, NKT cells participate in regulation of the immune response; they can skew it into either T_H1 or T_H2 direction and so substantially contribute to the development of autoimmune processes. Really, it was found that NOD mice were numerically and functionally deficient in NKT cells well before the onset of diabetes [71, 72]. Similar findings were reported in humans, too. Wilson et al. (1998) found that the diabetic siblings had lower frequencies of NKT cells compared with their non-diabetic sibling. Moreover, all NKT clones isolated from the diabetics secreted only IFN-γ upon stimulation; in contrast, most of clones from the non-diabetic sibling and healthy persons secreted both IL-4 and IFN-γ.

The role of B cells in insulitis is undefined. Since B cells can transfer neither insulitis nor diabetes, they have been considered to fulfil a non-destructive, perhaps antigen-presenting function, in the insulitis lesion. Nevertheless, autoantibodies can be detected in the sera of patients; their appearance reflects the progression of humoral autoimmunity to β cells. But however, there is no evidence that autoantibodies play a direct role in the immunopathogenesis of DM 1A [74, 75].

Pancreatic β cell components that are recognized by autoantibodies include insulin, proinsulin, GAD65, and two protein tyrosine phosphatases, IA-2 and IA-2β, respectively. Other islet antigens include carboxypeptidase H, ICA, and GM gangliosides [3, 74].

Insulin was the first autoantigen identified in DM 1A. Antibodies against insulin (IAA) are one of the earliest clinical markers of prediabetes. The levels of IAA are associated with the rate of the autoimmune destruction of the β cells making their detection an important aspect of diagnosis and prevention [46].

Glutamic acid decarboxylase of M_r 65,000 (GAD65) is an enzyme that converts glutamate to γ-amino butyric acid (GABA). GAD65 is expressed in human β cells and is a major target autoantigen in type 1A diabetes. Antibodies to GAD65 can be detected in the sera of 75% of new-onset DM 1A patients, one-third of whom test negative for ICAs. When, however, GAD65 antibodies coexist with additional ICAs, they indicate a high risk of DM 1A [75].

The membrane-associated protein tyrosine phosphatase, IA-2, is expressed in islets, brain and pituitary gland. Some 60-75% of new-onset DM 1A patients have autoantibodies against the protein, compared with only 2% of healthy controls. Intra-islet expression of a homologous protein, IA-2β, is restricted to β cells and displays an autoreactivity that is generally a subset of IA-2-positive persons [74].

Islet cell autoantibodies recognize multiple cytosol antigens. They are identified by indirect immunofluorescence of frozen human pancreatic sections and are the most sensitive single predictor of future DM 1A. However, the ICA procedure is technically demanding and simpler procedures for detecting autoantibodies against defined autoantigens are more reproducible [74].

Treatment

Current therapy of DM 1A relies on insulin application. However, insulin cannot prevent all of the late complications of diabetes, and life expectancy can be reduced by 10 to 15 years due to serious complications including retinopathy, nephropathy, cardiovascular diseases, or neuropathy. Systemic immunosuppression, e.g. with cyclosporine A, can halt β cell destruction [1, 76]. However, the protection only lasted as long as the drug was present and moreover, extended therapy was not feasible due to side effects. A more promising attitude is an induction of immune tolerance. Really, several biological agents were developed and tested, e.g. by lymphotoxin-beta receptor immunoglobulin fusion protein [66, 77], a marine-sponge-derived glycolipid, α-galactosylceramide [78], and others. The latest, very promising, attitude was reported by Bresson et al. recently [79]. They were successful in reverting clinically overt symptoms in two murine diabetes models (NOD and RIP--LCMV-NP mice, respectively) by simulataneous intranasal application of human proinsulin II B24-C36 peptide and intravenous administration of non-Fc-binding fully humanized IgG1 anti-CD3ε monoclonal antibodies. The co-administration of both biological agents resulted in expansion of $CD25^+Foxp3^+$ and insulin-specific Tregs producing IL-10, TGF-β, and IL-4. This observation is highly relevant for future clinical studies in humans.

Conclusion

Type 1 diabetes mellitus is an autoimmune disorder characterized by the destruction of insulin-producing β-cells of the pancreatic islets of Langerhans´ and lack of endogenous insulin. Susceptibility to type 1 diabetes mellitus is influenced by both genetic and environmental factors. It is generally believed that the environmental agents, such as viral infections, trigger disease development in genetically susceptible individuals. Despite the great complexity and difficulties we have faced in the field of immunopathogenesis, genetics, epidemiology of DM 1A etc., the arrival of sophisticated technologies, techniques, refinement of statistical methods will surely bring new knowledge and enable better to comprehend and to treat this modern plague of the mankind.

Acknowledgement

The Research Program of the Ministry of Education of the Slovak Republic, VEGA 1/3437/06, financially supported the study.

References

1. Robles DT, Eisenbarth GS: Diabetes and related autoimmune diseases. In: Clinical Imunology, Principles and Practice. Ed. RR Rich. Mosby. London, Edinburg, New York, Philadelphia, St. Louis, Sydney, Toronto. 2001, 82.1-82.18.
2. Rewers M (1991): The changing face of the epidemiology of insulin-dependent diabetes mellitus (IDDM): research designs and models of disease causation. Ann Med 23: 419-426.
3. Libman I, Songer T, LaPorte R (1993): How many people in the U.S. have IDDM? Diabetes Care 16: 841-842.
4. Devendra D, Liu E, Eisenbarth GS (2004): Type 1 diabetes: recent developments. BMJ 328: 750-754.
5. Pugliese A (2004): Genetics of type 1diabetes. Endocrinol Metab Clin North Am 33: 1-16.
6. Onkamo P, Vaananen S, Karvonen M, et al. (1999): Worldwide increase in incidence of Type I diabetes – the analysis of the data on published incidence trends. Diabetologia 42: 1395-1403.
7. Bao F, Yu L, Babu S, et al. (1999): One third of HLA DQ2 homozygous patients with type 1 diabetes express celiac disease-associated transglutaminase autoantibodies. J Autoimmun 13: 143-148.
8. Hoffenberg EJ, Bao F, Eisenbarth GS, et al. (2000): Transglutaminase antibodies in children with a genetic risk for celiac disease. J Pediatr 137: 356-360.
9. Pinhas-Hamiel O, Dolan LM, Daniels SR, et al. (1996): Increased incidence of non-insulin-dependent diabetes mellitus among adolescents. J Pediatr 128: 591.
10. Acton RT: The major histocompatibility complex. In: Clinical Immunology. Principles and Practice. Ed. RR Rich. Mosby. London, Edinburg, New York, Philadelphia, St. Louis, Sydney, Toronto. 2001, 6.1-6.13.
11. Mordes JP, Greiner DL, Rossini AA: Animal models of autoimmune diabetes mellitus. In: Diabetes mellitus: a fundamental and clinical text. Ed. D LeRoith, SI Taylor, JM Olefsky. Lippincott-Raven. Philadelphia. 1996, 349-360.
12. Bach JF (2002): The effect of infections on susceptibility to autoimmune and allergic diseases. N Engl J Med 347: 911-920.
13. Geographic patterns of childhood insulin-dependent diabetes mellitus. Diabetes Epidemiology Research International Group (1988): Diabetes 37: 1113-1119.
14. Green A, Gale EA, Patterson CC (1992): Incidence of childhood-onset insulin-dependent diabetes mellitus: the EURODIAB ACE Study. Lancet 339: 905-909.
15. Karvonen M, Viik-Kajander M, Moltchanova E, et al. (2000): Incidence of childhood type 1 diabetes worldwide. Diabetes Mondiale (DiaMond) Project Group. Diabetes Care 23: 1516-1526.
16. Gale EA, Gillespie KM (2001): Diabetes and gender. Diabetologia 44: 3-15.
17. Vandewalle CL, Coeckelberghs MI, De Leeuw IH, et al. (1997): Epidemiology, clinical aspects, and biology of IDDM patients under age 40 years. Comparison of data from Antwerp with complete ascertainment with data from Belgium with 40% ascertainment. The Belgian Diabetes Registry. Diabetes Care 20: 1556-1561.
18. Maier LM, Wicker LS (2005): Genetic susceptibility to type 1 diabetes. Curr Opin Immunol 17: 601-608.
19. Kantarova D, Buc M (2006): Genetic susceptibility to type 1 diabetes mellitus in humans. Physiol Res, in press.
20. Concannon P, Erlich HA, Julier C, et al. (2005): Type 1 diabetes: evidence for susceptibility loci from four genome-wide linkage scans in 1,435 multiplex families. Diabetes 54: 2995-3001.
21. Buc M (1993): The major histocompatibility complex in man. Folia Biol (Prague) 39: 223-242.
22. Buc M: Autoimmunity and autoimmune diseases. Veda, Bratislava. 2005 [in Slovak].
23. Zalkovicova M, Buc M, Niks M, et al. (1988): Genetic determination of phagocytic activity of polymorphonuclear leucocytes. Folia Biol (Praha) 34: 240-247.
24. Chowdhury TA, Mijovic CH, Barnett AH (1999): The aetiology of Type I diabetes. Clin Baillieres Best Pract Res Clin Endocrinol Metab 13: 181-195.
25. Nepom GT: HLA and type I diabetes. In: HLA in health and disease. Ed. R Lechler, A Warrens. Academic Press. New York, Boston, London, Sydney, Tokyo, San Diego, San Francisco. 2000, 231-237.
26. Buc M, Bucova M, Javor J, et al. (2006): Associations between HLA class II alleles and type 1 diabetes mellitus in the Slovak population. Endocr Regul 40: 1-6.
27. Redondo MJ, Kawasaki E, Mulgrew CL, et al. (2000): DR- and DQ-associated protection from type 1A diabetes: comparison of DRB1*1401 and DQA1*0102-DQB1*0602*. J Clin Endocrinol Metab 85: 3793-3797.
28. Greenbaum CJ, Gaur LK, Noble JA (2002): ICA+ relatives with DQA1*0102/ DQB1*0602 have expected 0602 sequence and DR types. J Autoimmun18: 67-70.
29. Noble JA, Valdes AM, Thomson G, et al. (2000): The HLA class II locus DPB1 can influence susceptibility to type 1 diabetes. Diabetes 49: 121-125.
30. Valdes AM, Noble JA, Genin E, et al. (2001): Modeling of HLA class II susceptibility to type I diabetes reveals an effect associated with DPB1. Genet Epidemiol 21: 212-223.
31. Stuchlikova M, Kantarova D, Michalkova D, et al. (2006): Association of HLA-DPB1 alleles with type I diabetes mellitus in Slovak population. Bratisl Lek Listy 107: 73-75.
32. Bennet ST, Lucassen AM, Gough SC, et al. (1995): Susceptibility to human type 1 diabetes at IDDM2 is determined by tandem repeat variation at the insulin gene minisatellite locus. Nat Genet 9: 284-292.
33. Barrat BJ, Payne F, Lowe CE, Hermann R, et al. (2004): Remapping the insulin gene/IDDM2 locus in type 1 diabetes. Diabetes 53: 1884-1889.
34. Kristiansen OP, Larsen ZM, Pociot F (2000): CTLA-4 in autoimmune diseases – a general susceptibility gene to autoimmunity? Genes Immun 1: 170-184.
35. Ueda H, Howson JM, Esposito L, et al. (2003): Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. Nature 423: 506-511.
36. Paust S, Lu L, McCarty N, Cantor H (2004): Engagement of B7 on effector T cells by regulatory T cells prevents autoimmune disease. Proc Natl Acad Sci USA 101: 10398-10403.
37. Bottini N, Musumeci L, Alonso A, et al. (2004): A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. Nat Genet 36: 337-338.
38. Smyth D, Cooper JD, Collins JE, et al. (2004): Replication of an association between the lymphoid tyrosine phosphatase locus (LYP/PTPN22) with type 1 diabetes, and evidence for its role as a general autoimmunity locus. Diabetes 53: 3020-3023.
39. Vella A, Cooper JD, Lowe CE, et al. (2005): Localization of a type 1 diabetes locus in the IL2RA/CD25 region by use of tag single-nucleotide polymorphisms. Am J Hum Genet 76: 773-779.
40. Nakagawa Y, Kawaguchi Y, Twells RC, et al. (1998): Fine mapping of the diabetes-susceptibility locus, IDDM4, on chromosome 11q13. Am J Hum Genet 63: 547-556.
41. Esposito L, Hill NJ, Pritchard LE, et al. (1998): Genetic analysis of chromosome 2 in type 1 diabetes: analysis of putative loci IDDM7, IDDM12, and IDDM13 and candidate

genes NRAMP1 and IA-2 and the interleukin-1 gene cluster. IMDIAB Group. Diabetes 47: 1797-1799.
42. Copeman JB, Cucca F, Hearne CM, et al. (1995): Linkage disequilibrium mapping of a type 1 diabetes susceptibility gene (IDDM7) to chromosome 2q31 – q33. Nat Genet 9: 80-85.
43. Bergholdt R, Ghandil P, Johannesen J, et al. (2004): Genetic and functional evaluation of an interleukin-12 polymorphism (IDDM18) in families with type 1 diabetes. J Med Genet 41: e39.
44. Bucova M (2002): Role of cytokines of local and systemic inflammation and septic shock. Vnitr Lek 48: 755-762.
45. Kristiansen OP, Karlsen AE, Larsen ZM, et al. (2004): Identification of a type 1 diabetes-associated CD4 promoter haplotype with high constitutive activity. Scand J Immunol 59: 582-591.
46. Buc M, Shawkatova I (2002): Immunopathogenesis of type 1A diabetes mellitus. Czech-Slovak Pediatry 57: 299-305 [in Slovak].
47. Honeyman M (2005): How robust is the evidence for viruses in the induction of type 1 diabetes? Curr Opin Immunol 17: 616-623.
48. Forrest JM, Menser MA, Harley JD (1969): Diabetes mellitus and congenital rubella. Pediatrics 44: 445-447.
49. Menser MA, Forrest JM, Bransby RD (1978): Rubella infection and diabetes mellitus. Lancet 1: 57-60.
50. Hummel M, Fuchtenbusch M, Schenker M, et al. (2000): No major association of breast-feeding, vaccinations, and childhood viral diseases with early islet autoimmunity in the German BABYDIAB Study. Diabetes Care 23: 969-974.
51. Hyoty H, Leinikki P, Reunanen A, et al. (1988): Mumps infections in the etiology of type 1 (insulin-dependent) diabetes. Diabetes Res 9: 111-116.
52. Parkkonen P, Hyoty H, Koskinen L, et al. (1992): Mumps virus infects beta cells in human fetal islet cell cultures upregulating the expression of HLA class I molecules. Diabetologia 35: 63-69.
53. Hyoty H, Hiltunen M, Reunanen A, et al. (1993): Decline of mumps antibodies in type 1 (insulin-dependent) diabetic children and a plateau in the rising incidence of type 1 diabetes after introduction of the mumps-measles-rubella vaccine in Finland. Childhood Diabetes in Finland Study Group. Diabetologia 36: 1303-1308.
54. Honeyman MC, Stone NL, Harrison LC (1998): T-cell epitopes on type 1 diabetes autoantigen tyrosine phosphatase IA-2: potential for mimicry with rotavirus and other environmental agents. Mol Med 4: 231-239.
55. Salminen K, Sadeharju K, Lonnrot M, et al. (2003): Enterovirus infections are associated with the induction of beta-cell autoimmunity in a prospective birth cohort study. J Med Virol 69: 91-98.
56. Yoon JW, Austin M, Onodera T, et al. (1979): Isolation of a virus from the pancreas of a child with diabetic ketoacidosis. N Engl J Med 300: 1173-1179.
57. Kukreja A, Maclaren NK (1999): Autoimmunity and diabetes. J Clin Endocrinol Metab 84: 4371-4378.
58. Horwitz MS, Bradley LM, Harbertson J, et al. (1998): Diabetes induced by Coxsackie virus: inition by bystander damage and not molecular mimicry Nat Med 4: 781-785.
59. Heino L, Lonnrot M, Knip P, et al. (2001): No evidence of abnormal regulation of antibody response to coxsackievirus B4 antigen in prediabetic children. Clin Exp Immunol 126: 432-436.
60. Sandor F, Buc M (2005): Toll-like receptors I. Structure, function and their ligands. Folia Biol (Praha) 51: 148-157.
61. Wen L, Peng J, Li Z, et al. (2004): The effect of innate immunity on autoimmune diabetes and the expression of Toll-like receptors on pancreatic islets. J Immunol 172: 3173-3180.
62. Zipris D, Lien E, Xie JX, et al. (2005): TLR activation synergizes with Kilham rat virus infection to induce diabetes in BBDR rats. J Immunol 174: 131-142.
63. Martin JM, Trink B, Daneman D, et al. (1991): Milk proteins in the etiology of insulin-dependent diabetes mellitus (IDDM). Ann Med 23: 447-452.
64. Wills-Karp M, Santeliz J, Karp CL (2001): The germless theory of allergic disease: revisiting the hygiene hypothesis. Nat Rev Immunol 1: 69-75.
65. Haskins K, Bradley B, Powers K, et al. (2003): Oxidative stress in type 1 diabetes. Ann NY Acad Sci 1005: 43-54.
66. Wu Q, Salomon B, Chen M, et al. (2001): Reversal of spontaneous autoimmune insulitis in nonobese diabetic mice by soluble lymphotoxin receptor. J Exp Med 193: 1327-1332.
67. Gommerman JL, Browning JL (2003): Lymphotoxin/light, lymphoid microenvironments and autoimmune disease. Nat Rev Immunol 3: 642-655.
68. Hoglund P, Mintern J, Waltzinger C, et al. (1999): Initiation of autoimmune diabetes by developmentally regulated presentation of islet cell antigens in the pancreatic lymph nodes. J Exp Med 189: 331-339.
69. Harrison LC, Schmidli RS, Petrovsky N, et al. Insulin-dependent diabetes mellitus In: Clinical Immunology. Ed. J Bradley, J McCluskey. Oxford Univ Press. Oxford, New York, Melbourne. 1997, 147-159.
70. Godfrey DI, Hammond KJ, Poulton LD, et al. (2000): NKT cells: facts, functions and fallacies. Immunol Today 21: 573-583.
71. Godfrey DI, Kinder SJ, Silvera P, et al. (1997): Flow cytometric study of T cell development in NOD mice reveals a deficiency in alphabetaTCR+CDR-CD8-thymocytes. J Autoimmun 10: 279-285.
72. Baxter AG, Kinder SJ, Hammond KJ, et al. (1997): Association between alphabetaTCR+CD4-CD8- T-cell deficiency and IDDM in NOD/Lt mice. Diabetes 46: 572-582.
73. Wilson SB, Kent SC, Patton KT, et al. (1998): Extreme Th1 bias of invariant Valpha24JalphaQ T cells in type 1 diabetes. Nature 391: 177-181.
74. Muir A, Maclaren N: Insulin-dependent diabetes mellitus, human. In: Encyclopedia of Immunology. Ed. PJ Delves, IM Roitt. Academic Press. San Diego, London, Boston, New York, Sydney, Tokyo, Toronto. 1998, 1399-1404.
75. Notkins AL (2004): Type 1 diabetes as a model for autoantibodies as predictors of autoimmune diseases. Autoimmun Rev 3 Suppl 1: S7-S9.
76. Mahon JL, Dupre J, Stiller CR (1993): Lessons learned from use of cyclosporine for insulin-dependent diabetes mellitus. The case for immunotherapy for insulin-dependent diabetics having residual insulin secretion. Ann NY Acad Sci 696: 351-363.
77. Levisetti MG, Suri A, Frederick K, et al. (2004): Absence of lymph nodes in NOD mice treated with lymphotoxin-beta receptor immunoglobulin protects from diabetes. Diabetes 53: 3115-3119.
78. Van Kaer L (2005): alphaGalactosylceramide therapy for autoimmune diseases: prospects and obstacles. Nat Rev Immunol 5: 31-42.
79. Bresson D, Togher L, Rodrigo E, et al. (2006): Anti-CD3 and nasal proinsulin combination therapy enhances remission from recent-onset autoimmune diabetes by inducing Tregs. J Clin Invest 116: 1371-1381.