***TEACHING UNIT 10***



**IMMUNE BASIS OF THYROID GLAND DISEASE**

Functional disorders of the endocrine glands are caused by their increased activities which results in excessive hormone production, or as a result atrophy endocrine glands, due to which the production of relevant hormones is absent. There are many causes of glandular dysfunction, while an autoimmune reaction to endocrine tissue is one of the most common.

Most autoimmune endocrine disorders are clinically silent until features of organ failure develop. At this stage, the gland is often irreversibly damaged with little chance of recovery even if the autoimmune process is stopped.

**1.1.** **Mechanisms of autoimmunity in endocrine diseases**

Autoimmune reactions can be directed against endocrine cells, their receptors, hormones or receptors on target cells.

Autoantibodies to endocrine cells are organ-specific and are detected only by tests based on the use of antigens of a specific endocrine gland. On the contrary, in systemic diseases (eg systemic lupus erythematosus) antigens are not organ-specific (eg nuclear antigens) but are present in all organs and tissues.

There are several mechanisms of autoimmune damage, and usually, multiple mechanisms may be involved in the development of the disease. Research results indicate that T lymphocytes and antibodies often participate together in the pathogenesis of autoimmune endocrine diseases. T lymphocytes (CD4+ and CD8+ T lymphocytes) are responsible for the destruction of glandular tissue, while antibodies act to interfere with the physiological function of the gland.

In autoimmune syndromes in newborns, caused by transplacental transfer of IgG, the disruption in endocrine function is transient and disappears after 3 weeks (corresponding to the half-life time of antibodies) without significant damage in the target organ.

Antibodies can affect the function or growth of an endocrine gland by reacting with hormone receptors. These antibodies can be stimulating or neutralizing. Patients may have a mixture of antibodies directed at the receptors, with some of these antibodies stimulating and some blocking the receptors. The shift (change) of one type of antibody to another can explain why in some people there are fluctuations in the function of the gland from hyperactivity to hypoactivity.

**1.2. Diseases of the thyroid gland**

Several thyroid antigens have been identified such as thyroglobulin, thyroid peroxidase (thyroid microsomal antigens), Na+ and I- symporter (responsible for iodine uptake in thyroid cells), surface and other cytoplasmic thyroid antigens. Of these antigens, thyroid peroxidase is available and used clinically in antibody detection assays (Table 1). This enzyme catalyzes the iodination of tyrosyl groups in thyroglobulin resulting in the synthesis of thyroid hormones, T3 and T4. The presence and especially a high titer of antibodies to thyroid peroxidase are a reliable sign of an autoimmune process in the gland. They are not directly proportional to the activity of the process, but they are certainly a sign of an autoimmune reaction.

**Table 1. Antibodies directed at thyroid peroxidase which are usually detected in various thyroid diseases**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |
|  | **Clinical manifestations** |  | **Thyroid-directed antibodies** |  |
|  |  |  |  | **peroxidase** |  |
|  |  |  |  |  |  |
|  | |  |  |  |  |
|  | ***Thyrotoxicosis*** |  |  |  |  |
|  |  |  |  |  |  |
|  | **Graves' disease** |  |  | **Positive (low titer)** |  |
|  |  |  |  |  |  |
|  | **"Hot" nodules** |  |  | **Negative** |  |
|  |  |  |  |  |  |
|  | ***Goiter*** |  |  |  |  |
|  |  |  |  |  |  |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | |  |  |  |
| **Hashimoto thyroiditis** | | **Positive (high titer)** |  |
|  |  |  |  |  |
| **Simple goitre** |  | **Negative** |  |
|  | |  |  |  |
| **De Quervain's thyroiditis** | |  | **Transiently positive** |  |
|  |  |  |  |  |
| **Cancer** |  |  | **Negative** |  |
|  |  |  |  |  |
| **1.2.1.** | **Thyrotoxicosis** |  |  |  |

Thyrotoxicosis is common and represents a hypermetabolic state that is related to excessive concentration of thyroid hormone in the blood, usually caused as a result of hyperthyroidism. It occurs in people of any age, but with the highest peak of incidence in the third or fourth decade of life. Compared to men, it occurs ten times more often in women. Thyrotoxicosis most often occurs due to Graves' disease, toxic adenoma and toxic multinodous goiter.

There is strong evidence that a circulating factor is responsible for Graves' disease. Thyroid stimulating factor (TSH, eng. Thyroid-Stimulating Hormone) and serum of patients with Graves' disease both stimulate thyroid secretion, with the serum having a longer-lasting effect. This is due to the presence of IgG antibodies directed at the TSH receptor on the surface of human thyroid cells. Most people with Graves' disease have antibodies directed at the TSH receptor that stimulates thyroid cells (so-called thyroid-stimulating antibodies ).

In autoimmune thyroiditis, the thyroid gland is characteristically infiltrated with T lymphocytes (both CD4+ and CD8+ T lymphocytes are present). These lymphocytes express a limited number of T cell receptor genes compared to peripheral blood T lymphocytes of the same individual. This finding indicates that intrathyroidal T lymphocytes express less diverse receptors, because T lymphocytes that are specific for peptides originating from the thyroid are more represented in this place. In Graves' disease, Th lymphocytes become sensitized to antigens in the thyroid gland and stimulate B lymphocytes to secrete antibodies. These antibodies target the TSH receptor on the surface of thyroid cells resulting in increased growth and function of the thyroid gland

Two out of 1,000 pregnant women have thyrotoxicosis, and occasionally it occurs in newborns of affected mothers - neonatal Graves' disease. This is a consequence of the transplacental passage of thyroid-stimulating IgG from mother to fetus. Graves' disease in infants can be severe. Affected children develop goiter (goiter), exophthalmos, feeding problems, pyrexia, and tachycardia, and heart failure may also occur if these children are not treated. Spontaneous recovery can occur after 2-3 months, which corresponds to the time of the mother's IgG metabolism (the half-life of IgG is three weeks).

Anti-thyroid antibody levels in Graves' disease and Hashimoto's thyroiditis patients tend to decrease during pregnancy and then rise again later.

In many pregnant women without visible signs of thyroid disease, fluctuations in the new anti-thyroid autoantibodies develop, which results in a transient disorder of thyroid function, ie.postpartum thyroiditis. The prevalence of this disease is about 5-10%. Thyroid dysfunction in the first year after pregnancy should be carefully treated. Some women with postpartum thyroiditis may later develop a noticeable autoimmune disease.

Half of the people with Graves' disease develop exophthalmos (bulging of the eyes), which may precede, appear at the same time or may follow the hyperthyroid phase. Exophthalmos can even occur occasionally in euthyroid individuals, or it can be associated with Hashimoto's thyroiditis. Smoking is an important risk factor. Exophthalmos is the result of two pathological processes: myositis and proliferation of retroorbital tissue. Myositis is accompanied by infiltration of lymphocytes. The serum of affected persons contains antibodies that bind to eye muscle extracts, and some of these antibodies cross-react with other antigens of the orbit, as well as with antigens of the thyroid gland. It is assumed that the TSH receptor expressed on fibroblasts and in the muscle tissue of the orbit contributes to the sensitization of lymphocytes. Lymphocytes sensitized to the TSH receptor in this way secrete cytokines, which causes an inflammatory process in the orbital tissue. In people with severe exophthalmos and compression of the optic nerve ("malignant exophthalmos"), high doses of corticosteroids are useful, sometimes given together with immunosuppressive drugs. If there is deterioration, surgical decompression is indicated. Orbital radiation is also used, although the benefit of this therapeutic procedure is controversial.

A few Graves' disease patients (3-5%) develop pretibial myxedema; these people tend to develop exophthalmos. Pretibial myxedema is clearly limited, subcutaneous thickening on the antero-lateral side of the leg. The swelling is hard when pressed, and the skin in these places is shiny and reddish brown. The development of pretibial myxedema was not associated with the duration or degree of hyperthyroidism. The pathogenesis of this myxedema is unknown, although, as in eye disease caused by thyroid dysfunction, aberrant expression of the TSH receptor is also present in this affected tissue.

Genetic factors are important in the etiology of Graves' disease. A history of hyperthyroidism in the family is registered in about 50% of affected persons. HLA-DR3 and CTLA-4 gene polymorphisms are closely related to Graves’ disease in Caucasians and together contribute to about 50% of genetic susceptibility in this ethnic group. The environmental triggers for Graves’ disease are unclear. There are some limited findings of viral infection caused by retroviruses in the thyroid tissue of affected individuals. There is a link between the onset of Graves’ disease and psychological stress. Treatment of patients with multiple sclerosis, which involves the use of a monoclonal antibody (antiCD52Abs) to remove lymphocytes, can induce Graves’ disease in about 10% of cases, which is probably a consequence of the depletion of regulatory T lymphocytes.

Graves’ disease can be successfully treated with antithyroid drugs, radioactive iodine, or surgery. It has been shown that immunosuppressive therapy is not necessary to reduce the level of causative antibodies.

**1.2.2. Hashimoto thyroiditis**

Hashimoto thyroiditis is a chronic inflammation of the thyroid gland and is a common cause of hypothyroidism and goiter. 5% of sufferers are hyperthyroid and develop a disease reminiscent of Graves' disease (also known as "Hashitoxicosis"). About 50% of patients eventually become hypothyroid, which is a consequence of the destruction of the thyroid gland. It is the main cause of goiter in children and young people and also causes idiopathic myxedema, which is the last stage of Hashimoto thyroiditis. Hashimoto thyroiditis is a familial disease and is associated with other organ-specific autoimmune diseases.

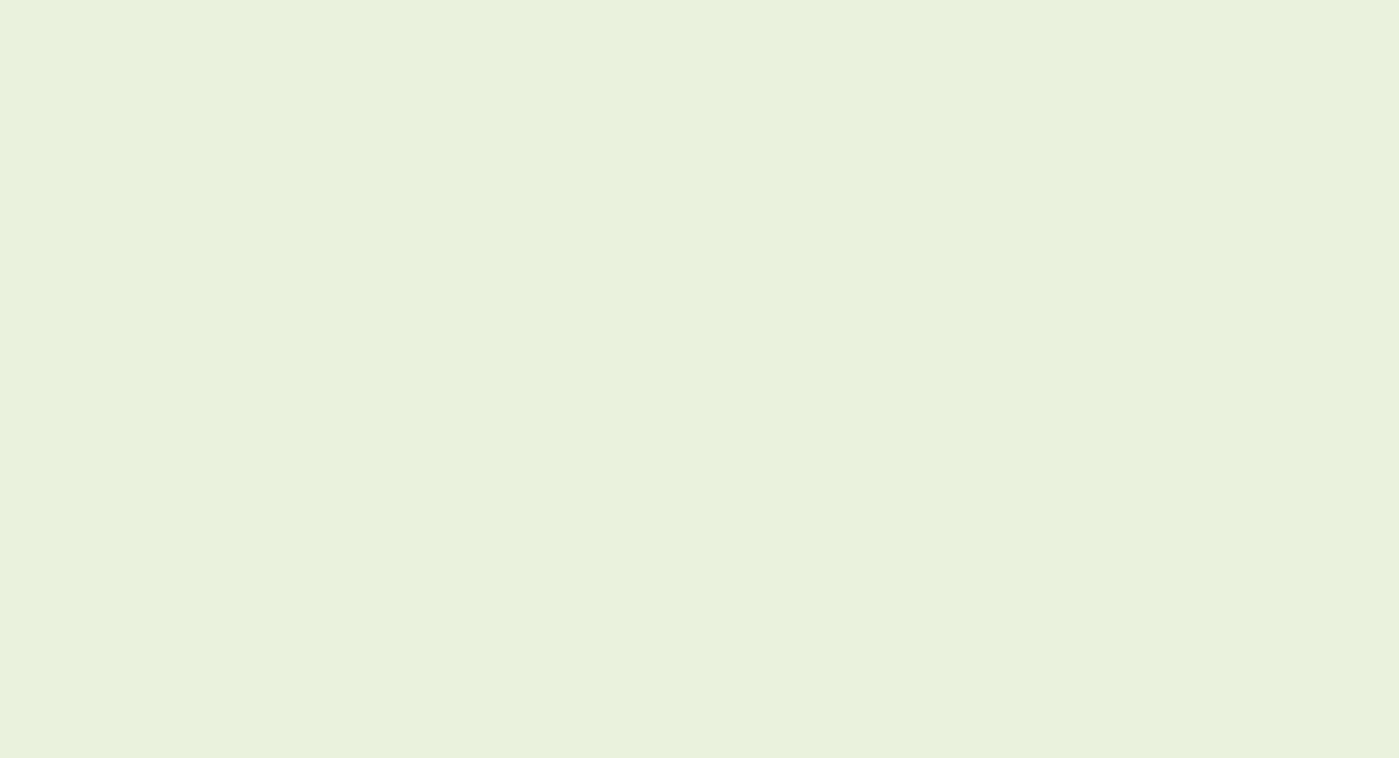
Pathogenesis Hashimoto's thyroiditis (table 2) involves T lymphocytes that are specifically sensitized to thyroid antigens, as well as the unclear participation of autoantibodies that also react to thyroid antigens. Goiter is the result of marked infiltration of the thyroid gland by T lymphocytes that disturb the normal architecture of the thyroid gland. The cellular infiltrate includes CD4+ and CD8+ T lymphocytes, and some B lymphocytes that form lymphoid follicles and germinal centers in this place. These cells express activation markers, while cytokines can be detected in the inflamed tissue. The destruction of thyroid cells is probably a consequence of apoptosis mediated by the Fas molecule and induced by cytotoxic T lymphocytes. This destruction of the thyroid gland results in a decreased concentration of T3 and FT4 in the serum, and an increase in TSH.

In Hashimoto's thyroiditis, antibodies directed at thyroglobulin and thyroid peroxidase are registered, as well as an antibody that blocks the TSH receptor. The main complication of Hashimoto's thyroiditis is progressive hypothyroidism.

**Table 2**



**Evidence of autoimmune reactions in the pathogenesis of Hashimoto's thyroiditis**



* **T lymphocytes specific for thyroid antigens are present in the circulation. Clones of T lymphocytes derived from these cells can kill thyroid cells in culture**
* **Serum autoantibodies have been shown to stimulate or block proliferation of thyroid cells**
* **Infiltration of the thyroid gland by T lymphocytes (CD4+ and CD8+ T lymphocytes) and**

**plasma cells**

* **By injecting thyroid antigen, experimental cell- mediated autoimmune thyroiditis**
* **Association with other autoimmune diseases both in the individual and in family**

Differential diagnosis of Hashimoto's thyroiditis includes simple goiter and subacute (De Quervain's) thyroiditis. De Quervain's thyroiditis is granulomatous inflammation of the thyroid gland caused by viruses (*Mumps, Coxsackieviruses, Influenza, Adenoviruses* and *Echoviruses*). Initially, the destruction of the follicle occurs with the release of thyroglobulin, T3 and T4, which results in hyperthyroidism. Then follows the recovery phase with the development of transient hypothyroidism. Patients present with pain on the front of the neck that radiates to the lower jaw, elevated body temperature, systemic signs of infection, initially signs of hyperthyroidism and then hypothyroidism. About 70% of patients with subacute thyroiditis have HLA antigen B35, which indicates that the tendency to the disease is partially regulated by MHC genes.

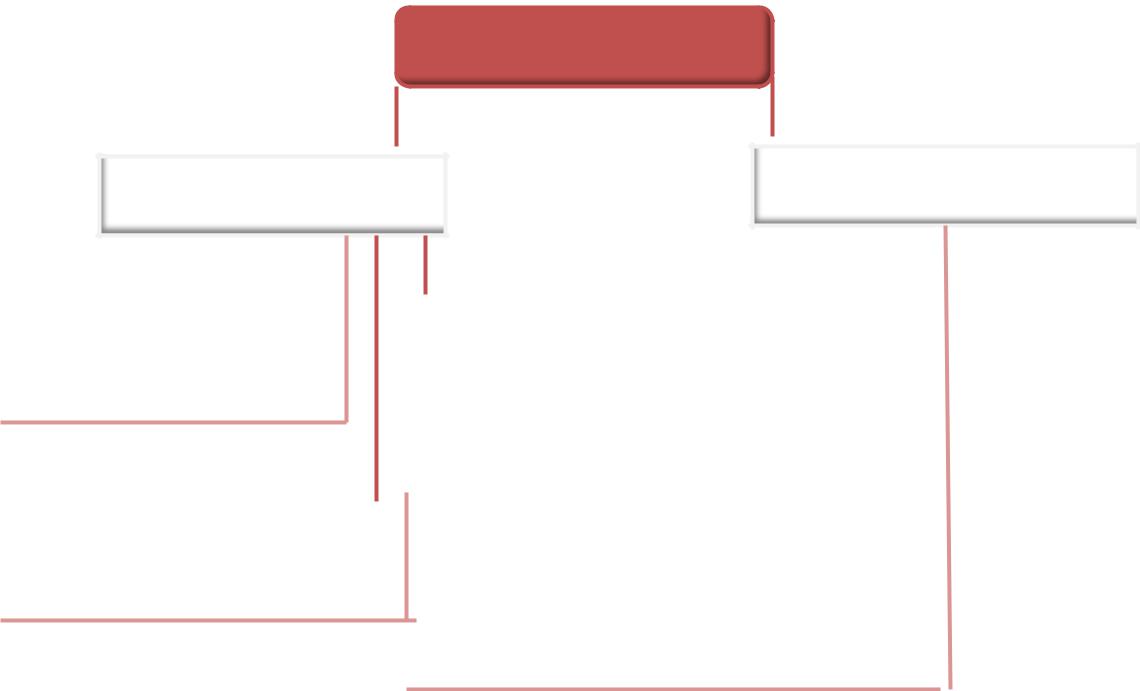
**1.2.3.** **Idiopathic thyroid atrophy (myxedema)**

Myxedema (formerly synonymous with hypothyroidism) today refers to skin and subcutaneous tissue changes seen in patients with severe hypothyroidism. The deposition of a mucinous substance (mucopolysaccharide) affects the thickening of the skin and subcutaneous tissue. The causes of this disease are different (Figure 3). Idiopathic thyroid atrophy, similar to Hashimoto's thyroiditis, is more common in women. Infiltration, fibrosis and atrophy are observed in the tissue of the thyroid gland. Antithyroid antibodies are present in approximately the same titer as in patients with Hashimoto's thyroiditis.

The pathogenesis of idiopathic thyroid atrophy involves antibodies that block both the growth and metabolism of thyroid cells. Primary antibodies that react with the TSH receptor or with another antigen on the membrane are thought to be generated for an unknown reason. Maternal growth-blocking antibodies may play a role in the absence of intrauterine thyroid development, resulting in thyroid cretinism.

As with Graves' disease, genetic predisposition to autoimmune hypothyroidism and Hashimoto's thyroiditis is associated with gene polymorphisms in the MHC locus as well as the CTLA-4 gene.

Triggers from the external environment are unknown. Smoking, infections, and exposure to high and low iodine levels are all associated with hypothyroidism. Treatment with certain drugs (eg, lithium and IFN-α) can induce autoimmune hypothyroidism. Exposure to radiation (eg, the Chernobyl disaster) is associated with an increased incidence of autoimmune processes in the thyroid.



**Thyroid diseases**

**Inhibition of function**

**Iodine deficiency**

**Antithyroid drugs**

**Dyshormonogenesis**

**Growth inhibition Autoimmune thyroiditis**

**Hypothyroidism**

**Diseases of the pituitary gland**

**and hypothalamus**

**Destruction of glands**

**Idiopathic thyroid atrophy**

**Autoimmune thyroiditis**

**(Hashimoto's thyroiditis)**

**Postviral typhoiditis**

**(De Quervain's thyroiditis)**

**Surgical removal**

**Radiation**

**Transient postpartum**

**hypothyroidism**

**Figure 3. . Causes of hypothyroidism**

**THE IMMUNE BASIS OF DIABETES MELLITUS TYPE 1**

* 1. **Introduction**

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia, i.e. permanent increase in blood glucose level. Hyperglycemia causes classic symptoms and signs of diabetes, namely polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). Diabetes occurs due to reduced secretion or reduced biological effect of insulin.

There are two basic types of diabetes. Insulin-Dependent Diabetes Mellitus (IDDM, English Insulin-Dependent Diabetes Mellitus, juvenile diabetes or type 1 diabetes) which occurs as a result of reduced insulin production in the pancreas and Insulin-Independent Diabetes Mellitus (NIDDM, English Non-Insulin-Dependent Diabetes Mellitus) , or type 2 diabetes). which occurs due to the reduced biological effect of insulin as a result of the development of insulin resistance. The characteristics of these two types of diabetes are shown in table 1.

***Table 1.***

|  |  |  |
| --- | --- | --- |
| **Characteristics** | **Type 1** | **Type 2** |
|  |  |  |
| **Prevalence** | **1:3000** | **3:100** |
| **Age** | **Usually <30 years.** | **Usually >40 years.** |
| **The course of the disease** | **Acute** | **Insidious** |
| **Association with autoimmune diseases** | **Yes** | **Not** |
| **Antibodies directed at islet cells Other** | **Yes** | **Not** |
| **autoantibodies** | **Sometimes** | **Not** |
| **Percentage of diabetes mellitus cases** | **10-20%** | **80-90%** |
| **Association with MHC genes** | **DR3 and DR4** | **Not** |
|  |  |  |

Within type 1 diabetes, two main forms are further distinguished: type 1A, which usually begins in childhood and is characterized by immune-mediated destruction of β-cells in pancreatic islets; type 1B in which severe β-cell destruction occurs in the absence of a detectable immune response directed at the pancreatic tissue. Antibodies directed against pancreatic islet cells can be detected in type 1A diabetes. but not in type 1B.

**1.2. Immunopathogenesis of diabetes mellitus type 1**

Diabetes mellitus type 1 is a chronic disease characterized by the cessation of insulin production as a result of autoimmune destruction of the β-cells of the islets of Langerhans.

Diabetes mellitus type 1 is an organ-specific autoimmune disease and is associated with other organ-specific autoimmune diseases such as thyrotoxicosis. Insulin production is absent due to a specific immune response to the β-cells of the pancreatic ducts of Langerhans. Histopathological sign of diabetes is infiltration of pancreatic islets with mononuclear cells - insulitis. Histological analysis shows an intense islet infiltrate in which activated CD4+ and CD8+ T lymphocytes and macrophages are present, a reduced number of insulin-producing β-cells and a relatively low number of glucagon-producing α-cells. Islet cell infiltration and subsequent β-cell damage may precede clinically manifest diabetes by many years, sometimes decades.

The antigens most often associated with this disease are GAD (Glutamic Acid Decarboxylase) and IA-2 (tyrosine phosphatase). It is believed that cytotoxic T lymphocytes kill β-cells, while autoantibodies play a minimal role in this. Thus, anti-islet cell antibodies (ICA) react to membrane and cytoplasmic antigens of islet cells. These antibodies (IgG2 and/or IgG4) can be detected months and years before the onset of clinical symptoms. Insulin autoantibodies are detected in one third of IDDM patients who are not treated with insulin. Their significance is not clear, but the simultaneous presence of insulin autoantibodies and ICA may indicate the future development of diabetes. During the destruction of islet cells, insulin may be present in a form that the immune system recognizes as foreign. This explains the presence of anti-insulin antibodies in people with type 1 diabetes who develop insulitis (inflammation of islet cells).

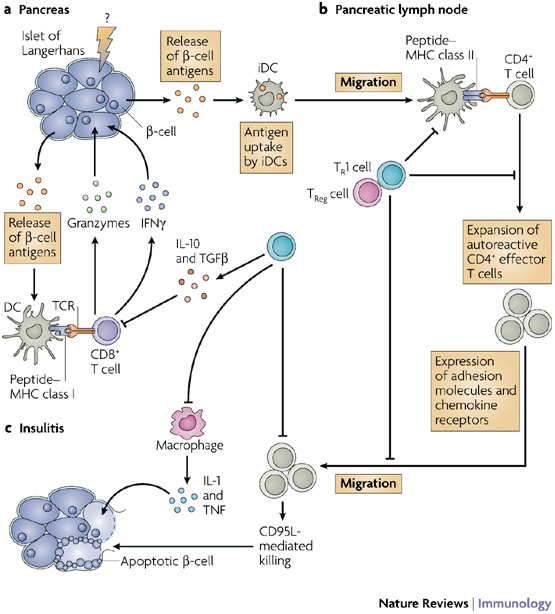
It is customary to divide the pathogenesis of type 1 diabetes into two phases, the first being called the initial phase and the second the effector phase.

***Initial phase*.** The processes in the initial phase of the disease can be briefly described asfollows: under the influence of unknown pathogenic agents, most likely viruses, modified β cell antigens are first released, which are then taken over by tissue APCs, processed and presented in the context of MHC class I molecules. Antigens displayed in this way are recognized by CD8+T lymphocytes, which activate and damage β cells that recognize the same combination of antigens and MHC class I molecules. CD8+ T lymphocytes damage β cells both by the secretion of cytotoxic cytokines (IFN-γ, TNF-α) and by direct contact (Fas/FasL interaction and perforin-granzyme mechanism). Cell antigens are released from damaged β cells, probably in an immature and/or altered form: insulin, GAD 65/67 (GAD from Eng. - glutamic acid decarboxylase), IA-2 (ICA512) (IA-2 from Eng. - insulinoma -associated- protein 2; ICA from Eng. – islet cell antigen). These antigens are taken up by immature dendritic cells in the pancreas and transported to the parapancreatic lymph nodes, where they are processed and presented to CD4+ T lymphocytes. Antigen presentation causes a clonal expansion of autoreactive effector CD4+ T lymphocytes, which express different adhesive molecules (ICAM1, LFA1, and chemokine receptors: CCR4, CCR5, CXCR3) on their surface, which now allows them, according to the antigenic gradient and chemokines produced at the site of initial inflammation under the influence of CD8+ T lymphocytes, migration the pancreatic tissue.

***Effector phase*.** Effector CD4+ T lymphocytes activate and trigger cells of innate andacquired immunity, which enables the development of insulitis. The first cells that infiltrate the islets in the early stage of insulitis are macrophages and dendritic cells, followed by the infiltration of T, B lymphocytes and NK cells. Macrophages are by far the most numerous infiltrate cells in the early stage of insulitis. Infiltrating cells secrete pro-inflammatory cytokines. The synergistic and cumulative effects of the mentioned cytokines show a strong cytocidal effect on β cells. IL1-β, TNF-α and IFN-γ cause selective destruction of β cells by inducing proapoptotic signals while simultaneously stimulating the expression of CD95 (Fas) molecules on these cells. Selective damage to β cells by cytokines conducts through the intracellular accumulation of reactive oxygen species intermediate products (ROS from Eng. – reactive oxygen species) that damage DNA and proteins. β cells are particularly sensitive to oxidative stress due to their relatively weak potential for the expression of antioxidant enzymes, which predisposes them to the selective action of these cytokines, i.e. ROS. These free oxygen radicals trigger the NFκB and MAPK (MAPK from Eng. - mitogen activated protein kinase) pathways of β cell apoptosis activation. In addition, it has been shown that IL1-β can directly and potently activate MAPK and this activation is dose-dependent. Therefore, IL1-β is thought to be the most important proinflammatory cytokine that damages β cells. In addition to the secretory form of these cytokines and the membrane form of IL1 and TNF-α expressed on macrophages and CD4+ T lymphocytes, they contribute to cytotoxicity. On the other hand, increased expression of the CD95 (Fas) molecule on β cells enables effector T lymphocytes to directly kill these cells by binding their CD95L (FasL) to CD95 (Fas) expressed on β cells. Although both Fas/FasL interaction and the perforin-granzyme pathway are involved in β-cell killing, the first mechanism seems to be more important.

In summary, the T cell immune response occupies a central role in the pathogenesis of diabetes and is responsible for the destruction of pancreatic islet cells. The dominant cells in this infiltrate are T lymphocytes, especially activated CD8+ T lymphocytes, which are involved in the active secretion of cytokines. Still undefined pathogenic conditions modify islet cell antigens. These antigens are taken up by APCs and presented to CD8+ T lymphocytes that damage those cells that express the peptide as part of MHC class I molecules. Released β cell antigens are taken up by immature dendritic cells (iDCs) in pancreatic ducts and transported to regional lymph nodes where they present antigens to CD4+ T lymphocytes. In doing so, autoreactive CD4+ T lymphocytes activate and migrate to the pancreatic islets. By producing cytokines, these cells stimulate migration and activate inflammatory cells, causing insulitis (Figure 1).

***Figure 1. Immunopathogenesis of diabetes mellitus type 1***



*Genetic factors* increase the risk for development of IDDM type 1A. About 95% of diabeticsin Northern Europe have HLA-DR3 and HLA-DR4 and about 40% of Caucasians with IDDM type 1A are DR3/DR4 heterozygotes. These alleles are associated with HLA-DQ variant alleles. The critical factor has been suggested to be the amino acid at position 57 on the HLA-DQβ chain. Genetic variants of DQV encoding the amino acid aspartate at this position appear to protect against the development of IDDM, while those variants encoding other amino acids increase the risk of the disease. The mechanism behind this very specific molecular bond is unknown, but the amino acid at position 57 in the HLA-DQβ chain is located in the active site (groove) of MHC class II molecules and can potentially affect the binding of a critical autopeptide. No genetic link has been identified for IDDM type 1.

*Factors of the external environment* play an important role in initiation of the NIDDM butnot IDDM. In a small number of cases, diabetes may be associated with specific infections, especially those viruses (mumps and coxsackie) known to show a tropism for the pancreas. However, the infection that is unequivocally related to type 1 diabetes is congenital rubella, which is very rare today. Most newly diagnosed cases of diabetes are not linked to any specific infection.

**1.3. Therapy of diabetes mellitus type 1**

Treatment of type 1 diabetes is based on insulin replacement therapy. Attempts to halt β-cell destruction involve intensive immunosuppressive therapy (eg, cyclosporine and monoclonal antibodies) initiated after diagnosis, when probably less than 10% of β-cells are preserved. In these cases, induction of diabetes remission is limited.

**Insulin**

Insulin modifications: Rapid-acting insulin has been modified to disrupt the natural ability of insulin to form a hexamer, causing it to be rapidly absorbed after subcutaneous administration. They are not associated with increased production of anti-insulin antibodies. Resistance to applied insulin is rarely present. Occasionally, hypersensitivity reactions occur at the site of insulin application:

**Immune suppression and corticosteroids**

Cyclosporine A inhibits the synthesis of IL-2. It is continuously prescribed for at least 6-12 months. The best results are achieved if the application is started within the first 6 weeks after the diagnosis of the disease. The drug is nephrotoxic. Azathioprine inhibits the proliferation of T and V lymphocytes and NK cells.

**New therapeutic approaches**

Modified anti-CD3 antibodies (TRX4, otelixizumab, teplizumab) block the proliferation and differentiation of T lymphocytes and stimulate the formation of Tregs. Two dosage cycles at 6-12 months.

Anti-CD20 antibodies (rituximab)-It inhibits the presentation of autoantigens to T lymphocytes. The therapy lasts 4 weeks.

Anti-CTLA-4 antibodies (abatacept)-Monthly infusion over a period of 2 years.

Anti-thymocyte antibodies (Thymoglobulin, Atgam)-It reduces the number of T lymphocytes. TNF-R IgG fusion protein (etanercept), IL-1 receptor antagonist (IL-1ra) (anakinra)