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# Common Mechanisms of Pathogenesis of Tissue-Specific Autoimmune Diseases: The Edited Model to Illustrate Those for IDDM and Multiple Sclerosis

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Additional information is available at the end of the chapter

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## 1. Introduction

Autoimmune diseases result from specific immune response to structures of the self. Such a response, resulting from activation of self-specific lymphocytes, is an inevitable side effect of the work of the immune system. However, mechanisms of central and peripheral tolerance normally prevent damage to tissues of the organism, blocking activation and proliferation of autoreactive lymphoid cells. Thus, it would be more accurate to say that autoimmune diseases result from breakdown of tolerance mechanisms that leads to chronic self-sustained response against the structures of the self. Autoimmune diseases should be discriminated from autoimmune reactions. The latter are associated with immune response against infectious pathogens and stop immediately after the pathogenic agent is eliminated. Autoimmune diseases are also quite frequently associated with cross-reactive immune response to exogenous pathogens. In fact, such a link is implicated into pathogenesis of most of those diseases. However, autoimmune diseases continue to progress even if the pathogen is cleared.

The key feature that distinguishes “normal” autoimmunity from pathological conditions is breakdown of tolerance that takes place in the latter case. This difference is illustrated by comparing characteristics of autoantibodies present in normal organism with characteristics of autoantibodies in patients with autoimmune diseases. Apart from higher titers in the patients with autoimmune diseases, antibodies in those patients also show higher avidity to target antigen and mono\oligoclonal structure, as opposed to polyclonal structure in normal samples. In normal organism, detected autoantibodies typically belong to IgM isotype,

while in pathology they usually belong to IgG isotype. This has an important implication, as production of IgG antibodies can not be mediated by B-lymphocytes alone. It practically always requires involvement of antigen-specific CD4+ T-cells, which illustrates that pathogenesis of autoimmune diseases is a complex multistep process, requiring breakdown of tolerance on several levels.

Normal samples		Samples from patients with autoimmune conditions
Isotype	IgM	IgG
Affinity	Low	High
Clonal structure	Polyclonal	Usually oligoclonal or monoclonal
Titer	Low	High

**Table 1.** Autoantibody characteristics in normal individuals compared to those in individuals having a tissue-specific autoimmune disease.

Etiology of autoimmune diseases remains obscure, along with factors that serve as triggers for the disease. Nowadays it seems obvious that neither genetic predisposition nor environmental factors alone are sufficient for causing the disease. The common concept is that autoimmune diseases arise from complex interaction between genetic factors and environment. The conception of post-infectious autoimmune syndrome (PIFAS) defines infection as an important component of pathogenesis (Foy et al., 1996). According to this conception, autoimmune diseases develop in genetically predisposed organisms as a result of specific infection causing cross-reactive immune response affecting own cells and tissues of the organism. Later this response becomes self-sustained and can continue even in the absence of infectious agent that caused it in the first place. In accordance with this conception, evidence arises that in most cases of autoimmune conditions, the primary event of pathogenesis is infection. Arguments that support role of infection in specific autoimmune diseases can be divided into several categories: clinical (clinical findings, as in the case of acute rheumatic fever following streptococcal infections), epidemiological (obtained in epidemiological studies that fit criteria of evidence-based medicine), laboratory (based on laboratory diagnostics of infection in the past, i.e. by accessing antibody levels in biological fluids or obtaining DNA of the pathogen from tissues affected by immune response).

These criteria are applied to show correlation between presence of an infectious agent in the organism and an autoimmune condition. Once such correlation has been found, the next step is studying mechanisms that mediate induction of disease. In relation to pathogens in question, such mechanisms can be divided into specific (those that include stimulation of antigen-specific clones of lymphocytes) and non-specific (based on causing appropriate inflammatory environment for disease induction) (Goodnow et al., 2007). Deeper insight into the problem shows that several of these mechanisms have to be active to initiate an autoimmune response, and that both specific and non-specific mechanisms typically contribute to pathogenesis equally.

## 2. Mechanisms of tolerance: An overview

A number of mechanisms is at work to prevent negative effects associated with autoimmune reactions. The conception of tolerance has been introduced to explain those mechanisms. Tolerance is lack of reactivity of the immune system to antigens of the body. Tolerance is an active process that can be induced through central and peripheral mechanisms. Central tolerance refers to processes taking place during lymphocyte differentiation in central lymphoid organs, before activation (in the process of antigen-independent differentiation of lymphocytes). A classic example of central tolerance is negative selection of T-lymphocytes that takes place during their maturing in the thymus. Selection of lymphocytes in the thymus includes two stages. On the first stage cells that have low ability to react with own molecules of major histocompatibility complex (MHC) undergo apoptosis. Ability to react with own MHC is necessary for proper T-cell function, since T-cells respond only to antigen fragments presented by antigen-presenting cells (APCs) in complex with MHC molecules (B-cells, on the other hand, respond to full molecules of antigens and don't require interaction with MHC). On the second stage cells that undergo apoptosis are those that strongly react with fragments of own antigens of the body presented by MHC (the so-called negative selection). Therefore, cells that survive do have ability to react with own MHC, but not too strong an ability. Those cells also have low potential to respond to own antigen fragments. Such a complex process of selection is necessary, because T-cells have a central role in specific immune responses, and consequences of a tolerance breakdown at T-cell level would be quite grave. However, it should be noted that possibility of response to own pathogens is an inevitable side effect of the mechanisms T-cells utilize in their receptor mechanisms (requirement of interaction with own MHC molecules). In B-cells the mechanisms of selection don't differ principally from those in T-cells, however they are much less strict.

The described mechanisms aren't sufficient to fully prevent appearance of autoreactive clones in the blood. However, activation of T-cells requires several conditions to be met (apart from interaction of the antigen-specific receptor with its target). Appropriate contact between T-cell and APC is called immune synapse. A key condition for a successful activation is interaction of CD28 molecule of the T-cell with costimulatory molecules B1/B7 of the APC. Without such interaction, activation of TCR causes anergy or apoptosis of the lymphocyte. This interaction is unlikely in the absence of appropriate costimulation, which is induced by presence of non-specific molecular signals of "presence of danger" (DAMPs – Danger associated molecular patterns) or "presence of an infectious agent" (PAMPs – Pathogen associated molecular patterns). The latter include fragments of bacterial cellular wall, exogenous DNA, flagellin, etc. Those factors cause activation of APCs, increasing expression of MHC-antigen complexes and costimulatory molecules. In the event of interaction of an autoreactive T-lymphocyte with its target, the described costimulation is not expected to be active. That means that APCs would not be activated, and won't express necessary amounts of B1/B7. Expression of MHC molecules would also remain low. This mechanism of control ensures that T-cell activation seldom takes place without activation of innate immunity. As a result, autoreactive T-lymphocytes that

interact with self-antigens more often than not undergo apoptosis or become anergic as a result of TCR stimulation without appropriate costimulation.

Activation of B-cells doesn't require antigen presentation in association with MHC. These cells have the ability to recognize antigens directly through BCR. However, as well as in the case of T-cells, stimulation of BCR has to be coupled with costimulatory mechanisms, or the cell would most likely undergo apoptosis. Signals inducing B-cells proliferation include interaction with PAMPs. Those are mediated by toll-like receptors (TLRs) that interact with PAMPs, and complement C3 receptor (CD21), that binds to C3 component of the complement. C3 binds to specific components of the microbial cell wall (alternative complement activation), or to pathogens opsonised with antibodies (classic complement activation). Those mechanisms direct immune response towards reaction to microbial pathogens bearing signs of "danger".

Before the start of B-cell antigen dependent differentiation and antibody production, B-cells have to pass another checkpoint (Goodnow et al., 2007). That is interaction with T-cells specific for the same antigen, through CD40/CD40L system (Foy et al., 1996). Therefore, interaction of two antigen-specific cells, both of which have undergone multiple points of selection, is required before B-cell mediated immune response with highly-affined antibody production and memory cells formation can take place. Functional activity of the immune system is preciously regulated to minimize chances of autoimmune reaction.

Another mechanism controlling the activity of immune response is activity of regulatory T-cells (Treg). Natural Tregs have the phenotype of CD4<sup>+</sup>CD25<sup>low</sup>, and express a specific transcription factor – Foxp3. Foxp3 mediates suppressor function by inducing factors such as TGFbeta, GITR, IL-10. Upon activating, Treg cells specifically inhibit immune response. An important feature of their cells is that their TCRs show significantly higher affinity to own antigens than those in other T-cells. It is thought that cells with affinity to self-antigens higher than in most T-cells but lower than is required for negative selection may differentiate into Treg (Wan et al., 2007). There are also other types of regulatory lymphoid cells with suppressor function, such as induced Treg that form in vivo in conditions of activated CD4<sup>+</sup>-cells stimulation with IL-10, and B-regulatory cells.

An important function of mechanisms of tolerance is degenerative feedback for countering uncontrolled immune cells activation. Continuous stimulation of TCR causes negative regulation of CD 28 receptor needed for T-cell costimulation through B1 and B7 molecules of the APCs. Instead, T-cells start producing CTLA (CD 28), a molecule that is an antagonist for B1 and B7. This cuts costimulation of T-cells and causes induction of anergy.

### 3. Role of infection: The conception of PIFAS

Seeing that autoimmune diseases arise from lack of balance between stimulation of the immune system and activity of suppressor mechanisms, infection, especially chronic, proposes to be an important factor in the induction of autoimmune process. Even by itself,

chronic antigen stimulation accompanied by lymphocyte proliferation and activation of effector mechanisms significantly magnifies the chance of breakdown in tolerance mechanisms. A parallel can be drawn between autoimmune conditions and tumors of the immune system. The latter, rising from uncontrolled proliferation of lymphocytes, have been firmly associated with excessive chronic antigen stimulation (chronic infection with HIV, Hepatitis C, Epstein-Barr virus).

The conception of PIFAS distinguishes the following 5 steps in the pathogenesis of autoimmune diseases (Cherepachina et al., 2009):

1. Genetic predisposition.
2. Infectious process causing cross-reactive activation of the immune system.
3. Latent stage of the autoimmune disease characterized by production of autoantibodies and antigen-specific clones of lymphocytes. It is accompanied by morphological signs of immune inflammation (i.e., latent autoimmune insulinitis preceding manifestation of autoimmune diabetes mellitus) and can be detected by accessing the proteome of the patient. Markers that can be detected in the proteome are divided into markers of immune inflammation and markers of tissue degeneration.
4. Latent stage of the disease accompanied by impairment of functional activity of the affected tissues which can be detected by functional diagnostics or by accessing the metabolome of the patient.
5. Manifest stage of the disease.

## 4. Pathogenetic mechanisms of autoimmune diseases

Now we shall give a more detailed description of mechanisms that have a role in the pathogenesis of autoimmune diseases.

### 4.1. Molecular mimicry

The conception of molecular mimicry is one of the most common concepts in immunology. It is also the simplest hypothesis that can be applied to explain the etiology of autoimmune diseases. The conception is built on laboratory findings that show homology between amino acid sequences of infectious agents and those of proteins of the body. Activation of T-cells with TCR specific for the pathogen causes cross-reactive reaction to own antigens of the organism, and as a result, impairment of functions of target organs. For the pathogen this serves as a mechanism of evading the immune response, since self-reactive clones of lymphocytes are typically less active due to mechanisms of negative selection, and are also prone to suppression by mechanisms of peripheral tolerance. This interferes with elimination of the pathogen (Sherbet, 2009). And long-term persistence of the pathogen contributes even further to the ongoing autoimmune reaction. Such a reaction may or may not come to an end after and if the infectious agent is finally eliminated. In the latter case, the process



becomes self-supporting, recruiting other mechanisms of disease progression. If secondary production of self-reactive T-lymphocyte clones takes place, as opposed to just antibody production, this usually marks the onset of autoimmune disease. An important factor here is the phenomenon of epitope spreading, which is caused by changes in conformation of antigens that are subject to immune response (see later).

#### 4.2. Superantigens

As opposed to specific monoclonal stimulation of immune system caused by antigens, stimulation by superantigens causes polyclonal activation of cells of the immune system. Mechanisms of superantigen-mediated activation primarily affect T-cells activation. Stimulation by antigens causes activation of T-cells as a result of presentation of processed antigen by MHC molecules of the APCs. TCR binds to the peptide presented by MHC. Superantigens react directly with MHC molecules, binding to beta-unit of the molecule (Conti-Fine et al., 1997). They cause non-specific polyclonal activation of T-cells, reacting with all cells that can bind to appropriate MHC-molecule (all cells that are restricted to the haplotype of that MHC molecule). Participation of superantigens in the pathogenesis of autoimmune diseases seems to be indirect. One of the aspects is their contribution to inflammatory response and antigen presentation (mostly due to stimulation of macrophages by activated CD4<sup>+</sup> Th1-cells). Pathogenic agents producing superantigens can also contribute to pathogenesis of autoimmune diseases by increasing the possibility of activation of autoreactive T-cells as a component of polyclonal activation.

#### 4.3. Altered presentation of own antigens

The next component of pathogenesis is abnormal presentation of self-antigens caused by inflammatory response to infectious pathogens. This can refer to increased expression of HLA-molecules (Conti-Fine et al, 1997), aberrant expression of HLA molecules, presentation of antigens that are normally invisible for the immune system. These phenomena are frequently seen as a component of normal immune reactions (hence another important implication of infectious agents in the pathogenesis) or autoimmune inflammatory reactions.

It is a well known fact that stimulation with inflammatory cytokines causes an increase in expression of MHC molecules. This refers both to professional APCs (macrophages, B-lymphocytes, dendritic cells) and most other cell lines (in the latter case, expression of HLA I is increased, making the cells vulnerable to autoreactive CD8<sup>+</sup> cytotoxic lymphocytes). Inflammatory signaling also stimulates aberrant expression of MHC, causing expression of HLA II by endothelial cells, fibroblasts and other cell lines that normally only express HLA I and don't function as antigen-presenting cells. Such aberrant expression of HLA molecules may lead to presentation of antigen determinants of the self that were previously unknown to the immune system.

Presentation of normally invisible antigens may occur as a result of breakdown of specific blood-brain barriers of "immunologically privileged" organs. This mechanism plays an important role in the pathogenesis of autoimmune uveitis, multiple sclerosis, etc.

#### **4.4. Presentation of altered self-antigens. Epitope spreading**

Disclosure of cryptic antigenic epitopes is an important mechanism in the progression of autoimmune diseases (Lehmann et al., 1997). Normal conformation of the body's proteins makes some epitopes invisible to antigen-recognizing receptors of the immune system. Conformation of peptides prevents those epitopes from being recognized by lymphoid cells. However, that also means that lymphocyte clones specific to those antigens do not get eliminated effectively during antigen-independent differentiation. Disclosure of those epitopes which may take place as a result of changes in protein conformation that result from immune inflammation may open up these epitopes for effective immune response. Those new targets for autoimmune reaction may have significantly higher affinity to antigen-recognizing receptors in comparison to the original ones. Clinically such an event is frequently associated with the disease taking rapidly progressive course, for example, with multiple sclerosis reaching secondary progressive type. The described phenomenon is known as epitope spreading – a process that is tightly associated with autoimmune diseases progression.

### **5. Conception of PIFAS as applied to explaining pathogenesis of autoimmune insulin-dependent diabetes mellitus**

#### **5.1. Introduction**

Insulin-dependent diabetes mellitus (IDDM) is a chronic autoimmune disorder that results from autoimmune destruction of insulin-producing pancreatic beta cells. In IDDM, the autoimmune process is steadily progressive, inevitably leading to total destruction of Langerhans islet beta-cells and ceasing of production of insulin. Clinical manifestation of the disease, presented by symptoms of insulin deficiency, takes place quite late into the autoimmune inflammatory process, when about 90% of islet cells have been destroyed (Epstein, 1994). Such a gap between the status of the autoimmune process and clinical symptoms makes immunosuppressive therapy ineffective in most patients: although application of such therapy can low the intensity of immune inflammation or in some cases cause a relapse of the immune inflammation, patient almost always becomes insulin-dependant anyway. This is largely due to lack of functional reserves in the population of beta-cells at the time of the diagnosis. Early detection of the autoimmune process in IDDM and identification of risk factors for the disease would greatly broaden the grounds for pathogenetic therapy, but this problem has yet to be effectively solved.

Pathogenesis of IDDM includes following stages.

1. Genetic predisposition,
2. Primary immune response aimed at the mimicking infectious pathogen.
3. Latent autoimmune insulinitis.
4. Asymptomatic impairment of beta-cell function (impairment of oral glucose tolerance).



## 5. Clinical manifestation of the disease.

Diagnostic markers vary for those stages: in the first stage, only markers of genetic predisposition can be detected (and used to calculate the risk of disease), while in the third stage it is possible to detect markers of autoimmune inflammation. In the manifesting stages of the full-term clinical illness canonical (routine) clinical diagnostic protocols would become possible and fruitful. It is evident that the key stage of pathogenesis in which the autoimmune process becomes irreversible and acquires self-progressive course is the third stage. An important task is diagnosing the disease at this stage, when there is room for pathogenetic therapy aimed at quenching immune inflammation.

### 5.2. Role of genetic factors in pathogenesis of IDDM

Important peculiarity of T1D pathogenesis is genetic predisposition that conditions the development of the disease. MHC (major histocompatibility complex) often elicits autoimmune responses by predetermining the inadequate behavior of immunocompetent cells. MHC represents a large family of genes encoding molecules of three major HLA (human leukocyte antigen) classes.

#### HLA class I

The HLA class I compartment contains both diabetoprotective genotypes and highly associative genes. HLA class I initiate and potentiate autoimmune destruction of beta cells. The diabetogenic alleles of MHC class I genes display age-related features. For example, HLA-E\*0101 is predominant in patients in whom T1D developed during the first 10 years of life, while HLA-E\*0103 is found in children under 10. (Kordonouri et al, 2010)

#### HLA class II

HLA class II constitute a family of genes which encode glycoproteins with an Ig-like structure and are predominantly localized on the APCs (antigen presenting cells) surface. Their functional role covers the presentation of antigen peptides to CD4 (+) T helper cells type I. Several autoimmune diseases (including T1D) are supported by promoting effects of HLA class II Ags. (Murdock et al., 2004).

#### HLA class III

The contribution of HLA class III to background predisposition of T1D is far fewer (compared to HLA classes I and II) but there are several HLA class III genes manifesting a diagnostically significant association with T1D. As an overall trend, HLA classes II and III provoke diabetes at the highest levels of the odds ratio, while the effect of HLA class I on T1D is much less expressed. (Lipponen et al., 2010).

Non-MHC genes also may play a prominent role in the development of autoimmune diseases.

There is quite a vast repertoire of non-MHC genes with a multitude of SNPs (single nucleotide polymorphism) that determine the attacks at some structural components of the pancreas or directly at insulin. Under certain conditions, e.g., under negative impacts of environmental factors these genes are “switched on” giving rise to immune disorders.

An immense variety of genes responsible for susceptibility to T1D are known, but their functional capabilities are either obscure or poorly investigated. Some genes whose role in etiology and pathogenesis of T1D leaves no doubt are described below.

### **TNFAIP3**

Tumor necrosis factor, alpha-induced protein 3 is inhibitor of TNF-induced apoptosis. This gene realizes miscellaneous functions to provide protection of beta cells from programmed cell death, inactivation of NF-kappa B signals, prevention of inflammatory lesions of pancreatic cells, deceleration or delayed recruitment of immunocompetent cells into target organs, and so on. Mutations in this gene represent the most common mechanism of dysregulation and disorganization of immune reactions resulting in autoimmunity. (Petrone et al., 2008)

### **INS**

INS (insulin) gene is a key participant in the synthesis of insulin molecules. Proinsulin molecules are formed during transcription of INS. Mutations in INS are manifested as insulino-pathies, e.g., enhanced production of "odd" insulin with impaired amino acid sequences and atypical conversion of proinsulin into insulin. The latter abnormality is unrelated to T1D; however, any change in the amino acid sequence may lead to immune failure and secretion of autoAbs. (Pociot et al., 2002)

### **ERBB3**

ERBB3 (Erythroblastic Leukemia Viral Oncogene Homolog) modulates the presentation of Ags and increases the risk of T1D. Mutations in ERBB3 lead to immunoregulatory collapses coupled with continuous emergence of autoreactive cells.

### **IL2RA**

It regulates immune and inflammatory responses, exerts negative control over cell proliferation and favors differentiation of T cells. In addition, IL2RA (interleukin-2 receptor-alpha) controls apoptosis via a positive feedback mechanism.

Mutation in IL2RA predetermine the susceptibility to T1D by interfering with the transcription and/or splicing of mRNA. In this way, IL2 and IL2RA exert genetic control over protein expression in different cell subpopulations.

### **IFIH1**

Interferon induced with helicase C domain 1(IFIH1) gene is involved in innate immune defense against viruses. Upon interaction with intracellular dsRNA(double-stranded RNA) produced during viral replication, triggers a transduction cascade involving MAVS/IPS1, which results in the activation of NF-kappa-B, IRF3 and IRF7 and the induction of the expression of antiviral cytokines such as IFN-beta and RANTES (CCL5). IFIH1 is directly involved in the destruction of Langerhans islets due to pooling and mobilization of autoreactive cells in response to viral invasion. This circumstance aggravates immune dissonance and promotes self-restructuring of targeted organs by provoking persistent deficiency of the pancreas and accelerating insulin failure.

CD226 (rs763361) SNPs regulate the activity of certain cells involved in immune mechanisms mediating beta cell destruction.

### 5.3. Pathology of IDDM

Morphologic substrate of the disease is autoimmune insulinitis. The characteristic feature of insulinitis in patients with IDDM is complete lack of beta cells. Other types of cells in the Langerhans islets remain intact. The inflammatory infiltrate consists mostly of CD8<sup>+</sup> cells with some other lymphocytes, macrophages and plasmocytes. Beta-cells of the islets demonstrate increased expression of HLA I, while the APCs and endothelial cells show increased expression of HLA II. This is a characteristic feature of immune inflammation with cell-mediated immune response.

The key factor in beta-cells destruction is thought to be CD8<sup>+</sup>-cells-mediated cytotoxicity. However, CD4<sup>+</sup> cells are also crucial for pathogenesis, since blocking their proliferation in mice hampers the disease progress. This is quite logical, since, CD8<sup>+</sup> cells activation requires participation of CD4<sup>+</sup> cells. Furthermore, it seems that apart from cell-mediated cytotoxicity, another mechanism playing just as important role in the disease progression is inflammatory cytokine production by macrophages and CD4<sup>+</sup> cells. Cytokines that have been implicated in the pathogenesis of IDDM are interleukin-1 (IL-1) and interferon-alpha (Fabris et al., 1998, Waguri et al., 1994). There have been reports of patients developing fulminant IDDM in the course of interferon-alpha therapy (Fabris et al., 1998). These and other cytokines contribute to inflammatory response and promote CD8<sup>+</sup> cells activity. IL-1 causes increased NO production in beta cells which leads to excessive synthesis of free radicals. Beta-cells are quite sensitive to damage caused by free radicals, which makes them especially vulnerable to inflammatory responses. This data shows that even non-specific inflammation can cause beta-cell destruction (and become the foundation for specific autoimmune response to emerge). Apart from creating a condition of increased susceptibility to specific immune reaction against beta-cells, inflammatory status by itself may cause beta-cells destruction, as well as serve as a mechanism of stimulation of the immune system with altered antigens of the destroyed cells.

Autoantibodies present in IDDM include:

- GAD(Glutamic acid decarboxylase) 65 and GAD67 autoantibodies to glutamate decarboxylase (an enzyme that catalyzes the conversion of glutamic acid into  $\gamma$ -aminobutyric acid and CO<sub>2</sub>;
- IA-2(insulinoma antigen 2) autoantibodies to membrane-bound proteins IA-2 and IA-2 $\beta$ ;
- ICA (islet cell antibody) autoantibodies to a heterogeneous cluster of antigens expressed in beta cells;
- ZnT8 autoantibodies to a member of the zinc transporter protein family;
- IAA - autoantibodies to insulin.

Serving as markers of the autoimmune inflammatory process, these antibodies can be present before clinical onset of the disease, and can be used for detecting IDDM at latent stages. The ICA autoantibodies, reacting with cytoplasmic antigens of islet cells, are found in IDDM patients in 0.5 % of normal subjects and in 70-80 % of patients with newly diagnosed IDDM. Those oligoclonal antibodies react with a variety of antigens. One of those is pancreatic enzyme glutamate decarboxylase. Presence of ICA autoantibodies in healthy subjects increases the risk for future development of the disease. The risk is lower for the patients with ICA autoantibodies that react with glutamate decarboxylase than for those who have antibodies specific for other targets. The link is age-dependent, which is attributed to the risk of autoimmune IDDM being higher in younger people. The disease also takes more rapid course in younger patients, with less time passing between clinical onset and complete loss of insulin production.

The anti-insulin antibodies are found in about 50% of patients with newly diagnosed IDDM. Those antibodies may also originate from insulin administration, which is a case in patients receiving non-human insulin, and interferes with efficiency of treatment. However, in IDDM anti-insulin antibodies may represent autoimmune reaction to own tissues, before any insulin administration. They can be used in combination with islet-cell cytoplasmic autoantibodies for prediction of IDDM development. Combined use of both autoantibodies greatly increases diagnostic value of the test.

In vitro, mononuclear cells of patients with IDDM proliferate in response to glutamate decarboxylase. This reaction can be detected earlier than diagnostically significant levels of anti-glutamate decarboxylase Abs. So, in vitro reaction of mononuclear cells with glutamate decarboxylase is considered to be one of the earliest markers of autoimmunity against beta-cells.

#### **5.4. Role of infection in the pathogenesis of IDDM**

One of the conceptions proposed to explain the pathogenesis of IDDM is the conception of molecular mimicry. Pathogens having antigen determinants homologous to those of beta-cells include Coxsackie virus (common epitopes with glutamate decarboxylase – an enzyme characteristic for beta-cells), mumps virus and *Chlamidia pneumoniae*. There are several mechanisms that allow viral infection to contribute to pathogenesis. In case of rubella virus and cytomegalovirus, there is evidence of direct induction of autoimmunity. Infection with those agents is associated with presence of autoantibodies in newly diagnosed patients with IDDM. Also, cytomegalovirus has been shown to induce production of antibodies to 38 kDa protein of the islet cells (Yoon et al., 1990). Those antibodies are frequently detected in patients with IDDM. However, as of now there is little reliable data on amino acid sequences homology between proteins of rubella virus and cytomegalovirus and islet cell antigens.

Another way infectious pathogens can contribute to pathogenesis of IDDM is through direct cytolytic effect on islet cells. An example is Coxsackie B virus, which mediates immune-independent cytolysis of beta-cells (Andreoletti et al., 1998). Coxsackie B virus infection has been shown to be a risk factor for IDDM (Andreoletti et al., 1998), and homology has been shown between viral protein 2C and islet cell antigen GAD 65 (Huang et al., 2011). Cytolytic

effect even further amplifies the contribution of this agent to pathogenesis, as antigen release from beta-cells and non-specific inflammatory response greatly facilitate the conditions for autoimmune reaction.

## **6. Conception of PIFAS as applied to explaining pathogenesis of multiple sclerosis**

### **6.1. Introduction**

Multiple sclerosis (MS) is a tissue-specific autoimmune disease, characterized by immune inflammation in the central nervous system (CNS) and chronic processes of demyelization. In MS, the primary targets of immune response are myelin antigens, with the myelin basic protein (MBP) usually described as the main target. Degradation of myelin impairs conductive function of neurons and causes specific symptoms of the disease. The disease develops in genetically predisposed individuals as a result of cross-reactivity of the immune system to exogenous pathogenetic agents. According to PIFAS conception, the following stages can be described in pathogenesis of MS:

1. Genetic predisposition.
2. Induction of immune response to antigens of myelin in genetically predisposed individuals, mediated by infectious processes in the CNS or by other factors.
3. Latent autoimmune inflammation in the CNS.
4. Latent impairment of neurological functions.
5. Manifestation of the disease in the form of clinically independent syndrome (CIS) or primary progressive multiple sclerosis.

### **6.2. Genetic factors in pathogenesis of multiple sclerosis**

For developing genetic markers of increased susceptibility to MS, three groups of genes are usually studied: HLA genes, cytokine genes (IRF8, TNFa, CD6) and genes taking part in metabolism of myelin (MBP, CTLA1). (Dujmovic, 2011). Among genetic risk factors for MS, human leukocyte antigen (HLA)-class II alleles, specifically the HLA DR and DQ loci, are the best studied. It is currently thought that susceptibility to MS is defined not by individual alleles, but rather by their interaction (conception of epistasis – interaction between certain alleles of HLA). Lincoln and colleagues reported epistasis among 3 HLA-class II alleles (DRB1, DQA1, and DQB1) in 2 independent Canadian cohorts. For example, HLADQ1\*0102 increased MS risk when combined with HLADRB1\*1501, thereby implicating the HLA-DQ molecule in susceptibility to MS. Some alleles of HLA seem to reduce the risk of MS, an example being HLA-DRB1\*01 (Fernandez-Morera et al., 2008, Isobe et al., 2010). In De Luca's study, HLA-DRB1\*01 has been shown to be notably underrepresented in patients with malignant cases of MS in comparison to those with benign cases (DeLuca et al., 2007), implicating its role in determining the severity of the disease.



Prognostically, some HLA alleles correlate with higher incidence of neutralizing Abs to IFN  $\gamma$  (HLA-DRB1\*0408) (Caminero et al., 2011).

TNFRSF1A (TNF $\alpha$ ) gene mutations also play a role in genetic predisposition to MS. A correlation has been found between MS and another autoimmune disease - TNF receptor-associated periodic syndrome. The latter is firmly associated with TNFRSF1A mutations (IMSGC, 2011). Also, a link has been shown between MS and variants of several other cytokines, most notably IL8 and IL6 (Yoon et al., 1989).

### **6.3. Clinical classification of multiple sclerosis. Natural history of multiple sclerosis**

The first manifestation of MS is termed as clinical isolated syndrome – CIS. Diagnosis of MS can be maintained after two distinct exacerbations of the disease, diagnosed clinically or instrumentally – by MRI. Extensive data shows that about 80% of patients with CIS that have changes in their MRI develop MS in 20 years. However, disease modifying treatment can in certain cases prolong latent stages of the disease. Research of biomarkers determining risk of progression to MS in the groups of risk is, therefore, of much importance. One of such markers is Protein 14-3-3, which is thought to act as a chaperone in neurons and oligodendrocytes. Protein 14-3-3 is a sensitive marker of damage to neural tissue, and it has been shown to be an independent factor in predicting conversion of CIS to MS.

An important moment in pathogenesis of MS is loss of protective function of the blood-brain barrier (BBB). Normally, low permeability of BBB makes the CNS immunologically privileged, preventing immune response to its antigens. However, in MS this protective function of the BBB is lost as a result of primary inflammatory response to infection or other factors leading to damage to neurons and glial cells. Immunoregulatory defects including reduced levels of regulatory T cells (Tr1, Th2, Th3) in MS patients allow myelin-reactive Th1-cells to extravasate, cross the blood brain barrier (BBB) and enter the CNS. This barrier is not normally accessible to T-cells, unless it is affected by a virus, which reduces the strength of the junctions forming the barrier. Within the CNS, myelin-reactive Th1-cells interact with microglia (localized APCs) presented antigens and secrete inflammatory cytokines (IL-2, IFN  $\gamma$ , TNF $\alpha$ ) which initiate inflammatory cascades. Mononuclear phagocytes, T-cells and microglia containing the RANTES (CCL5) (regulated on activation, normal T-cell expressed and secreted) receptor CCR5 and T-cells containing the interferon- $\gamma$ -inducible protein of 10 kDa (IP-10) (CXCL10); monokine induced by interferon- $\gamma$  (Mig) (CXCL9) receptor CXCR3 are targeted to the inflammation, demyelination sites by the RANTES and IP-10/Mig chemokines, respectively.

An important mechanism mediating lymphoid cells migration in inflammatory conditions increased expression of integrins, such as VLA-4. Interacting with ICAM receptor of endothelial cells, VLA-4 plays an important role in the process of lymphocytes passing the BBB. That makes VLA-4 one of potential targets for MS therapy. Immunomodulatory therapy with interferon- $\beta$  also affects BBB permeability, reducing migration of CD8<sup>+</sup> lymphocytes.

Increased permeability of BBB allows antigen-specific lymphocytes and antibodies to reach the CNS. However, clinically the disease may remain silent for some time: as it is common with autoimmune diseases, MS is characterized with extended latent stage (third stage according to the conception of PIFAS). The silent autoimmune process can be identified by presence of specific markers of the disease.

Clinically MS is categorized as either primary progressive MS (PPMS) (15% of cases at onset) or relapsing-remitting MS (RRMS). The latter can evolve into secondary progressive MS (SPMS), which takes place in about 65% of patients who presented with RRMS. Morphologically progressive forms of the disease are characterized by diffuse inflammatory changes in the CNS, while in RRMS inflammatory changes are local (Leech et al., 2007). Even healthy-looking white matter of patients with progressive forms of MS shows increased permeability of the BBB, and this may be an evidence of diffuse inflammatory process in those forms of the disease (DeStefano et al., 2001). Research of factors affecting clinical course of the disease is of much importance. Disease evolution to secondary progressive variant may be mediated by changes of the immune response, as well as by changes in target tissues. One of the mechanisms involved in disease progression is transportation of the antigens to cervical lymph nodes, with activation of new antigen-specific T-cells and expansion of antigen repertoire. This doesn't explain, however, why evolution to secondary-progressive variant is seen only in certain subjects. The key role in this process is given to a previously discussed factor - epitope spreading, taking place as a result of changes in conformation of myelin leading to opening of previously inaccessible epitopes. Those epitopes may show higher affinity to antigen-binding sequences of autoantibodies and autoreactive TCRs. Epitope spreading is accompanied by changes in biomarker profiles, with occurrence of new antibody types. This may be seen before clinical signs of secondary progressive MS. Another factor that might play a role in MS transformation to secondary progressive type is degree of excitability of neurons (Kutzelnigg et al., 2005). There is data that the CSF of patients with RRMS inhibits activity of Na<sup>+</sup>-canals of neurons, which allows to presume it might show an *in vivo* effect of reducing excitability of neurons. This might reduce the degree of neuronal damage and serve as a "protective" factor preventing the development of SPMS.

An independent hypothesis of MS progression explains the development of SPMS as a naturally determined result of continuous disease progression (DeStefano et al., 2001). After damage to CNS reaches a certain threshold, a break of compensation occurs after which further disease progression results in equivalent progression of functional disability. Before this point, the clinical symptoms don't fully reflect the severity of autoimmune process. This hypothesis explains low efficiency of disease-modifying treatment at the stage of SPMS. In the light of such point of view, RRMS should be viewed as a state of partial compensation preceding manifestation of the disease in the form of SPMS.

#### **6.4. Pathology of multiple sclerosis. Role of infection in pathogenesis of multiple sclerosis**

The characteristic morphological feature of relapsing-remitting MS are MS lesions disseminated in location and age. Typically, the plaques are located in the white matter of the CNS.

The most frequent locations of the plaques are periventricular white matter, optic nerve and the spinal cord. Another demyelization process can also usually be detected, affecting individual nerve fibers in the spinal cord. Even areas that are not affected by demyelization process show abnormalities that some researchers believe may contribute to the pathogenesis of progressive forms of the disease. MS plaques can be detected by functional diagnostics, and their localization correlates with clinical findings. Acute MS plaques show signs of immune inflammation and degradation of myelin. The mechanisms active in MS plaques are not completely understood. It is clear that damage to myelin, glial and neural cells is mediated by classic immune effector mechanisms: cell-mediated cytotoxicity and antibody production. Cerebrospinal fluid and MS lesions of the patients show high levels of cytotoxic CD8<sup>+</sup> cells specific to MBP, and neural and glial cells have been shown to increase production of MHC I molecules in conditions of immune inflammation, becoming targets for CD8<sup>+</sup> cytotoxic activity. How those mechanisms act together is, as of yet, unclear. Recent results of have shed some light on this problem. They have described four morphological types of inflammation in MS plaques (Luchinetti et al., 2000). Three of these mechanisms are typical for RRMS, and the fourth type is characteristic for PPRS.

1. Demyelination associated with T-cell and macrophages-mediated inflammation.
2. Demyelination associated with T-cell and macrophages-mediated inflammation with extensive antibody deposition in tissues and in glial cells. This pattern seems to be the most common, and is associated with morphological signs of remyelination.
3. Demyelination associated with an infiltrate of T-lymphocytes and activation of macrophages and microglia.
4. Demyelination associated with infiltration with T-cells. This pattern is found in PPRS and is characteristic for this type of the disease.

This shows heterogenic nature of effector mechanisms active in MS. Authors of the study hypothesize that different pathological patterns can represent different pathogenetic pathways of the disease which may be associated with different prognosis and respond to treatment.

In chronic plaques, the inflammatory process becomes inactive. Activated inflammatory cells leave central areas of the plaque, while in the periphery, they retain their activity for some time. During relapses of the disease, reactivation of some chronic plaques can follow. This is accompanied by an increase in permeability of the BBB and by migration of macrophages and antigen-specific T-cells.

The inflammatory process in MS plaques is accompanied by axonal degeneration. Acute axonal degeneration can be morphologically detected by presence of axonal "ovoids". Later on, ovoids disappear, and morphological picture starts pointing at Wallerian degeneration. Axonal damage in MS is thought to be irreversible, and to be responsible for chronic disability in MS patients. It has been shown that markers of neurodegeneration correlate well with state of functional disability.

As with IDDM, it is yet unclear (Anderson et al., 2009) whether the disease is initiated by cross-reactivity of the immune system to MBP, or the primary event is morphological damage to the cells caused by infection or other factors. The immune initiation conception is the more widely accepted one. It is supported by events of experimental transfer of MS to laboratory animals by one of the following ways: a) immunizing the animals with antigens of myelin b) transplanting antigen-specific CD4<sup>+</sup> cells. The latter experiments have become the basis for viewing CD4<sup>+</sup> cells as a key factor in disease initiation (as opposed to IDDM, where CD8<sup>+</sup> lymphocytes seem to be the key mediator of pathogenetic events, although they are supported by CD4<sup>+</sup> Th1-cells). In accordance with these views, blood of patients with MS contains increased numbers of CD4<sup>+</sup> cells specific to MBP.

The neural initiated conception views alteration of glial cells caused by chronic infection as the initiating mechanism of the disease. While the speculation of the infectious factor being the main etiological agent would seem too awkward, there is enough evidence to consider some viruses persisting in the CNS as a factor initiating immune response. Cells of microglia have been shown to raise expression of MHC molecules and inflammatory cytokines in conditions of tissue injury and viral infection. They are able to serve as APCs, playing an important role in primary recruitment of lymphocytes specific to antigens of myelin.

That is quite logical, seeing as antigen mimicry is believed to play quite an important role in the pathogenesis of MS. MS is believed to initiate in genetically susceptible hosts, when common microbes that contain protein sequences cross-reactive with self-myelin antigens activate antigen presenting cells (APC) in the blood. The most studied as a MS risk factor is Epstein-Barr virus infection. In Ascherio et al study, all 100% of patients with MS were infected by EBV (Rudick, 2001). Frequency of MS in patients that had infectious mononucleosis is higher than in general population. It has been shown that BCRs of B-lymphocytes active towards MBP show homology with antibodies specific to EBV latent membrane protein 1 (LMP1) (Gabibov et al., 2011). However, evidence for correlation between DNA load and frequency of MS remain conflicting, as well as that for similarity of geographic patterns of EBV infection and MS. In contrary, antiEBV antibodies seem to be a perfect risk marker, as their level in serum strongly correlates to frequency of MS (Andreoletti et al., 1998). This means that latent EBV infection seems to be the most significant risk factor. The key role is thought to belong to patterns of EBV genes expression in latent state. Changes in methylation of the viral DNA might result in increase in production of certain proteins involved in autoimmune response (Niller et al., 2011). Other viruses that show high expression in tissues of MS patients are HHV-6 and HERV. Increase of expression of those agents might contribute to disease pathogenesis by augmenting the inflammatory response against oligodendrocytes.

Another agent thought to be involved in MS pathogenesis is *Acinetobacter*. There is statistically significant increase of anti-*Acinetobacter* antibodies in the serum of patients with MS. There is also correlation between distribution of *Acinetobacter* sinusitis and MS. Also, DNA sequences of *Acinetobacter* show homology with sequence of myelin.



Current data suggest that infectious triggers are most likely ubiquitous, i.e., highly prevalent in the general population, and that they require a permissive genetic trait which predisposes for MS development.

As the disease progresses, the protective function of the BBB becomes chronically impaired. Morphological investigation of tissues of patients with secondary progressive MS shows formation of lymphoid follicle-like structures in the meninges. Those follicles are hypothesized to be the place of production of autoantibodies specific to various antigens of CNS. Those include previously discussed anti-neurofilament antibodies.

Pathogenetic role of autoantibodies in MS remains unclear. While some researchers claim that autoantibodies in MS just serve as a “witness” of pathological process mediated by other mechanisms (such as cell-mediated cytotoxicity), there is data (Lincoln et al., 2010) showing that some antibody types may play a role in the disease progression through modifying normal antigens of myelin and provoking specific immune response. This refers to catalytic antibodies (abzymes) with proteolytic activity (antibody proteases). The typical mechanism of catalysis for these antibodies is nucleophilic catalysis. Recently abzyme-dependent catalytic degradation of an autoantigen, MBP (myelin basic protein), was associated with the course of the neurodegenerative disease MS and its rodent model, experimental autoimmune encephalomyelitis (EAE). Autoantibody-mediated degradation of MBP was shown to be site specific, with cleavage sites localized to the immunodominant epitopes of the protein. These findings were supported by studies from others. Interestingly, this reaction was inhibited in vitro by glatiramer acetate (Copaxone), an established treatment for MS. (Belogurov et al., 2008)

### **6.5. Prediction and prognosis: changes in the proteome characteristic for multiple sclerosis**

Main diagnostic criteria for MS are based on dissemination of clinical, instrumental, laboratory findings. The three clinical stages of MS are preclinical phase, phase of immune inflammation and degenerative stage. In the preclinical phase there are no morphological changes, but biomarkers of immune inflammation might already be found, although at low titers. At this stage, abs to the “mimic” epitopes of microbial pathogens are found, supporting the view that the disease is triggered by reaction of the immune system to such epitopes. The process becomes self-supporting after immune response shifts towards determinants of the CNS. Preclinical diagnostics of MS aims at discovering autoantibodies before morphological and/or clinical manifestation of the disease.

Markers of the autoimmune process in MS can be divided into two main categories: markers of immune inflammation and markers of neurodegenerative processes. Biological fluids used for sampling include blood serum and the cerebrospinal fluid (CSF). Advantages of using blood serum include higher availability of the material, lower risk of the procedure and economical advantages. However, proteome of blood serum is less selective to pathology of the central nervous system than that of the CSF. Certain markers are difficult to detect, and there are technical problems associated with high-abundance proteins of the blood serum. These have to be removed from the sample in order to reach diagnostically applicable levels



of sensitivity and specificity. CSF is much more convenient in terms of sample preparation, sensitivity and specificity. However, obvious reasons prevent widely using it for screening, especially in healthy individuals.

Non-specific markers of immune inflammation include oligoclonal IgG bands, light Ig chains, chromogranin A, clusrerin, CC3.

Markers specific to autoimmune processes taking place in MS are autoantibodies to myelin basic protein (anti-MBP) and to myelin oligodendrocyte protein (anti-MOG). Antibodies found in MS patients include anti-myelin basic protein antibodies, antiganglioside antibodies, anti-myelin oligodendrocyte glycoprotein antibodies (Yoon et al., 1990). Anti-MBP antibodies are the most common markers used for preclinical diagnostics. To raise the specificity of the method, selective reactivity of autoantibodies to certain MBP fragments can be measured. It should be noted that anti-MBP antibodies are also found in some healthy people.

Antiganglioside (AGM)-antibodies are found in patients with various neurological disorders and are not specific for MS. However, they can be used for prognostic purposes. There are several subtypes of anti-AGM antibodies. Presence of several common AGM-antibodies as opposed to typical AGM-1 antibodies reflects high degree of disease progression. Clinically this correlates with secondary-progressive form of MS and bad prognosis.

Myelin oligodendrocyte protein is a superficial component of myelin. In animal models, this protein has been shown to be quite vulnerable to both cell and humoral immune response. It is thought to be one of the primary targets in initiation of MS. Antibodies to this protein might offer a promising perspective for preclinical diagnostics of MS. MOG is the most interesting candidate B-cell autoantigen in MS. Because of its location it is an ideal target for antibody-mediated demyelination. Anti-MOG antibodies are indeed able to cause myelin destruction in EAE models, while other antibodies against major myelin proteins such as MBP or PLP, which are both not located on the myelin surface, do not cause myelin destruction on their own. Anti-MOG Abs mediate a characteristic vesicular transformation of compact myelin in acutely demyelinated lesions that also has been documented in human MS lesions strongly suggesting a role of anti-MOG Abs in MS. The B-cell response to MOG is enhanced in MS also supporting the pathogenic importance of anti-MOG Abs.

Antiganglioside antibodies (anti-GM) are more of prognostic value, as there is a correlation between anti-GM antibody type and clinical type of the disease (primary progressive, back-and-remitting, secondary progressive).

Prognostic use of antibodies is based on the fact that correlation can be made between types of main determinants and antibodies and prognosis/ clinical stage of the disease. In the course of the disease, autoantibodies cause changes in myelin structure, which leads to formation of new, more immunogenic epitopes, and changes in autoantibodies specter. Clinically this is accompanied by transformation of MS into secondary-progressive form. While the disease progresses, immune inflammation becomes less specific, with initiation of im-

immune response to various determinants of the CNS as opposed to primary response which affects just the components of myelin.

It is necessary to note, however, that markers of immune inflammation do not show exact correlation with degree of axonal damage, although changes in antibody profiles can be useful in predicting evolution to secondary progressive clinical type. That is why markers of neurodegeneration are just as important.

Markers of neurodegeneration are components of structures altered during the clinical attack of MS. The body fluid that reflects their level most accurately is the CSF. However, it is often inconvenient to use CSF, making it necessary to use blood as the more accessible material, although its proteome doesn't reflect proteome of the CNS as accurate as CSF does.

One of such markers is NF-L – the light chain of neurofilaments constituting cytoskeleton of axons. NF-L is raised in a number of neurodegenerative diseases, reflecting axonal damage. There is also correlation between NF-L levels and exacerbation of the disease, and an increase in NF-L levels has been detected in patients with CIS prior to manifestation of MS. (3 years). In patients with MS it is also not uncommon to detect antibodies against neurofilaments – anti-neurofilament antibodies. The best studied marker is the main target of autoimmune reactions in MS – myelin basic protein (MBP). MBP is one of the most abundant proteins of myelin, and is present in both central and peripheral nervous system. High levels of MBP have been shown to correlate with upcoming relapses of MS, as well as with the disease attaining the progressive clinical type. In combination with anti-MBP and anti-MOG antibodies, detection of fragments of MBP can be used for predicting relapses in the course of the disease (Huang et al., 2011). Another prognostic marker is acidic calcium-binding protein – a component of axons damaged during the course of the disease. Tau-protein – a cytoskeleton protein involved in formation of microtubules, is another marker of neurodegeneration. Changes in phosphorylation of the protein lead to impairment of axonal transport, and to formation of insoluble neurotoxic aggregates. In patients with MS, levels of abnormally phosphorylated tau-protein have a tendency to rise with time (Lincoln et al., 2009).

## 7. Conclusion

The pathogenesis of autoimmune diseases is a complex process, with both genetic and environmental factors playing equally important role. In genetically predisposed organisms, the key event promoting the start of autoimmune response is often infection, usually with a viral pathogen. There is a variety of specific patterns by which infections can contribute to autoimmunity. Those patterns were analyzed in the light of fundamental conceptions of autoimmunity. Also, models of infectious pathogens inducing autoimmune processes were discussed on the base of two autoimmune conditions: IDDM and MS, both of which seem to fit the introduced model.

## Author details

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