REVIEWS

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The Molecular Background of Autoimmune Endocrine Disorders

Molekularne podłoże chorób autoimmunologicznych układu dokrewnego

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Abstract

Recent data concerning the molecular background of autoimmune endocrine disorders are presented. Apart from a few rare syndromes caused by single gene mutations, most endocrine autoimmune diseases are complex polygenic traits triggered by environmental factors. The search for the genetic component concentrates on polymorphisms in genes involved in immune tolerance and immune response. The DNA polymorphisms may result from single nucleotide substitutions (SNPs) or from a variable number of tandem repeats of a short sequence, as in the IDDM2 locus associated with type 1 diabetes. The HLA system, especially class II, was the first confirmed source of alleles conferring susceptibility to autoimmune disorders. Currently, major attention is turning to numerous SNPs which may influence the structure or function of proteins taking part in immune mechanisms. Polymorphisms in *CTLA-4*, *PTPN22*, *PDCD1*, *FCRL3*, and *SUMO4* as well as those in genes associated with vitamin D action have been hitherto investigated. Some of these have shown significant association with type 1 diabetes, Graves' disease, chronic thyroiditis, and/or autoimmune Addison's disease. There are now many other gene polymorphisms under investigation in autoimmune endocrine disorders. The progressing exploration of this field will enable future practical use in forecasting the development of autoimmune disorders and possibly in preventing their onset (**Adv Clin Exp Med 2007, 16, 4, 549–559**).

Key words: autoimmunity, polymorphism, endocrinology.

Streszczenie

Artykuł w skrócie przedstawia aktualne dane dotyczące molekularnego podłoża autoimmunologicznych chorób układu dokrewnego. Poza kilkoma rzadkimi zespołami spowodowanymi mutacjami pojedynczego genu, większość autoimmunologicznych chorób endokrynnych jest mutacją wielu genów, ujawniającą się pod wpływem czynników środowiskowych. Poszukiwania składowej genetycznej koncentrują się na polimorfizmach genów zaangażowanych w procesy tolerancji immunologicznej i odpowiedź odpornościową. Polimorfizmy DNA mogą być skutkiem zmian jednonukleotydowych albo różnej liczby tandemowych powtórzeń krótkiej sekwencji, jak w przypadku *locus* IDDM2, sprzężonego z cukrzycą typu 1. Układ HLA, zwłaszcza klasy II, był pierwszym potwierdzonym źródłem allelów sprzyjających rozwojowi zaburzeń autoimmunizacyjnych. Obecnie uwagę skupiają liczne polimorfizmy jednonukleotydowe mogące wpływać na strukturę i funkcję białek biorących udział w mechanizmach immunologicznych. Dotychczas badano polimorfizmy genów *CTLA-4*, *PTPN22*, *PDCD1*, *FCRL3*, *SUMO4*, a także genów związanych z działaniem witaminy D w organizmie. Niektóre z nich wykazały znaczący związek z rozwojem cukrzycy typu 1, choroby Graves-Basedowa, przewlekłego zapalenia tarczycy i/lub choroby Addisona. Obecnie w autoimmunologicznych chorobach układu dokrewnego analizuje się różne polimorfizmy genetyczne. Ich coraz lepsza znajomość umożliwi w przyszłości zastosowanie praktyczne – przewidywanie ryzyka chorób autoimmunologicznych i, być może – zapobieganie ich rozwojowi (**Adv Clin Exp Med 2007, 16, 4, 549–559**).

Słowa kluczowe: autoimmunizacja, polimorfizm, endokrynologia.

Autoimmune disorders are caused by uncontrolled reaction of the immune system against self antigens of the organism. According to a 2005 National Institutes of Health report, these diseases affect 5–8% of the population; however, the authors admit that for many disorders, reliable epidemiological data are still lacking [1]. A thorough statistical analysis is even more difficult due to the unclear classification of this group of disorders, which belong to different medical disciplines and variously fulfill the criteria of an autoimmune condition (Rose & Bona, 1993). The American Autoimmune Related Diseases Association distinguishes about 80 diseases with an autoimmune component, including those as common as rheumatoid arthritis and type 1 diabetes as well as very rare diseases such as scleroderma. Autoimmune disorders present a spectrum from organ-specific to systemic conditions, also known as connective tissue diseases. The former subgroup contains the autoimmune endocrine disorders: type 1 diabetes, Graves' disease, chronic Hashimoto's thyroiditis, adrenocortical insufficiency (Addison's disease), hypoparathyroidism, lymphocytic hypophysitis, and primary hypogonadism, especially premature ovarian failure of autoimmune origin.

Immunological Tolerance

Autoimmunity is due to alterations in immunological tolerance, defined as the lack of a specific immune response after exposure to a particular antigen, in this case a self antigen. Immunological tolerance involves lymphocyte development and function, and its mechanisms may be divided into central and peripheral ones. The central phase takes place during lymphocyte maturation in the thymus (or, in case of B cells, in the bone marrow) and consists of negative selection. The T lymphocytes with surface TCRs (T-cell receptors) presenting strong affinity to self antigens are clonally deleted [2]. Nevertheless, this process is not fully efficient, and even healthy individuals have some autoreactive cells, found on the periphery, with lower affinity to self antigens. This is why additional peripheral mechanisms are necessary to prevent the expansion of autoreactive lymphocytes which failed to be eliminated during their development. These mechanisms comprise clonal anergy, apoptosis, mechanical barriers, and the function of regulatory T cells.

The specific activation of a T lymphocyte by an antigen requires not only interaction between its TCR and the MHC (major histocompatibility complex) molecule on the antigen-presenting cell (APC), but also a second, co-stimulatory signal. This second signal is essential for the initiation of the lymphocyte's response to the antigen, and its lack, even in a situation of antigen recognition, leads to T-cell anergy. An example of the co-stimulatory pathway is the interaction between the CD80 (B7-1) and CD86 (B7-2) molecules expressed on activated APCs with the CD28 molecule on T cells. Instead of CD28, another molecule, CD152, also known as CTLA4 (Cytotoxic T Lymphocyte Antigen 4), may interact with B7. Expressed exclusively on activated T cells, CTLA4 presents very high affinity for CD80/CD86, and by binding them, inhibits the second signal transmission [3]. In this way an autoreactive lymphocyte, on encounter with a specific self antigen, does not become activated. Mice with *Ctla4* knock-out died within a month after birth due to massive lymphoproliferative changes.

The apoptosis of autoreactive lymphocytes is induced by interaction between the Fas death receptors (CD95) and their ligands, FasL, on the surfaces of APCs and activated lymphocytes, respectively. This association attracts the intracellular adaptor protein FADD (Fas-associated death domain-containing protein), which binds procaspase 8 molecules, transforms them into active cytosolic enzymes, and subsequently provokes a cascade activation of consecutive caspases, initiating the apoptotic process. Fas-FasL interaction is also possible between neighboring lymphocytes and even within the same T cell (cell suicide) [2].

Some self antigens are additionally shielded by special anatomical barriers protecting certain privileged places, such as the eyeball, testis, and brain. Under physiological conditions, autoreactive cells have no chance of encountering their specific autoantigens, which are thus ignored by the immune system. The destruction of these protective barriers, through injury or inflammation, is associated with increased risk of autoimmunity [2].

An important element of peripheral immunological tolerance are regulatory T cells, which display potent inhibitory effects on T-cell function. The best described hitherto is the $CD4^+CD25^+$ subpopulation, comprising 5–10% of the $CD4^+$ T lymphocytes. During their development they undergo positive selection in the thymic cortex. Their TCR receptors have high affinity for self antigens presented by the thymic cells [2]. It is still unknown in what way $CD4^+CD25^+$ cells become anergic and how they are protected from negative selection. The mechanism of their tolerant action has also not been revealed. According to some authors, regulatory T cells secrete cytokines, such as TGF-β and interleukin-10, that inhibit T-cell responses. Some other data indicate cytokine-independent

direct contact with activated T cells, particularly Th1 [4]. Experiments with NOD mice, an animal model of type 1 diabetes, have demonstrated that transfer of $CD25^+$-depleted $CD4^+$ T cells to immunodeficient mice had a strong diabetogenic effect, while co-transfer of $CD4^+CD25^+$ T cells significantly inhibited the development of the disease and diminished lymphocytic infiltration of pancreatic islets [5]. When comparing the populations of regulatory T lymphocytes in healthy individuals and patients with autoimmune polyglandular syndrome type 2 (Addison's disease together with concomitant type 1 diabetes and/or autoimmune thyroid disease), no differences in number or surface markers were discovered; however, the suppressor activity of the regulatory T cells from the patients was impaired [6].

Thus it can be assumed that the presence of some autoreactive cells is a normal situation in the organism. Autoimmune disorders seem to develop in individuals with inefficient mechanisms protecting against the activation of these cells. Mutations and polymorphisms in genes involved in the functioning of the immune system as well as environmental influences (infections, diet, different chemicals, drugs, etc.) are among the possible causes of impaired immunological tolerance. The frequent familial susceptibility to autoimmune diseases proves the role of genetic factors.

Monogenic Autoimmune Endocrine Disorders

Only a few rare autoimmune endocrine disorders are caused by a single gene mutation. Type 1 autoimmune polyglandular syndrome (APS1), also known as APECED (Autoimmune PolyEndocrinopathy–Candidiasis–Ectodermal Dystrophy) is due to a mutation of the *AIRE* gene (AutoImmune REgulator) located on the long arm of chromosome 12. *AIRE* is mainly expressed on epithelial cells of the thymic medulla, where it activates the transcription of genes encoding specific peripheral antigens [7]. Thus it is involved in the education of thymocytes and in the negative selection of autoreactive cells. *AIRE* mutations may impair this process, promoting autoimmunity. Over 50 different *AIRE* mutations have already been described, the most common being the so-called "Finnish mutation" R257X. The clinical onset usually takes place in childhood and is characterized by chronic mucocutaneous candidiasis, autoimmune hypoparathyroidism, and adrenocortical insufficiency [8]. The presence of two of these three disorders is sufficient to establish a diagnosis of APS1. Moreover, any other autoimmune disorder may be found in these patients, as well as dental enamel hypoplasia, nail dystrophy, and keratopathy. The syndrome has an autosomal recessive mode of inheritance and its highest incidence is observed in Finns and Iranian Jews [8].

Another, extremely rare autoimmune disorder with endocrine dysfunction is the IPEX syndrome (Immune dysregulation, Polyendocrinopathy, Enteropathy, and X-linked inheritance), the effect of a mutation in the *FOXP3* gene (Forkhead box protein P3) located on chromosome Xp11.23. *FOXP3* encodes a transcription factor expressed on $CD4^+CD25^+$ T cells and necessary for their development and appropriate regulatory function [9]. Foxp3-deficient mice present with severe lymphoproliferative autoimmune syndrome and generalized overproduction of cytokines [10]. In humans, *FOXP3* mutation manifests as watery diarrhea difficult to control, insulin-dependent diabetes, thyroid disorders, and eczema. Most affected individuals die within their first year of life [9]. The preliminary reports on an association between *FOXP3* polymorphisms and isolated type 1 diabetes cases have not been confirmed although the gene maps within the locus linked to this disease.

Polygenic Background of Autoimmune Endocrine Disorders

Most autoimmune endocrine disorders are complex polygenic traits. There is no single gene responsible for their development and, consequently, no classical Mendelian inheritance pattern. Numerous predisposing genes play a role in the pathogeneses of these diseases, which are triggered by environmental factors. The importance of a genetic background is supported by studies on mono- and dizygotic twins and by comparative genotyping of affected individuals and the general population [11]. In type 1 diabetes, for instance, the concordance between monozygotic twins is about 20–40%. Genetic factors are also considered responsible for nearly 80% of the susceptibility to Graves' disease [11].

Recent progress in molecular biology has enabled the exploration of the genetic backgrounds of autoimmune disorders, which are currently connected mainly with DNA polymorphisms. The term "polymorphism" (derived form Greek *polymorphos*, meaning multiform) refers to the presence of two or more alternative alleles in a given chromosomal locus in the population (Vogel and Motulsky, 1986). In contrast to a mutation, a polymorphism is recognized when the less

frequent allele is observed in more than 1% of the population. DNA polymorphism may result from point changes in a nucleotide sequence (SNP, single-nucleotide polymorphism) or from variable numbers of short nucleotide sequence repeats. Polymorphisms are common phenomena, within the normal range, responsible for inter-individual variation. They may influence gene expression, alter protein structure, or provoke no evident effect at all. Polymorphic genetic variants are not the direct cause of a complex disease, but they may be associated with increased (or diminished) predisposition to developing this type of disorder. If an allele appears statistically more frequently in the affected group than in the general population, there is a strong probability that it confers a higher risk of the disease, whereas a less frequent allele may be a protective one.

The HLA Region

In the human genome, the Major Histocompatibility Complex (MHC) is characterized by exceptional polymorphism. This system is encoded by HLA (Human Leukocyte Antigen) genes located on the short arm of chromosome 6 and divided into three classes. The highly polymorphic class I and II genes encode surface glycoprotein molecules directly involved in antigen presentation to T lymphocytes. Class I HLA gene products are expressed on virtually all nuclear cells, while class II are typically present on professional APCs, i.e. macrophages, dendritic cells, and B cells.

Several hundred allelic variants have so far been described within HLA classes I and II in humans, and their number gradually increases as different populations are investigated. Moreover, each class II MHC molecule is composed of two non-covalently bonded chains, alpha and beta, encoded by separate genes. In some HLA regions, several genes encoding alpha and beta chains have been found; for example, at locus DR there are 3–4 functional genes for the beta chain, all of which can be expressed together with the product of the alpha chain gene. The human DR region contains a single alpha chain gene, whereas the DP and DQ regions have two genes each. The total number of DRB alleles exceeds 500, while the DRA chain is highly conservative: only 3 alleles were observed [12]. This abundance of genes and alleles increases considerably the possible number of synthesized proteins. The cell surfaces in a single person may display several different MHC molecules.

The HLA system has been associated with the risk of autoimmune disorders for a long time. The allelic variants of the HLA system may differently induce an immune response because of the variable efficiency of antigen presentation or the diverse ability of T cells to recognize an antigen presented by a particular allele. About 50% of genetic susceptibility to type 1 diabetes is attributed to class II HLA alleles. The development of this disease is particularly promoted by HLA-DR3 and DR4 (especially HLA-DR3/DR4 heterozygotes: 2% of the general population and 40% of patients with type 1 diabetes) as well as HLA-DQ57βnonAsp status, i.e. lack of aspartic acid at position 57 of the HLA-DQ beta chain [13]. Addison's disease associates particularly with DR3(HLA-DRB1*03)-DQ2(HLA-DQA1*0501-DQB1*0201) and DR4-DQ8 (especially in heterozygotes). The haplotype DR4(0404)-DQ8 is found in 30% of affected subjects, but in less than 1% of the general population. Autoimmune thyroid disease (Graves' disease or chronic Hashimoto's thyroiditis) is associated with HLA-B8, DR3, and DR5. Some alleles promoting autoimmunity are shared among several disorders. DQA1*0501 is more frequent in patients with type 1 diabetes, Graves' disease, or Addison's disease [14], while DRB1*0301 is found significantly more often in those with type 1 diabetes, Addison's disease, or celiac disease [15]. The HLA alleles which are less frequent in the affected population may present a protective influence, as HLA-DR2 in the case of type 1 diabetes.

Molecular mapping of the HLA region has revealed the existence of several genes homologous to the classical HLA system encoding proteins of unknown function, for example the family of *MIC* genes. *MIC-A* gene is the best described; in its exon 5 a microsatellite polymorphism was discovered which consists of different numbers of GCT trinucleotide repeats. Its A5.1 allele is characterized by five GCT repeats and an insertion of a single nucleotide, guanine, resulting in a frameshift and premature termination of transcription [15]. The transmembrane domain of this new protein has lower hydrophobic amino-acid content and its anchorage to the cell membrane is therefore less stable, which in turn favors the soluble form of the protein. The presence of the A5.1 allele, especially in homozygotes, was found to be associated with several autoimmune disorders: type 1 diabetes, Addison's disease, Graves' disease, and celiac disease. On the other hand, the MIC-A6 and -A9 alleles seem to present a protective influence on autoimmune disease development. As the protein encoded by *MIC-A* gene is a ligand of the NKG2D receptor on NK cells, there were some attempts to associate disturbed *MIC-A* expression with initiation of the autoimmune

process; however, no definite proof has yet been found.

Insulin Gene

Another example of simple sequence-length polymorphism is the so-called variable number of tandem repeats (VNTR) in the IDDM2 locus on the short arm of chromosome 11. In the 11p15.5 region are located the genes encoding tyrosine hydroxylase (*TH*), insulin (*INS*), and insulin-like growth factor IGF2. A region of high variability of minisatellite fragments extends approximately 600 bp upstream from the site of *INS* transcription initiation. According to the number of 14-nucleotide-sequence repeats, the alleles in this locus are divided into three classes: I (26–63 repeats), II (about 80 repeats), and III (149–209 repeats). The class I alleles predispose recessively to type 1 diabetes, while the class III alleles have a dominant protective effect. In order to explain the significance of the variable number of 14-nucleotide repeats, insulin gene transcription *in vitro* and its expression *in vivo* were analyzed. In pancreatic beta-cells, class I alleles were associated with a higher steady-state insulin expression than class III, while in human fetal thymus the level of INS mRNA was two to threefold higher in the presence of class III than for class I alleles [16]. The VNTR alleles, located in the *INS* promoter region, may modulate the thymic expression of this gene, thereby influencing the induction of immunological tolerance to proinsulin, a specific beta-cell antigen. The protective action of class III alleles might be due to a more efficient negative selection of autoreactive thymocytes. On the other hand, an attempt to associate the VNTR alleles with the presence of anti-insulin antibodies in patients with recent onset of type 1 diabetes failed, as there was no significant difference between patients with I/I and III/X genotype [17]. No associations between VNTR polymorphism and Addison's disease, Graves' disease, celiac disease, or multiple sclerosis were found.

CTLA4 Gene

The genomic regions linked to increased risk of type 1 diabetes used to be described as consecutive IDDM, beginning with IDDM1 (HLA region). IDDM1 and IDDM2 were identified while searching for functional candidate genes and comparing healthy and affected populations. Subsequently, IDDM3-18 were associated with diabetes risk based on linkage analyses in affected families. Each IDDM locus may include several genes. Studies aimed at understanding their influence on diabetes development are still underway.

One of the best-described regions is IDDM12, on the long arm of chromosome 2 (2q33), containing genes encoding the CD28, CTLA4, and ICOS (inducible co-stimulator) molecules. Especially *CTLA4* gene was the object of numerous studies. Initially, three polymorphisms were analyzed: C(-318)T within the gene promoter, microsatellite $(AT)_n$ polymorphism in the 3'UTR region, and, above all, A49G in exon 1, substituting threonine for alanine [3]. The association between CTLA4 and autoimmune disorders was confirmed in a number of studies, also concerning endocrine diseases. However, the results of analyses carried out in different populations were confusing. Thus a whole segment of approximately 300 bp in the 2q33 region has been sequenced, revealing more than 100 SNPs. A study in patients with Graves' disease demonstrated that the polymorphisms presenting the highest differences in frequency between healthy and affected individuals were mainly localized in the 3' region of *CTLA4* gene [18]. Interestingly, none of the three previously studied polymorphisms presented strong association with Graves' disease. Seven SNPs, plus A49G, were chosen for further analyses, with the genotyping of another several hundred patients. The strongest association with Graves' disease was ascertained for the G allele of the CT60 polymorphism [18]. Similar results were obtained in Hashimoto's thyroiditis. The analysis of *CTLA4* markers in type 1 diabetes-affected families revealed that alleles which positively correlated with autoimmune thyroid disease were also associated with the development of diabetes. The significance of the MH30/G, CT60/G, JO30/G, and JO31/G polymorphisms was likewise confirmed in patients with Addison's disease, independently of its form: isolated or as part of type 2 autoimmune polyendocrine syndrome [19]. To explain these observations, the role of CT60 alleles in *CTLA4* expression, particularly in the splicing of its transcript, was suggested. Examination of the mRNA levels of the two CTLA4 isoforms, i.e. a full-length (flCTLA4, exons 1–4) and a soluble isoform (sCTLA4 without exon 3, which encodes the tansmembrane domain), demonstrated a diminished ratio of sCTLA4 to flCTLA4 splice forms in CT60G disease-susceptible individuals [18]. The soluble form retains its ability to interact with CD80/CD86 molecules, so its lower expression may attenuate their inhibition, thus promoting T-cell activation, typical for autoimmunity. On the other hand, a study of patients with autoimmune thyroid disease revealed increased amounts of

sCTLA4 in plasma compared with a healthy group [20]. This relationship was explained by competition between both isoforms to bind CD80/CD86 molecules. However, the results of a recent Swedish study failed to confirm an association between CT60 and the expression of CTLA4 isoforms [21].

The properties of CTLA4 are already used in therapy. A fusion protein combining the CTLA4 extracellular domain and the Fc fragment of human IgG1 was created (abatacept). It binds CD80/CD86 on antigen-presenting cells and therefore blocks their interaction with CD28 molecule on T lymphocytes, preventing T-cell activation [22]. Abatacept therapy has given positive results in animal models of systemic lupus erythematosus and diabetes. It has also proven its efficiency in clinical trials in human rheumatoid arthritis and psoriasis. The US Food and Drug Administration has admitted abatacept in rheumatoid arthritis therapy. The example of the *CTLA-4* gene demonstrates the difficulties with unequivocal determination of the polymorphisms associated with complex diseases, but at the same time it confirms that persistent investigation of the molecular background of autoimmunity may bear fruit, permitting the practical use of acquired knowledge.

PTPN22 Gene

Currently, an object of increasing interest is *PTPN22* gene, located on chromosome 1 (1p13.3–1p13.1), encoding the cytoplasmic protein tyrosin phosphatase LYP. The gene is expressed in the thymus, spleen, bone marrow, and all types of peripheral blood mononuclear cells [23]. LYP phosphatase, together with Csk kinase, prevents the spontaneous activation of T cells. Csk phosphorylates tyrosine residues at the carboxyl terminus of Src family kinases, which take part in the initiation of lymphocyte activation. The Src kinase conformation changes and it becomes inactivated. In this way the signal cascade is attenuated and T-cell stimulation is suppressed. Efficient blockade of the activating signal at the level of Src kinases requires an interaction between Csk kinase and LYP phosphatase which, in turn, dephosphorylates the appropriate tyrosine residues in Src kinases and in their substrate, ZAP-70 protein [24]. C1858T polymorphism of the *PTPN22* gene results in a substitution of arginine for tryptophan (R620W) in the LYP P1 domain, responsible for the binding of the phosphatase with Csk kinase. As the interaction between the two enzymes becomes weaker, the inhibition of Src kinases is also impaired. This may result in excessive activation of T lymphocytes, as found in autoimmune disorders. A study on the 293T cell line cotransfected with Csk cDNA clones and *PTPN22* R620 or W620 variant, respectively, provided indirect evidence of this mechanism. The co-immunoprecipitation of *PTPN22* revealed that at similar expression levels of both constructs, the co-precipitation of Csk with W620 protein was 2.5- to 3-fold lower than with R620 [23]. Thus it seems that the lower affinity of the PTPN22 W620 variant to the Csk kinase may promote increased T-lymphocyte activation. Mice with a knock-out of the murine equivalent of the *PTPN22* gene demonstrated increased numbers of T cells, lymphadenopathy and splenomegaly, and high concentrations of immunoglobulins; however, no autoantibodies were detected.

First reports on the C1858T polymorphism in *PTPN22* described the association of the T allele and type 1 diabetes [25] as well as an increased occurrence of this allele in patients with rheumatoid arthritis [23]. Subsequent publications confirmed the association of this polymorphism with different rheumatoid disorders, type 1 diabetes, and Graves' disease, including one Polish study which revealed a dose-dependent effect of the T allele influencing the age at disease onset [26]. Studies of the relationship between C1858T and Hashimoto's thyroiditis, Addison's disease, Sjögren's syndrome, vitiligo, psoriasis, and celiac disease are still underway. In the meantime, Japanese researchers have identified another *PTPN22* polymorphism which might be associated with type 1 diabetes susceptibility: a G(-1123)C substitution in the gene promoter [27]. The DNA sequence comprising this polymorphism is typical for the transcription factor AP-4 (activator protein-4) binding site and may influence *PTPN22* expression.

The increasing amount of data concerning the role of gene polymorphisms in the pathogenesis of complex disorders stimulates attempts of the practical use of this knowledge. A study of Swedish blood donors revealed that the presence of the *PTPN22* 1858T allele together with antibodies to cyclic citrullinated peptide has 100% specificity in detecting rheumatoid arthritis before its clinical onset [28]. The possibility of early diagnosis is particularly important at the beginning of the disease, when the clinical symptoms may be still ambiguous, while it is the best time to introduce an effective and resolute therapy.

A prospective study confirming the marker role of *PTPN22* in endocrinology is part of the Finnish Diabetes Prediction and Prevention Project. Neonates at three university centers are screened for the HLA-DQB1 risk genotypes.

Those with confirmed risk alleles are then regularly monitored for the appearance of serum autoantibodies specific to the preclinical stage of type 1 diabetes [29]. Nearly 9000 children have been enrolled so far, and over 100 have already progressed to clinical diabetes. An enormous, well-described risk group enables an analysis of the influence of several genetic and environmental factors. Apart from the confirmation that *PTPN22* 1858T is a type 1 diabetes-predisposing allele, the researchers managed to prove its significance in the progression of the autoimmune process. Among the children with positive pancreatic islet cell autoantibodies, those with the TT genotype at position 1858 were characterized by a fourfold increase in the risk of the appearance of consecutive antibodies specific to pancreatic autoimmunity [29]. Patients with a high risk of type 1 diabetes are invited to participate in a clinical trial assessing the efficacy of nasally administered insulin in the prevention of the disease.

PDCD1 Gene

Another gene which seems to be associated with autoimmunity is *PDCD1* (programmed cell death 1), formerly known as PD-1, located on chromosome 2q37.3. It encodes an immune receptor, a member of the immunoglobulin superfamily, found on the surface of activated B and T cells and their precursors. This receptor acts as a negative regulator of lymphocytic activation. It has two ligands: PD-L1, expressed on hematopoietic and parenchymal cells (pancreatic islet cells, for instance), and PD-L2, found exclusively on macrophages and dendritic cells. The interaction of *PDCD1*-encoded protein with its ligand inhibits T-cell proliferation and cytokine production [30]. NOD mice with *PDCD1* or PD-L1 blockade rapidly developed diabetes, and the lymphocytic infiltration of their pancreatic islets as well as the incidence of GAD-reactive splenocytes were significantly increased compared with controls [30]. Among the several SNPs found in the *PDCD1* gene, PD-1.3, substituting guanine for andenine at 7146 position of the nucleotide chain, deserves particular consideration. It is localized in intron 4 of the *PDCD1* gene within the RUNX1 (runt-related transcription factor 1) binding site sequence. RUNX1 is a transcription factor important for normal thymocyte development and crucial in the pathogenesis of acute myeloblastic leukemia. Electrophoretic studies with anti-RUNX1 antibodies have demonstrated specific binding of nuclear extracts from a human T-cell line in the presence of the *PDCD1* PD-1.3G allele, while the A allele hindered this interaction [31]. It seems that appropriate RUNX1 binding may influence the expression of *PDCD1*, significant for the inhibition of autoreactive cells.

The literature concerning *PDCD1* polymorphisms and autoimmune disorders is still scarce. In some populations, the association between the PD-1.3A allele and systemic lupus erythematosus, especially its renal complications, has been confirmed. In Asian groups, an association between PDCD1 and rheumatoid arthritis was found, while a European study revealed the significance of PD-1.3 in the seronegative form of this disease. In autoimmune endocrine disorders, *PDCD1* polymorphisms were investigated only in association with type 1 diabetes. The PD-1.3 SNP was significantly more frequent in Danish diabetic patients than in a control group [32]. A Japanese study failed to confirm an association between *PDCD1* and diabetes; however, this analysis was limited to polymorphisms within the exons and at the exon-intron junctions.

FCRL3 Gene

Linkage analysis has revealed an association between 1q21-23 region and a number of autoimmune disorders (rheumatoid arthritis, systemic lupus erythematosus, sclerosis multiplex). This region includes genes of the Fcγ immunoglobulin receptor and its homologues of unknown ligands and functions. One potential candidate gene in this group is *FCRL3* (*Fc receptor-like 3*), expressed mainly on B lymphocytes in germinal centers of the lymphatic nodes. Japanese researchers have discovered four SNPs within the *FCRL3* gene associated with the development of rheumatoid arthritis. Especially the FCRL3_3 polymorphism located at the transcription initiation site occurred statistically more often in those patients. The FCRL3_3*C allele was also associated with increased susceptibility to autoimmune thyroid disease in Japanese people [33]. The experimental data demonstrate that the C allele may facilitate the activation of the *FCRL3* gene promoter and the binding of the nuclear factor NF-κB, which regulates several genes of the immune system. Unfortunately, most analyses of Caucasian populations did not reveal any association between *FCRL3* polymorphisms and rheumatoid arthritis, type 1 diabetes, or Graves' disease. Only in one group of British patients with Addison's disease did the polymorphisms of this gene occur significantly more frequently than in a control group [34]. However, the FCRL3_3*C allele, promoting autoimmunity in the Japanese population, appeared to be a protective one in British patients. This may be an incidental finding, a reflec-

tion of real differences in the pathogenesis of those disorders, or evidence of the existence of another susceptibility allele in linkage disequilibrium with the polymorphisms analyzed so far. The explanation of these paradoxical results requires further studies of the 1q21-23 region.

Vitamin D-Associated Genes

Numerous data indicate that the role of vitamin D is not only restricted to calcium homeostasis, but also links to the function of the immune system. The wide spread of its receptors in the immune cells confirms this hypothesis. Vitamin D stimulates the production of the anti-inflammatory cytokines interleukin-4 and transforming growth factor-beta1 (TGF-β1). At the same time, the synthesis of interleukin-2 and interferon-gamma, characteristic of the type Th1 immune response, decreases in $CD4^+$ T cells [35]. The immunomodulatory action of vitamin D also inhibits HLA class II expression on thyroid follicular cells and pancreatic islet beta-cells *in vitro*. Vitamin D supplementation hampers autoimmunity in animal models of rheumatoid arthritis, systemic lupus erythematosus, type 1 diabetes, and inflammatory bowel disease, whereas its deficiency precipitates the progress of those disorders [35]. Decreased serum 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$, an active form of vitamin D) concentrations were found in patients with type 1 diabetes and those with Graves' disease. A Finnish study of over 10,000 children showed that daily supplementation of 2000 IU of vitamin D during infancy diminished the risk of type 1 diabetes, assessed 30 years later [36]. A one-year therapy with $1,25(OH)_2D_3$ together with intensive insulin treatment in children with recent diabetes onset did not reveal significant differences in C-peptide concentrations and glycated hemoglobin levels compared with their peers on insulin therapy only. However, the insulin doses in the vitamin D-supplemented group were lower.

The considerable environmental role of vitamin D in the development of autoimmune disorders has encouraged research aiming to find potential correlations with the genetic background of its action in the organism. Vitamin D receptor (VDR) polymorphisms were supposed to modulate the responsiveness to the vitamin, so they were studied in patients with multiple sclerosis, type 1 diabetes, inflammatory bowel disease, and rheumatoid arthritis. The results were highly variable, but a meta-analysis of 1997–2005 studies did not confirm an association of allelic VDR variants with the risk of type 1 diabetes [37]. A few studies concentrated on genetic variants of the vitamin D binding protein (DBP) responsible for vitamin's transport in the blood and into target cells. DBP genotypes, analyzed in specific populations such as Pima Indians and indigenous Colorado and Pacific islands inhabitants, appeared to be connected with some parameters of glucose homeostasis: fasting glycemia and insulinemia and response to the oral glucose tolerance test. Unfortunately, these results were not reproducible in Caucasian populations. A few analyses of DBP gene polymorphisms in intron 8 and exon 11 did not provide unequivocal results in Europeans with Graves' disease or type 1 diabetes.

The transformation of vitamin D into its active form, 1,25-dihydroxycholecalciferol, is catalyzed by 1α-hydroxylase. Any defects in the *CYP27B1* gene encoding this enzyme may therefore impair vitamin D function in the organism. Mutations altering the amino-acid sequence of 1α-hydroxylase cause hereditary vitamin D-dependent rickets. Polymorphisms discovered in the *CYP27B1* gene promoter and intron 6 might influence the enzyme's expression, which was also found in extra-renal tissues, such as pancreatic islets. Experiments on diabetic NOD mouse macrophages revealed diminished CYP27B1 induction in response to inflammatory cytokines. A mouse model deficient in 1α-hydroxylase exhibited calcium homeostasis disorders together with cervical lymphadenopathy and decreased number of peripheral $CD4^+$ and $CD8^+$ lymphocytes.

Analyses of *CYP27B1* polymorphisms in the context of autoimmune disease incidence are still rare. Initially, the substitution of cytosine for thymine in intron 6 was investigated, but among endocrine disorders, only an association with Hashimoto's thyroiditis was preliminary demonstrated. SNPs in the gene promoter seem more propitious, particularly C(-1260)A. According to one hypothesis, this substitution changes the CDX2 transcription factor binding site, which may influence gene transcription and consequently alter the enzyme activity and eventually impair vitamin D3 function. In Germans, the genotype CC was statistically more frequent in patients with Addison's disease, autoimmune thyroid disorders, and type 1 diabetes [38]. Similarly, a British analysis confirmed an association between the CYP27B1 gene C(-1260) allele and Addison's disease, but not Graves' disease.

SUMO4 Gene

Sometimes an investigated polymorphism is definitely associated with susceptibility to one or

more diseases in a particular population, while these results are impossible to reproduce in other ethnic groups. An example is the *SUMO4* (small ubiquitin-related modifier 4) gene located on chromosome 6q25 within intron 6 of the *MAP3K7IP2* gene. It encodes a little-known protein which probably influences the immune response by interacting with IκBα molecule, a negative regulator of the nuclear transcription factor NF-κB. An A163G polymorphism was found in the *SUMO4* gene, replacing methionine with valine in codon 55. According to some experimental data, the activity of transcription factor NF-κB is higher in GG homozygotes [39]. Both its location, corresponding to the IDDM5 locus, and its potential function made *SUMO4* an interesting candidate for analyses in type 1 diabetes. Japanese and Korean studies demonstrated a significant association between A163G polymorphism and diabetes, autoimmune thyroid disease, and rheumatoid arthritis. Unfortunately, analyses concerning type 1 diabetes in Lithuanian, Finnish, British, Norwegian, and American Caucasian cohorts failed to confirm a connection between the G allele and the development of the disease or even suggested the opposite, i.e. an unfavorable role for the A allele. Moreover, a British study did not reveal any significant difference in the frequencies of the two alleles of the A163G *SUMO4* polymorphism in patients with Graves' disease and Addison's disease versus controls [40]. Thus it seems that in non-Asian populations there is another candidate gene at the IDDM5 locus to search for.

Other Autoimmune Endocrine Disorders

Among the autoimmune endocrine disorders, type 1 diabetes and autoimmune thyroid diseases usually attract the most attention. This is mainly due to the relatively high frequencies of their incidence and, indirectly, to the increasing costs of their diagnostics and therapy. A rare disorder presents more difficulties in collecting large groups of patients for association studies. Thus data concerning genetic susceptibility to hypoparathyroidism, hypopituitarism, or primary hypogonadism of autoimmune origin are rather scarce. Hypoparathyroidism occurs typically as part of the APS 1 syndrome, caused by the *AIRE* gene mutations. Single publications on genetic polymorphisms in isolated hypoparathyroidism did not show any association with *CTLA4* or *PTPN22* gene variants. In some hypoparathyroid patients, activating autoantibodies against parathyroid calcium-sensing receptor are found in serum, but a few attempts to detect potential changes in its gene did not succeed.

In the case of hypopituitarism it is particularly difficult to establish an autoimmune background of the disease, i.e. lymphocytic hypophysitis. In practice, the diagnostics usually aims to exclude proliferative changes in this region and to determine the extent of hormonal deficiency. An MR image of extensive infiltration may even simulate pituitary adenoma. An autoimmune origin is strongly suggested by female sex, incidence during pregnancy or in the postpartum period (necessary to differentiate from Sheehan syndrome), and other concomitant autoimmune disorders. Little is known about pituitary autoantigens, and no routine serological diagnostics of this disease is performed. However, an association was found between autoimmune hypophysitis and certain HLA alleles: A2, Cw1, Cw2, DR2.

The authors conclude that better understanding of immune system function stimulates more detailed analyses of the genetic background of autoimmune disorders. Many studies concentrate on genes involved in the immune response: those encoding APC and T-lymphocyte surface molecules or elements of intracellular signal transduction within an activated lymphocyte. Some polymorphisms seem to promote autoimmunity generally, being associated with several disorders, while others are more specific, limited to susceptibility to a particular disease. Although the influence of a single gene in complex traits is relatively weak, this is still a promising field for future investigations. It is worth noting that most monogenic disorders are quite rare. In fact, these are polygenic diseases (i.e. cardiovascular, neoplastic, autoimmune, or psychiatric disorders) that constitute a major socio-economic burden to modern societies. Good knowledge of factors predisposing to their development will enable better delineation of risk groups, early introduction of strategies preventing or delaying disease onset, and more individualized therapeutic approaches.

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