

5. DIABETES AND AUTOIMMUNITY

Assist. Prof. Borut Božič, Ph.D.

University Medical Center, Department of Rheumatology
Ljubljana and University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia

1.1. Introduction

Autoimmunity is the reaction of the immune system against the body's own tissues. Tolerance and specifically self-tolerance is one of the most exciting (and controversial also) areas of immunology and remains a phenomenon that must be explained in any theory of immunity. To understand how autoimmune reactions can develop it is necessary to know the mechanisms by which self-tolerance is normally maintained. These include:

1. sequestration of autoantigen in inaccessible sites;
2. deletion of autoreactive T cells during thymic development;
3. failure to process and present particular self molecules;
4. induction of energy in autoreactive T cells, due to lack of co-stimulatory signals or specific cytokines;
5. suppressor cells and hormones.

Failure of any of these mechanisms could lead to autoimmune reaction. Many more individuals develop autoimmune reactions than autoimmune diseases. Autoimmune diseases occur when autoimmune reactions result in pathological tissue damage. Autoimmune diseases tend to distribute themselves within a spectrum. At one pole are non-organ specific diseases with autoimmune reaction to antigens, distributed throughout the body resulting in destructive lesions of skin, blood vessels, kidneys, joints, lungs; at another pole are the organ specific diseases with destructive lesions of a single organ in the body (skin, liver, gonads, thyroid, pancreatic islets). There is no clear border between these two poles.

Diabetes mellitus is a heterogeneous group of disorders, all characterized by hyperglycemia. Experts recommended one set of criteria for diagnosis and another set for classification. One purpose is to secure optimal treatment of the patients; another is to support research aimed at understanding the etiology and pathogenesis of diabetes mellitus. Etiologic classification of diabetes considers

- a. type 1 diabetes with β -cell destruction,
- b. type 2 diabetes with unknown etiology,
- c. other specific types which include uncommon forms of immune-mediated diabetes and
- d. gestational diabetes.

The practical approach is to distinguish between type 1, which is an immune-mediated disease (or idiopathic) and type 2, which is not immune-mediated. Some individuals develop a milder form of type 1 diabetes, characterized by the presence of auto-antibodies, but with clinical classification as type 2. This type is classified as the uncommon form, sometimes-

called type 1.5 diabetes.

Type 1 diabetes is the most severe type of diabetes, leading to life-long dependency on daily insulin injection. Type 1 or immune mediated diabetes (IMD) results from an organ-specific autoimmune mediated loss of insulin-secreting β cells. This chronic destructive process involves both cellular and humoral components detectable in the peripheral blood, months or even years, before the onset of clinical diabetes. Anyway we are still far from understanding its etiology: how and which genetic and environmental factors interact to initiate the immune-mediated process that results in β -cell destruction.

1.2. Genetic factors

Always, when self-recognition as part of self-tolerance is in the question, the genes of the major histocompatibility complex (MHC) are involved through their expressed products - HLA proteins. Some haplotypes predispose the IMD, whereas others protect. HLA class II region on the 6th chromosome is called immune mediated diabetes 1 region (IMD1), which consists from DR and DQ alleles. This HLA gene region plays a role in antigen presentation and initiation of immune response. The mechanisms by which HLA-DQ and HLA-DR allelic proteins elicit susceptibility and protection in IMD are not understood; however, it is reasonable to consider that the affinity of islet cell antigenic binding to their clefts is responsible. There are multiple loci in IMD1 region contributing to susceptibility: DRB1, DQB1, DQA1, and DPB1. There is a genetic associations hierarchy in human immune mediated diabetes: HLA-DRB1*04/DQA1*0301/DQB1*0302 is the predominant HLA haplotype associated with susceptibility in IMD. HLA- DRB1*15/DQA1*0102/ DQB1* 0602 are the predominant HLA class II alleles associated with protection. Analysis of the DRB1*04 subtype is particularly informative because a risk of IMD, associated with this subtype is greatly variable depending on the population: different alleles are important as IMD risk for Norwegians, French, Spaniards, and Australians. Similarly, association with strong protection is also provided by alleles of DRB1*04 subtype - and again: different alleles for different populations.

Susceptibility at MHC class II seems to be a necessary but not a sufficient predisposing factor. Or by other explanation, 60% of the genetic susceptibility to IMD is conferred by HLA. Near the insulin gene on chromosome 11 lies another locus (called IMD2), which seems to play a role in the level of gene transcript expression in the thymus. It presumably eliminates insulin-autoreactive T cells from escaping into the circulation.

Beside these two loci, several more have been found, which give better understanding of genetic predisposition for IMD. Some of them could influence the immune response in general, for example through the polymorphism of cytotoxic T-lymphocyte adhesion ligand (CTLA-4). This linkage is not understood well, but it has been speculated that a gene polymorphism, involving an AT repeat at the C terminus at the 3' end of the gene may affect the stability of CTLA-4 mRNA. Since CTLA-4 is involved in T-cell apoptosis, less stable CTLA-4 mRNA may lead to T cell survival because CTLA-4 protein is not formed.

1.3. Pathogenesis

The central role of T cells in the pathogenesis of IMD has been demonstrated in several ways, for example with neonatal thymectomy of non-obese diabetic mice, with transfer of T lymphocytes from diabetic into non-diabetic mice, in humans with immunosuppressive therapy or even with bone marrow transplantation from a diabetic donor. IMD results from cell-mediated autoimmune attack directed towards the insulin-producing islets of Langerhans, which leads to specific destruction of the pancreatic β -cells. The process of destruction of β -cells is chronic in nature, often beginning during infancy and continuing over the many months and years that follow. At the time of clinical diagnosis more than three quarters of the β -cells have been destroyed and islets are infiltrated with chronic inflammatory mononuclear cells, a process that has been called insulinitis. Among mononuclear cells have been CD8+ cytotoxic T cells.

Long before a person develops diabetes, autoantibodies to β -cells and their antigens are detected. The most important predictive markers for IMD are cytoplasmic islet cell antibodies (ICA), glutamic acid decarboxylase autoantibodies (GADA or GAD65), insulin autoantibodies (IAA) and autoantibodies to tyrosine-phosphatase (insulinoma associated antigen 2, IA-2 and IA-2 β). These antibodies are of significance in discriminating between diabetes type 2 and so-called type 1.5 diabetes. The spectrum of antibodies, their avidity and affinity distinguish individuals who develop diabetes from those who do not. Antigenic and epitope spreading of the autoantibody responses is one of the important markers of imminent progression: those with autoantibodies to multiple antigens most often progress rapidly, while the presence of any one of the antibodies alone may not be predictive of the disease. The Immunology of Diabetes workshop and Immunology of Diabetes Society have organized antibody standardization workshops since 1985. Such workshops are of invaluable importance, particularly because enzyme-linked immunosorbent assays, also used in the detection of autoantibodies in diabetes, are on first glance simple and non-problematic. The Combinatorial Islet Autoantibody Workshop has demonstrated that only the use of a combination of autoantibody assays has made it possible for several laboratories to achieve excellent discrimination between diabetic and control sera. It has been demonstrated that GAD65 and IA-2 have a high diagnostic sensitivity and specificity for IMD. It should be stressed that in situations where a serologic marker could precede the clinical features of the disease for several years, it is very difficult to decide whether such a marker belongs to a subset of the healthy population or is indeed a marker of the developing disease. So the data about diagnostic specificity and diagnostic sensitivity should be evaluated considering a follow up of serologically positive individuals. This view is even more important, if we know that autoantibodies are not suited to detect clinically overt diabetes. In such conditions, diagnosis is established on the basis of clinical and metabolic criteria.

The exact mechanism involved in the initiation and progression of β -cell destruction is still unclear. The presentation of β -cell-specific autoantigens by antigen presenting cells (APC) to CD4+ helper T cells in association with MHC class II molecules is considered to be the first step. Both subsets of CD4+ and CD8+ T cells are required for islet invasion and β -cell destruction. However, the relative contribution of each subset to trigger the diabetes is not clear. Macrophages stimulate the CD4+ T cells by interleukin (IL) 12 to secrete interferon (IFN) γ and IL2. IFN γ stimulates resting macrophages to secrete tumor necrosis factor and

free radicals, which are toxic to β -cells. Other cytokines stimulate migration of CD8+ cytotoxic T cells, which cause, recognizing the autoantigens on β -cells together with MHC class I molecules, their damage.

Defective MHC class I self-peptide presentation could be the result of wrong transcription and/or translation of an inducible protease subunit of the proteasome known as LMP2. The proteasome is a giant multisubunit ATP-dependent protease, one of whose functions is a degradation of intracellular antigen for presenting in the MHC class I. It has been shown in non-obese diabetic mice that the defective expression interrupts the proteolytic processing of NF- κ B, a transcription factor central to effective lymphocytic maturation, normal regulation of T cell cytokine production and protection of T cells from apoptosis. Diabetic human subjects have defective antigen presentation, defective in vitro proteasome processing of test substrates and interrupted MHC class I display of self-peptides with poor T cell selection.

1.4. Immunological models

Cytokines are very important in controlling the development of the immune response. They modulate the differentiation and division of haematopoietic stem cells and activation of lymphocytes and phagocytes. They are of crucial importance in triggering or perpetuating immune diseases including IMD. T helper cells are differentiable by their chemokine receptors and the cytokines they secrete. IFN α and IL 2, secreted by T helper cells 1 (Th1), promote cell-mediated immunity. IL 4 and IL 10 secreted by T helper cells 2 (Th2), down-regulate Th1 cell activity and are mainly involved in humoral immunity. Th1 cells might promote disease and Th2 might be protective in the process of autoimmune reaction. Unfortunately, some pathogenic Th1 cells have been shown to be diabetogenic after switching to Th2 type also. B-lymphocytes might have in this story more influence on the pathogenesis of diabetes by presenting autoantigens to CD4+ cells than by secreting autoantibodies.

Natural killer T cells (NK-T) are able to rapidly produce large amounts of cytokines, suggesting that these cells play a role in regulating the speed of immune responses. They can specifically recognize the human cluster of differentiation 1 (CD1) molecules. CD1 have been characterized as antigen-presenting molecules that not belong to MHC classes. They are similar to MHC class I molecules and are prominently expressed on specialized APC. The pocket of antigen-binding groove of CD1 is constituted by hydrophobic residues, suggesting that the antigens presented by CD1 are not peptides, but rather lipids and glycolipids. NK-T cells have been recognized as a major source of IL 4 on primary antigenic stimulation, and can be autoreactive in vitro for CD1 molecules in the absence of exogenous antigens. It has been suggested that NK-T cells are important as regulatory cells in autoimmune diseases. Early defect in NK-T cells could lead to the genesis of autoimmunity through a deficiency in Th2 cell function.

Loss of self-tolerance could allow autoreactive elements to escape the eradication by the process of negative selection occurring in the thymus. Studies in animal models have shown that any healthy immune system contains potentially auto-aggressive T cells. Even that self-tolerance is absolutely required for maintenance of good health, it has been inferred that the mechanism of thymic tolerance though sophisticated is not a perfect one. So, the ques-

tion is not “have we or have we not potentially auto-aggressive T cells” but how can their activation be prevented?

Apoptosis has been traditionally thought of as a non-inflammatory process, which does not induce an immune response. Apoptosis is involved in negative selection in the thymus, in deletion of structures that are needed exclusively during one stage of development, in tissue homeostasis, where apoptosis acts as a counterbalance of proliferation to maintain tissue size. However, recent studies indicate that apoptotic cells can be involved in immune processes. They can display autoreactive antigen in their surface blebs, they can activate dendritic cells and they can induce the formation of autoantibodies. These findings suggest that the neonatal wave of β -cell apoptosis may provide autoantigen necessary for triggering β -cell directed autoimmunity.

Molecular mimicry received considerable attention when the activation of autoreactive T cells was reported to be associated with the onset of several diseases. Molecular mimicry postulates that structural similarity occurs between pathogen epitopes and self-proteins of the host. Autoimmunity may occur when T cell reactivity to an infecting pathogen results in the activation and expansion of T cells cross-reactive against a biologically relevant epitope of an autoantigen. There is some evidence that could support the involvement of molecular mimicry. A stretch of 6 amino acids (PEVKEK) is shared by the islet cell antigen GAD65 and a protein involved in CVB4 virus replication. The shared cross-reactive epitope is immunodominant GAD epitope in 25% newly diagnosed diabetic patients. Immunodominant epitope on the protein IA-2 had 56% identity and 100% similarity for 9 amino acids of a major immunogenic protein of human rotavirus.

Unlike conventional antigens, superantigens bind outside the MHC-binding grooves on APCs connecting the V β portion of the T cell receptors (TCR). Because the TCR repertoire encompasses a limited number of families with V β elements very similar in sequence, any superantigen is capable to activate a large fraction of the circulating T cells - up to 30% what is several thousand times more than conventional antigens.

1.5. Environmental factors

With the last immunological models of IMD triggering some of the possible environmental factors were mentioned. Both superantigens and molecular mimicry include infection. Several studies have reported a viral etiology associated with IMD, of which congenital rubella is clinically established. Other candidates are rotaviruses, retroviruses, Coxsackie B, herpesviruses, cytomegalovirus, measles, hepatitis C and the bacterium *Haemophilus influenzae*. It could be connected with viral infection in very early childhood, and that children who were breast-feeding rarely get IMD in contrast with those who were not breast-feeding or were breast feeding for a very short time.

Non-antigenic specific mechanism of T cell activation has been proposed to be involved in the pathogenesis of IMD. Virus infection could locally create pro-inflammatory milieu favoring the differentiation of Th1 type cytokines and recruitment of macrophages and other immune responding cells.

1.6. Conclusion

The number of people afflicted by IMD continues to increase at an extremely high rate. The reasons for this can be not only genetic and not only environmental factors. The most important long-term goal of the research on IMD is to understand the immunopathological basis of this disease. A complete understanding of the genesis of IMD will be probably achieved from the comprehension of the complex interaction amongst different events that are still only partially known and not from the analysis of a single causative factor.

Recommended literature:

1. Thomas L (Ed). Clinical Laboratory Diagnostics. TH-Books 1998.
2. Roitt I, Male D, Brostoff J. Immunology. Mosby 1998.
3. Fauci AS (Ed). Harrison's Principles of Internal Medicine, McGraw-Hill 1998.
4. Parry SL, Hall FC, Olson J, Kamradt T, Sonderstrup G. Autoreactivity versus autoaggression: a different perspective on human autoantigens. *Current Opinion in Immunology* 1998; 10:663-8.
5. Lernmark A. Type I Diabetes. *Clin Chem* 1999; 45/8(B):1331-8.