Chapter 2

Immunologic and Genetic Factors in Type 1 Diabetes Mellitus

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1. Introduction

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, insulin action, or both (Kumar et al., 2002). In type 1 diabetes, the body does not produce insulin. Insulin is a hormone that is needed to convert sugar, starches and other food into energy needed for daily life. Insulin deficiency in turn leads to chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism (Kumar et al., 2002). As the disease progresses tissue or vascular damage ensues leading to severe diabetic complications such as retinopathy, neuropathy, nephropathy, cardiovascular complications and ulceration (Huang, Kim et al. 2002; Wallace, Reiber et al. 2002; Bearse, Han et al. 2004; Seki, Tanaka et al. 2004; Svensson, Eriksson et al. 2004). Thus, diabetes covers a wide range of heterogeneous diseases. Diabetes is the most common endocrine disorder and by the year 2015, it is estimated that more than 200 million people worldwide will have DM and 300 million will subsequently have the disease by 2025. Type 1 diabetes is usually diagnosed in children and young adults, and was previously known as juvenile diabetes.

The diagnostic criteria and the classification of diabetes was first put forward by the World Health Organization (WHO) in 1965 then by the National Diabetes Data Group (NDDG) in 1979, and the latest recommendations have been published by the American Diabetes Association (ADA) in 1997 and by the WHO in 1999(Genuth, Alberti et al. 2003). According to the ADA recommendation, the fasting glucose concentration should be used in routine screening for diabetes as well as epidemiological studies; the threshold for fasting glucose is fasting glucose = 7.0 mmol/L (126 mg/dl) and /or a 2-h glucose = 11.1 mmol/L (200 mg/dL). For the diagnosis of diabetes, at least one criteria must also apply:

- Symptoms of diabetes (polyurea, polydipsia, unexplained weight loss, etc) as well as casual plasma glucose concentration = 11.1 mmol/L (200mg/dL).
- Fasting plasma glucose = 7.0 mmol/L (126mg/dL), with no caloric intake for at least 8 h.

Diabetes Mellitus may be categorized into several types but the two major types are type 1 and type 2. The term type 1 and type 2 were widely used to describe Insulin-Dependent Diabetes Mellitus (IDDM) and Noninsulin-Dependent Diabetes Mellitus (NIDDM), respectively. On the basis of etiology, Type 1 (DM) is present in patients who have little or no endogenous insulin secretory capacity and who therefore require insulin therapy. The two main forms of clinical type 1 diabetes are type 1a (about 90% of type 1 cases) which is thought to be due to immunological destruction of pancreatic beta-cells resulting in insulin deficiency, and type 1b (idiopathic, about 10% of type 1 diabetes), in which there is no evidence of autoimmunity. Type 1a is characterized by the presence of islet cell antibody (ICA), anti-glutamic acid decarboxylate (anti-GAD), IA-2 or insulin antibodies that identify the autoimmune process with beta-cell destruction. Autoimmune diseases such as Grave's disease, Hashimoto's thyroiditis and Addison's disease may be associated with Type 1 (DM) (Betterle, Zanette et al. 1984; Atkinson and Maclaren 1994). There is no known etiological basis for type 1b diabetes mellitus. Some of Type1b patients have permanent insulinopaenia and are prone to ketoacidosis, but have no evidence of autoimmunity. This form is more prevalent among individuals of African and Asian origins.

Type 2 diabetes is the commonest form of DM and is characterized by disorders of insulin secretion and insulin resistance.

Type 1 (DM) is a multifactorial disease characterized by the autoimmune destruction of insulin-secreting pancreatic beta-cells causing tissue damage. The peak age of onset is about 12 years, and from then onwards daily injections of insulin are required by affected individuals. With a frequency of about 0.4% in Caucasians of European descent, Type 1 (DM) is second to asthma as the most serious chronic childhood disease in the Western world (Wan, Yang et al. 2010). There is a marked geographic variation of Type 1 (DM), with a higher incidence in the European and North American than the Asian and south American countries. The current global increase in incidence of 3% per year is well reported. This rapid rise strongly suggests that the action of the environment on susceptibility genes contributes to the evolving epidemiology of this disease(Wan, Yang et al. 2010).

Type 1 (DM) shows a complex mode of inheritance, with disease susceptibility caused both by genetic and by environmental components. The penetrance of disease genes being determined by unknown environmental factors. Identical twins of affected individuals have a risk of developing the disease of only 36% (Owerbach and Gabbay 1996), demonstrating the importance of the environmental factors. Nevertheless, genetic factors are essential, as measured by the quantity (i.e. the ratio of the risk to siblings of patients compared with the population prevalence). The disease is polygenic in humans and in mice, with a number of different susceptibility genes each accounting for a portion of the familial clustering of the disease (Pharoah, Dunning et al. 2004). Around the time of clinical presentation, insulitis, a chronic inflammatory infiltrate of the islets affecting primarily insulin containing islets, is present in the majority of cases. The mononuclear cell infiltrates in the islet, which results in the development of insulitis (a prerequisite step for the development of diabetes) are primarily composed of T cells. It is now well accepted that these T cells play important roles in initiating and propagating an autoimmune process, which in turn destroys insulin-

producing islet beta-cells in the pancreas (Toyoda and Formby 1998). Understanding insights of the mechanism of immune-mediated islet cell destruction and the interaction between the immune system and pancreatic islets provide new therapeutic means of preventing this chronic debilitating disease.

Before safe and rational therapies can be offered in a clinical setting, a detailed understanding of the immune-mediated process that results in Type 1 (DM) is required, as is the accurate identification of those at risk of the disease. The immunogenetics of type 1 diabetes has become the model upon which other complex disorders are studied, and in this chapter we focus on the importance of recent insights into the pathogenesis and natural history of Type 1 (DM) with consideration to current therapeutic strategies, and future perspective for the efficient treatment.

2. Diabetes mellitus clinical manifestation and diagnosis

The symptoms of diabetes are more readily recognizable in children than in adults, so it is surprising that the diagnosis may sometimes be missed or delayed. Those families with a strong family history of diabetes should suspect diabetes, especially if there is one child in the family with diabetes. Main manifestations are: polyuria, polydipsia, polyphagia, progressive cachexia, glucosuria, hyperglycemia, increasing of specific gravity of urine, blurred vision, fatigue, cramps and candidiasis. Diabetic retinopathy is a major complication of diabetes (Bakker, Tushuizen et al. 2012). Diabetes causes high blood sugar levels, which can damage blood vessels. The damaged vessels around the retina can leak protein and fats, forming deposits that can interfere with vision. The damaged blood vessels are also not as effective at carrying oxygen to the retina, which can also cause damage (Bakker, Tushuizen et al. 2012).

When blood glucose concentrations increase, more glucose is filtered by the glomeruli of the kidneys than can be reabsorbed by the kidney tubules, resulting in glucose excretion in the urine. High glucose concentrations in the urine create an osmotic effect that reduces the reabsorption of water by the kidneys, causing polyuria (excretion of large volumes of urine) (Katavetin 2009). The loss of water from the circulation stimulates thirst. Therefore, patients with moderate or severe hyperglycemia typically have polyuria and polydipsia (excessive thirst). The loss of glucose in the urine results in weakness, fatigue, weight loss, and increased appetite (polyphagia). Patients with hyperglycemia are prone to infections, particularly vaginal and urinary tract infections and an infection may be the presenting manifestation of diabetes (Katavetin 2009).

There are two acute life-threatening complications of diabetes: hyperglycemia and acidosis (increased acidity of the blood), either of which may be the presenting manifestation of diabetes. In patients with Type 1 (DM), insulin deficiency, if not recognized and treated properly, leads to severe hyperglycemia and to a marked increase in lipolysis (the breakdown of lipids), with a greatly increased rate of release of fatty acids from adipose tissue (Wajchenberg 2007). In the liver, much of the excess fatty acid is converted to the keto acids beta-hydroxybutyric acid and acetoacetic acid. The increased release of fatty acids and keto acids from adipose, liver, and muscle tissues raises the acid content of the blood,

thereby lowering the pH of the blood. The combination of hyperglycemia and acidosis is called diabetic ketoacidosis and leads to hyperventilation and to impaired central nervous system function, culminating in coma and death.

Many studies have also shown that hyperglycemia causes oxidative stress in tissues that are susceptible to complications of diabetes mellitus, including peripheral nerves (Ziegler, Sohr et al. 2004). The autonomic nervous system modulates the electrical and contractile activity of the myocardium via the interplay of sympathetic and parasympathetic activity (Lahiri, Kannankeril et al. 2008). An imbalance of autonomic control is implicated in the pathophysiology of Type 1 (DM). Cardiovascular autonomic neuropathy, a common form of autonomic dysfunction found in patients with diabetes mellitus (Maser and Lenhard 2005), as well, and causes abnormalities in heart rate control, as well as defects in central and peripheral vascular dynamics.

Symptoms are similar in both types of diabetes but they vary in their intensity. Longstanding Type 1 (DM) patients are susceptible to microvascular complications; and macrovascular disease (coronary artery, heart, and peripheral vascular diseases) (Saely, Aczel et al. 2004; Svensson, Eriksson et al. 2004)and end stage renal disease. Ketoacidosis is usually not a problem in patients with type II diabetes because they secrete enough insulin to restrain lipolysis.

Symptoms in type 2 DM are similar but usually milder and insidious in onset. Geographical differences exist in both the magnitude of these problems and their relative contributions to overall morbidity and mortality.

3. Composition of the islet infiltrates and the mechanism of beta-cell destruction

The histopathology of type 1 diabetes is defined by a decreased beta-cell mass in association with insulitis, a characteristic lymphocytic infiltration limited to the islets of Langerhans and prominent in early stage disease in children. It is considered to be pathognomonic for recent onset disease. The infiltrate consists predominantly of T cells, in which CD8+ lymphocytes dominate, but may also contain CD4+ lymphocytes, B-lymphocytes and macrophages (Willcox, Richardson et al. 2009).

The cellular response is accompanied by a humoral response that includes autoantibodies against a wide array of beta-cell antigens (which will be discussed later). However, the precipitating (auto)antigen against which the inflammatory response is directed has not been identified, nor has it been established whether the humoral response that is considered to be part of our current diagnostic criteria is a cause or a consequence of the disease. Although animal models for the disease exist, like the spontaneously non-obese diabetic (NOD) mouse, they are found to differ from the human disease in many key aspects and it is an open question whether data derived from such models will be applicable to patients. In fact, even after a century of research we know very little about the etiology and histopathology of the human disease.

The pancreas is a difficult organ to biopsy and most of the material is therefore post-mortem. The islets are scattered in a matrix of exocrine tissue and thus form only 1–2% of the parenchymal tissue. In addition the beta-cells are not homogeneously distributed throughout the gland and are often located within a few lobes. In a diabetic condition, the lesions are mainly found in islets in which beta-cells are still present and the lesions will largely disappear together with the beta-cells against which the reaction appears to be directed. In addition, the few cases that were brought to autopsy often died in ketoacidosis, they may thus represent a more fulminant version of the disease that is not necessarily characteristic for the disease process in the rest of the population. Lastly, and perhaps most importantly, the histopathological lesions that we observe in cases with recent onset disease will only show the final stages of a process that has been going on for a long period of time, and until recently, we had no material available of earlier stages of the disease. Identifying patients with pre-clinical disease and studying the immunological processes occurring at this stage may prove to be indispensable for a breakthrough in our quest for the etiology of the disease.

3.1. A Brief history of insulitis

Inflammatory infiltrates in the islets of Langerhans were first described in 1902 by the German pathologist Schmidt (Nagler and Taylor 1963), who found foci of small-cell infiltration in the periphery of islets of Langerhans from a 10 year old diabetic child with an unknown duration of disease. This islet-specific inflammation, later termed "insulitis" by the Swiss pathologist von Meyenburg, was long considered to be a rare event. Cecil (Cecil 1909) described leucocytic infiltration associated with islets in 9 out of 90 patients with diabetes, but often under conditions in which a more generalized pancreatitis was present; he observed islet-specific inflammation in only a single case, involving a young adult patient with recent onset disease. In 1928 Stansfield and Warren were the first to draw attention to the association between insulitis and the age of the patient; they described insulitis in a six year old girl who died in a diabetic coma two months after onset of the disease, and in an 11 year old girl who died in a diabetic coma within four weeks after the initiation of symptoms. In their view, the striking lymphocytic infiltration in the islets of both cases suggested a causal relationship between the inflammation and the diabetic condition in these two young patients with recent onset fulminant disease. On the other hand, it was clear from their studies in larger groups of children that insulitis was not always observed. These observations were revisited in 1958 by LeCompte (Lecompte 1958) who collected four cases with insulitis, all involving acute onset disease and short duration in children. He proposed four possible explanations for the presence of the cellular infiltrate: a direct invasion of the islets by an infectious agent, a manifestation of functional overstimulation or strain, a reaction to damage by some unknown nonbacterial agent and lastly an antigen-antibody reaction.

Fifty years later one could still make the same list, as none of these possibilities has been excluded. In a 1965 landmark study, Willy Gepts (Gepts 1965) reported the presence of the lesion in 15/22 (68%) recent onset cases below the age of 40 and noted that it was not present in patients with a disease duration of more than a year. He also noted that beta-cell mass

appeared to be reduced to approximately 10% of that in non-diabetic controls. Other authors supported the findings as well. Foulis et al (Foulis, Liddle et al. 1986) using a 25-year computerized survey of deaths in the UK to identify 119 young patients who died in ketoacidosis before the age of 20, in combination with immunohistochemistry to identify islets and infiltrating leucocytes, confirmed that insulitis was present in 47 out of 60 (78%) of young patients with recent onset disease (<1 year). These investigators, however, also pointed out that certain heterogeneity seemed to exist in their patient population, as it is observed that young-adult patients with a short duration of the disease showed no evidence of insulitis and in which all islets contained insulin. Together it appears that insulitis exist predominantly in (pre) diabetic patients in which it is limited to islets that were still insulin-containing.

3.2. Pathogenic autoantigen in type 1 diabetes

The major autoantigens in Type 1 (DM), identified by circulating autoantibodies, are glutamic acid decarboxylase (GAD), tyrosine phosphatase-like insulinoma antigen and (pro) insulin. It is not clear, however, which if any drive pathogenic T cells. So far, no antigen has emerged as dominant, although both glutamic acid decarboxylase and insulin have been postulated to be principal autoantigens (Pugliese, Brown et al. 2001).

With the possible exception of rare self-antigen-expressing cells in lymphoid tissue (Pugliese, Brown et al. 2001), proinsulin is expressed uniquely in beta-cells. Investigation on humans (Kent, Chen et al. 2005), and murine model (Nakayama, Abiru et al. 2005), highlight the pancreatic beta-cell hormone insulin as a major target for T cell attack. If insulin, or peptides of the β chain of insulin, is given orally (Bergerot, Fabien et al. 1994) intranasally (Harrison, Dempsey-Collier et al. 1996) or subcutaneously(Hutchings and Cooke 1998), diabetes is suppressed. In addition, when proinsulin is expressed in the NOD mice under the control of a MHC class II promoter, such that it is expressed on antigen-presenting cells and in the thymus, the incidence of diabetes is decreased (French, Allison et al. 1997). There are some reports demonstrating that insulin gene polymorphism is associated with predisposition to Type 1 (DM) (which will be discussed later). In some studies specificity of the T cell response was confirmed by isolation of $CD4^+$ and $CD8^+$ T cell clones specific for the insulin epitopes. The most convincing evidence of a pathogenic role of insulin specific $CD4^+$ T cells came from a study in which the insulin A1–15 specific T cells were expanded from pancreatic lymph nodes of deceased patients affected by Type 1 (DM) (Kent, Chen et al. 2005).

Moreover, Multiple T-cell epitopes against GAD65 (glutamate decarboxylase 65) have been associated with Type 1 (DM). GAD65 is expressed in the endocrine cells of the islets of Langerhans and in the central nervous system (Karlsen, Hagopian et al. 1991). The major autoantigens, in which there are evidence that are associated to the pathogenesis of Type 1 (DM) are listed below.

3.3. Phenotyping insulitis

Immunophenotyping of the infiltrate showed that most cells corresponded to T cells, with T cytotoxic/suppressor cells being most abundant, although helper $CD4^+$ T cells and NK cells

were also present (In't Veld 2011). CD8+ T lymphocytes (T cytotoxic/suppressor) were the main infiltrating cell type. In addition to lymphocytes, macrophages were a prominent feature of the infiltrate; although it is controversial and in some studies macrophages were not found in insulitic lesions. The composition of the cellular infiltrate is stratified according to the percent beta-cells present in islets collected across patients (In't Veld 2011). The percent beta-cells would be a surrogate marker for the stages of advent of the insulitic lesion, with 50–69% insulin-positive area taken as starting point and 0% insulin-positive area as end-stage (In't Veld 2011). In all stages, CD8+ T cells are predominant, increasing in number with decreasing insulin-positive area, but disappearing when insulin-positivity is completely lost. CD20+ B-cells were found to be the second most prominent cell type, following the dynamics of CD8+ cells, while macrophages were present at relatively constant levels becoming the most prominent infiltrating cell type in insulin-deficient islets.

autoantigen	Expression	Subcellular location	Involvement in the NOD mouse	Human T1D		
				autoantibodies	$CD4^+$ T cells	$CD8^+$ T cells
Insulin	β-cell, thymus	secretory granule	+	+	+	+
GAD 65	neuroendocrine	synaptic-like microvesicles	+	+	+	+
GAD 67	neuroendocrine	cytosol	+	+	+	+
IA-2 (ICA512)	neuroendocrine	secretory granule		+	+	+
IA-2 β/phogrin	neuroendocrine	secretory granule		+	+	+
IGRP	β-cell	endoplasmic reticulum	+	?	+	+
Chromogranin	neuroendocrine	Secretory granule	+	?	?	?
ZnT8	β-cell	secretory granule	?	+	?	?
HSP-60 HSP-70	Ubiquitous	mitochondria	+	+	+	?
Glima-38		secretory granule	?	+	?	?
Amylin/IAPP		secretory granule	?	?	?	+
CD38	Ubiquitous	?	?	±	?	?

Table 1. Autoantigens defined as recognized by T cells in human and NOD mice type 1 (DM).

Phenotyping the infiltrating cells in insulitic lesions is only a first step in a process to identify the antigen against which the infiltrate is directed. The key step will be to analyze their specificity. Direct analysis of insulitic T cell specificity has been reported to date is limited, although some studies suggest that CD4+ T cells isolated from pancreases of Type 1 (DM) patients are specific to some parts of the insulin molecule (Kent, Chen et al. 2005). Although the predominantly CD3+CD8+ phenotype of the infiltrating cells is compatible with a cytotoxic T cell mediated beta-cell destruction, it has not yet been proven that the cells in the insulitic lesion are the cells that are actually responsible for the destruction of the beta-cell component. However many studies suggest that a cytotoxic T cell mediated destruction of insulin-producing beta-cells is initiated by an unknown (auto)antigen, leading to the destruction of beta-cell mass.

It is equally well possible that a large part of such infiltrates are the consequence of beta-cell destruction rather than its cause, at least at this relatively late time point in the progression of the disease. What we observe at clinical presentation is the final stage of a process that may have been going on for a long period of time. If the presence of circulating autoantibodies against islet cell antigens is considered as a surrogate marker for beta-cell destruction, then the process may take years before the clinical threshold is reached. Determining the incidence and time course of insulitis prior to diagnosis and correlating it to the presence, and persistence of circulating immune markers, will be crucial for our understanding of the disease and for the development of immune intervention strategies. It is important to correlate such information to the regenerative capacity of the beta-cell mass. Not all individuals who are autoantibody-positive progress to overt disease and the process may well involve episodes of fulminant destruction followed by episodes of repair and regeneration.

Islets with insulitis contain replicating beta-cells, indicating that beta-cells retain a substantial capacity for growth that appears to be activated under conditions of inflammation. It cannot be excluded that such newly formed cells attract recurrent autoimmune attack and that islets with a low insulin-positive area represent islets with regeneration rather than islets in the last stages of beta-cell destruction.

3.4. Mechanisms of beta-cell destruction and initiation of pathogenesis

Although the pathogenesis of Type 1 (DM) has been extensively studied, the precise mechanisms involved in the initiation and progression of beta-cell destruction remain unclear. Animal models used in the study of Type 1 (DM), such as the (BB) rat and (NOD) mouse, have greatly enhanced our understanding of the pathogenic mechanisms involved in this disease. In these animals, macrophages and/or dendritic cells are the first cell types to infiltrate the pancreatic islets (Yoon and Jun 2005). Macrophages must be involved in the pathogenesis of Type 1 (DM) early on, since inactivation of macrophages results in the near-complete prevention of insulitis and diabetes in both NOD mice and BB rats (Yoon and Jun 2005). The activated macrophages secrete IL-12, which stimulates Th1 type CD4+ T cells. The

CD4+ T cells secrete IFN-γ and IL-2. IFN-γ activates other resting macrophages, which, in turn, release cytokines, such as IL-1β, TNF-α, and free radicals, which are toxic to beta-cells. During this process, IL-2 and other cytokines induce the migration of $CD8^+$ peripheral T cells to the inflamed islets, perhaps by inducing the expression of a specific homing receptor. The precytotoxic $CD8^+$ T cells that bear beta-cell-specific autoantigen receptors differentiate into cytotoxic effector T cells upon recognition of the beta-cell-specific peptide bound to MHC class I molecules in the presence of beta-cell-specific $CD4^+$ T helper cells. The cytotoxic $CD8^+$ T cells then affect beta-cell damage by releasing perforin and granzyme, and by Fas-mediated apoptosis. In this way, macrophages, CD4+ T cells, and CD8+ T cells synergistically destroy beta-cells, resulting in the onset of autoimmune Type 1 (DM).

Both direct and indirect killing of beta-cells, mediated by $CD8^+$cytotoxic T cells and $CD4^+$helper T cells respectively, are thought to occur by apoptosis following activation of caspases, but necrosis also might play some role (Rasche, Busick et al. 2009). Based upon animal models, it is now generally believed that multiple effector molecules and pathways are involved in beta cell killing. In addition to apoptosis being the main mechanism by which beta-cells are destroyed, beta-cell apoptosis has been implicated in the initiation of Type 1 (DM) through antigen cross-presentation mechanisms that lead to beta-cell-specific T cell activation (The term cross-presentation denotes the ability of certain antigen-presenting cells to take up, process and present extracellular antigens with MHC class I molecules to CD8+cytotoxic T cells). In mammals, there are 14 caspases, of which many participate in the apoptotic pathways. Caspase-3 is the major effector caspase involved in apoptotic pathways. Caspase-3 knockout mice were protected from developing diabetes in autoimmune diabetes model.

As explained before, autoantibodies have turned out to be excellent diagnostic and predictive markers for Type 1 (DM). However, it is generally thought that they play only a minor role, if any, in the actual pathogenesis of the disease. Instead, the cell-mediated immune response is believed to be responsible for beta-cell killing as explained above. Inflammatory cells are found in and around the pancreatic islets. However, human studies show that in some individuals these inflammatory cells are present for years without clinical symptoms. In fact, some individuals with autoantibodies and insulitis do not go on to develop clinical disease. The outcome appears to be related to the amount of beta-cell destruction. It is estimated from animal studies that between 80 and 90% of the beta-cells must be destroyed before the diabetes becomes clinically apparent. In humans, however, the temporal and quantitative relationships between inflammatory cells, beta-cell damage, and clinical diabetes have been difficult to determine because pancreatic biopsies are not easy to be performed. Finally, much of our information about cell-mediated immune pathogenesis and beta-cell killing comes from animal models. These animals spontaneously develop an autoimmune disease similar, although not identical, to human autoimmune Type 1 (DM).

The possible dysregulation of Regulatory T cells (Treg) suppressor activity is shown to association to Type 1 (DM) as well.

3.5. Immunoregulatory problems underlie loss to beta-cell tolerance

Regulatory CD4+ T-cells (Treg), whose development and function is dictated by the Foxp3 gene in mice and humans have the primary function of pouring a cold shower on inflammatory responses. They suppress and regulate the function of various immune responses to microbes, tumors, allergens and transplants (Sakaguchi, Setoguchi et al. 2006). It is suspected that defects in Treg number and activity are causally related to the development of Type 1 (DM). It is likely, that certain genetic predispositions, coupled with the possible contribution of external environmental factors or infections, could potentially alter regulatory T-cell function in susceptible individuals and trigger a full-scale diabetic autoimmune reaction in the pancreas.

Many studies have implicated Treg cells in the control of diabetes onset and progression, and that reduced Foxp3+ Treg cell frequencies or functions in NOD mice, represent a primary predisposing factor to diabetes. Whether thymic development of Treg cells is normal in NOD mice has been a contentious issue. Recent studies show that thymic development of Treg cells seems to be normal, as thymectomy in NOD mice up to 3 weeks of age results in exacerbated Type 1 (DM) due to a marked reduction in Treg cells (Dardenne, Lepault et al. 1989). Surprisingly, the NOD background proved superior in generating Treg cells in the thymus relative to non-autoimmune prone strain C57/BL6, suggesting that central tolerance mechanisms are intact. Furthermore, the frequency and function of single-positive Foxp3+ Treg cells in the thymus of NOD was comparable to diabetes-resistant C57/BL6 mice (Tritt, Sgouroudis et al. 2008).

It is believed that regulatory T cells may represent a kind of master switch, and by understanding how they are made, how they function and how they survive, we may be able to stop disease from occurring. The use of beta-cell specific Tregs is also leading to a tissue specific immunotolerance without perturbing the general immunocompetence. If subsequent studies show that Tregs represent a safe and efficient source for therapy, they could become an important weapon in the fight against immune mediated pathology.

4. Triggers of autoimmune cascade

A critical question, independent of the mechanism by which the immune response kills beta-cells, is what actually triggers the autoimmune cascade. Immunologic, genetic, and environmental factors have been implicated. Normally an individual's T lymphocytes are immunologically anergic or tolerant to self-antigens. T lymphocyte education and selection takes place in the thymus. T cells that do not receive a signal from an HLA-autoantigen complex die by neglect. T cells that receive a signal from an HLA-autoantigen complex that is too strong die by apoptosis. However, T cells that receive a weak, low affinity signal from an HLA-autoantigen complex are positively selected. These positively selected autoantigen-specific T cells, generally present in very low numbers, escape from the thymus and migrate to peripheral organs throughout the body including the pancreas. Under ordinary circumstances they remain dormant and are kept under strict regulatory control by still poorly defined regulatory mechanisms (*e.g.* CD4+CD25+Foxp3+ Regulatory T cells or other

regulatory pathway). If, however, these antigen-specific T cells come in contact with cognate autoantigens presented by beta-cells or APCs, through MHC-I and MHC-II respectively, in the pancreas and if the regulatory controls fail, these dormant, antigen-specific T cells will be activated and the autoimmune cascade of beta-cell killing will be initiated. Thus, immune dysregulation may serve as one of the triggers for autoimmunity. Genetic and environmental factors have been implicated as possible initiating triggers. The fact that in identical twins the concordance rate for Type 1 (DM) is less than 50% argues for genetic predisposition upon which an environmental insult is superimposed. More than 20 putative diabetes predisposing genes have been identified, but most of them have only a weak association, and in many cases the association has been difficult to confirm. The one exception is the HLA genes, which are thought to contribute as much as 50% of the genetic risk for Type 1 (DM) (Noble, Valdes et al. 2010). Although HLA genes may be necessary, by themselves they seem not to be sufficient to produce the disease. From a genetic point of view, all the evidence points to Type 1 (DM) as a complex disease involving a combination of several different genes. However, it may be that there is no specific "diabetes" gene(s). Instead, there may be the "wrong combination" of perfectly normal genes (*i.e.* alleles, haplotypes) that regulate, at the level of the beta-cell, processes such as apoptosis or antigen processing and presentation which, in turn, may trigger an autoimmune response.Various environmental triggers, e.g. certain viruses and dietary factors, are also thought to initiate the autoimmune process, leading to the destruction of pancreatic beta-cells and consequent Type 1 (DM). The major focus of the following parts is on genetic and environmental factors that predispose and triggers the autoimmune cascade.

4.1 Genetic etiology of Type 1 (DM)

To date, twelve separate chromosome regions have been implicated in the development of human Type 1 (DM). The major disease locus, *IDDM1* in the major histocompatibility complex (MHC) on chromosome 6p21, accounts for about 35% of the observed familial clustering and its contribution to disease susceptibility is likely to involve polymorphic residues of class II molecules in T-cell-mediated autoimmunity (Huber, Menconi et al. 2008). *IDDM2* is encoded by a minisatellite locus embedded in the regulatory region of the insulin gene. Familial clustering of disease can be explained by the sharing of alleles of at least 10 loci. *IDDM1* and *IDDM2* interact epistatically. For a multifactorial disease, such as Type 1 (DM), important information concerning the pathways and mechanisms involved can be gained from examining such interactions between loci, using methods that simultaneously take account of the joint effects of the various underlying genetic components.

The task of identifying susceptibility genes for complex human traits can be facilitated by first mapping susceptibility genes in an experimental species, such as the mouse or rat, and then performing mapping studies in humans by examining regions of disease in an animal model. However the animal models might not be same as the human susceptibility genes, in terms of the number of genes involved, interactions between loci and the physiological disease processes. For *IDDM*, the (NOD) mouse spontaneously develops Type1 (DM) with remarkable similarities to the human disorder. Moreover, *IDDM1,* the major genetic locus

contributing to human *IDDM* in the MHC has been shown to be conserved between the two species of human and murine. A second locus, *IDDM7* on chromosome 2q31, is homologous to a region containing the *NOD* locus, *Idd5,* on chromosome 1 (Copeman, Cucca et al. 1995). Thirdly, there is evidence that the gene region encoding the human interleukin 1 (IL-1) on chromosome 2q12-q21 is associated and linked to Type 1 (DM). This region would correspond to mouse chromosome 2, in which the region encoding IL-1 has also been linked to NOD diabetes (Serreze, Prochazka et al. 1994).

A key issue in the identification of disease susceptibility genes is that of testing association versus testing linkage. Only through demonstrating association between specific alleles at a disease locus and the disease can the causal role of particular polymorphisms in the physiological disease processes be investigated. For mapping of disease loci, however, demonstration of linkage and estimation of the recombination fraction between a disease locus and known marker loci is required. Having mapped a disease locus to a particular chromosome region, the position of the disease locus can be further localized using tests of linkage disequilibrium between particular marker alleles and the disease, which assumes a founder effect for the ancestral mutation or selection of the mutation in the study population.

The strongest genetic association for Type 1 (DM) is with the HLA class I and II genes, with a 30–50% of the genetic risk for progression of the disease. Therefor in the next section the contribution of HLA genes predisposing to Type 1 (DM) will be addressed more extensively.

4.1.1. HLA genes predisposing to Type 1 (DM)

From a genetic point of view, combinations of several different genes are involved in predisposition to Type1 (DM), and there is no specific diabetes gene(s). Human Leukocyte Antigen (HLA) genes are thought to contribute the massive part of the genetic risk for Type1 (DM). In humans, the MHC is known as the HLA complex and contains over 200 genes. It is located on chromosome 6 and encodes HLA class I and class II molecules. The main function of these molecules is to present antigens that have been processed into peptides to antigen-specific receptors on CD4 and $CD8^{+}$T lymphocytes. Class I molecules, expressed on most nucleated cells, are encoded by genes within the HLA-A, -B, and -C loci, whereas class II molecules, expressed primarily on antigen-presenting cells (*e.g.* macrophages and dendritic cells), are encoded by genes within the HLA-DP, -DQ, and -DR loci.

HLA class I and II genes are highly polymorphic and consist of many different alleles. In type 1 diabetes, certain HLA class II alleles or combinations of alleles (haplotypes) show a strong association with the development of diabetes, whereas other haplotypes show a weak or even protective association. It is well established that the HLA-DR3 and HLA-DR4 genes at the HLA-DR locus on chromosome 6 are strongly associated with increased susceptibility to insulin-dependent diabetes (Field and McArthur 1987), and that the predisposition is greatest among individuals who possess both of these genes (HLA-DR3/4 heterozygotes). Furthermore, individuals with the HLA haplotype DRB1*0302- DQA1*0301, especially when

combined with DRB1*0201- DQA1*0501, are highly susceptible (10–20-fold increase) to Type1 (DM) (Pociot and McDermott 2002). In contrast, individuals with the haplotype DRB1*0602-DQA1*0102 rarely develop type 1 diabetes. Many other high and low risk haplotypes have been identified, and the frequency of specific haplotypes differs among ethnic groups (Pociot and McDermott 2002). Other genes within the HLA complex, particularly class I genes, also have been linked to type 1 diabetes, but the strongest linkage by far is with the DQ and DR class II genes.

Experimental support for the importance of class II genes in the development of diabetes comes from a variety of sources including the deletion of specific MHC loci in mice and their replacement with human HLA homologs. Although the linkage of HLA class II molecules with Type1 (DM) is now well established and the binding of peptides to pockets within the groove of the HLA class II molecule understood, why the binding of peptides to certain HLA class II molecules, and not to others, is associated with autoimmune Type 1 (DM) remains unresolved (concerning the fact that CD8+ T cell have the largest contribution in the pathogenesis of Type 1 (DM)). Regardless of mechanism, HLA typing has proved useful in population screening for identification and follow-up of individuals at high risk for disease.

4.1.2. Non-HLA genes contributing to Type 1 (DM)

Multiple studies have recently linked Type 1 (DM) to 50 non-HLA gene polymorphisms (Pociot and McDermott 2002). Major efforts have therefore been made to identify non-HLA genetic risk factors for Type 1 (DM). Interestingly enough, many of the genetic factors are important to the function of the immune system. For instance *PTPN22* is a regulator of T-cell function and a genetic polymorphism results in a phosphatase variant that is increasing the risk not only for Type 1 (DM) but also for rheumatoid arthritis, juvenile rheumatoid arthritis, systemic lupus erythematosus, Graves' disease, generalized vitiligo, and other human autoimmune diseases (Herr, Dudbridge et al. 2000). The *PTPN22* polymorphism seems in particular to affect progression from pre-diabetes to clinical disease (Herr, Dudbridge et al. 2000) also in individuals with lower risk HLA genotypes. The variable nucleotide tandem repeat in the promoter region of the insulin gene *INS VNTR* seems to contribute to Type 1(DM) by the mechanisms of central tolerance (Takase, Yu et al. 2005). In newly diagnosed Type 1 (DM) patients the presence of insulin autoantibodies is associated with the *INS VNTR* polymorphism (Takase, Yu et al. 2005).

The majority of the non-HLA genetic factors seem to be associated with the immune system. We have also demonstrated the association of the polymorphism of Th1 type cytokines (IL-12, IL-18) as well as TGF-β in human patients with Type 1 (DM). It is therefore attractive to speculate that their contribution is related to the ability of the immune system to mount an autoimmune reaction specifically directed toward the islet beta-cells.

The existence of genes predisposing to Type 1 (DM) in the region of the insulin (INS) gene now also established. Association analysis has demonstrated an increased frequency of class 1 alleles of the 5' INS polymorphism in diabetics compared with controls. Interestingly, the

effect of INS region susceptibility on Type 1 (DM) cannot be detected by linkage analysis, suggesting that if a genetic marker locus is close to a disease susceptibility locus, association analysis may be a more sensitive method than linkage analysis for detecting the susceptibility locus.

4.2. Potential environmental triggers of beta-cell autoimmunity in Type 1 (DM)

The clinical presentation of type 1 diabetes is preceded by an asymptomatic period of highly variable duration. Aggressive beta-cell destruction may lead to disease manifestation within a few months in infants and young children, whereas in other individuals, the process may continue for years (in some cases, even for >10 years) before the eventual presentation of overt disease.

Several lines of evidence support a critical role of exogenous factors in the pathogenesis of Type 1 (DM). Studies in monozygotic twins indicate that only 13–33% are pairwise concordant for Type 1 (DM) (Barnett, Eff et al. 1981), suggesting that there is either acquired postconceptional genetic discordance or differential exposure to the putative environmental factor(s). The geographic variation in the incidence of Type 1 (DM) in children is conspicuous. This difference in incidence can hardly be explained by genetic factors. A substantial increase in the incidence of Type 1 (DM) among children has been documented over the last decades, particularly in Europe—for example, in Finland, the incidence has increased 4.5-fold from the early 1950s (Gale 2002). Such an increase cannot be the consequence only of enhanced genetic disease susceptibility in the population but must mostly be due to changes in lifestyle and environment. As well, available data indicate that the incidence of Type 1 (DM) has increased in population groups who have moved from a low-incidence region to a high-incidence area, emphasizing the influence of environmental conditions (Akerblom and Knip 1998). Accumulating evidence suggests that the proportion of subjects with high-risk HLA genotypes has decreased over the last decades among patients with newly diagnosed type 1 diabetes, whereas the proportion of people with low-risk or even protective HLA genotypes has increased (Resic-Lindehammer, Larsson et al. 2008). These data are compatible with an increased environmental pressure resulting in progression to clinical diabetes with less genetic susceptibility.

As mentioned earlier, the first signs of beta-cell autoimmunity and the autoantibodies may appear very early in life. Many studies have revealed that there is an unequivocal temporal variation in the appearance of the diabetes-associated autoantibodies reflecting the initiation of the disease process and paralleling the seasonal variation. Most initial autoantibodies appear during the cold period in the fall and winter but rarely in the spring or in the summer. There also seems to be some variation from one year to another in the timing and height of the autoantibody peaks.

The pattern of the autoantibody appearance strongly points to the role of infectious agents with conspicuous seasonal variation as triggers of beta-cell autoimmunity. Such variations are typical for viral infections, and the pattern of laboratory-confirmed enterovirus infections. In addition to viral infections, one should also consider other environmental variables with

seasonal variation. There is definitely seasonal variation in the amount of daylight and sunshine hours, especially in Northern Europe, which has the highest incidence of Type 1 (DM) in the world (Moltchanova, Penttinen et al. 2005). Without oral substitution, the sunlight-dependent synthesis of vitamin D in the skin is the most important source of this immunologically active hormone. Some studies have indicated that the lack of oral vitamin D substitution in infancy increases the subsequent risk of type 1 diabetes. Also improved insulin sensitivity in the spring and summer because of more physical exercise can be taken into account as well. Improved insulin sensitivity diminishes beta-cell stress, as the workload on the beta-cells decreases. However, it is unlikely that there should be substantial seasonal variation in physical exercise in very young children, the target group in whom the seasonal variation in the appearance of the first diabetes-associated autoantibodies has been observed.

Accordingly, we are left with viral infections as the most likely explanation for the seasonal variation in the emergence of the first signs of beta-cell autoimmunity. Taken into account the timing and profiles of the autoantibody peaks, such as enterovirus infections, appear to be the most probable trigger of beta-cell autoimmunity. The frequency of enterovirus infections has decreased over the last decades in the background population in developed countries, e.g., in Finland and Sweden (Viskari, Ludvigsson et al. 2005). Despite that, these countries have a high and increasing incidence of Type 1 (DM) among children. This appears to be paradoxical. The paradox can, however, be explained by the so-called "polio hypothesis" introduced by Viskari et al (Viskari, Koskela et al. 2000). The polioviruses comprise three serotypes among >60 enterovirus serotypes. When the frequency of acute poliovirus infections started to decrease at the beginning of the last century among the general population in countries with an increasing standard of hygiene, the incidence of paralytic polio being a complication of the acute infection began to increase. This was obviously the consequence of decreased levels of protective maternal poliovirus antibodies transferred transplacentally and through breast milk to the infant, leading to a situation where the risk increased that the infant would get his or her first poliovirus infection at the time of no maternal protection. Similarly, the decreasing frequency of enterovirus infections in the background population would increase the susceptibility of young children to the diabetogenic effect of enteroviruses. The same phenomenon may also contribute to the marked international variation in Type 1 (DM) incidence, because enterovirus infections seem to be rare in countries where the rate of type 1 diabetes is high.

The tropism phenomenon (the characteristic of a virus to infect a particular tissue or cell type), in which the attachment of the virus to the viral receptors on the cell surface together with other interactions with cellular proteins is a central feature, is thought to explain why some variants of enteroviruses may be diabetogenic and others not. It has been proposed that pancreatic beta-cell tropic variants of the coxsackie B virus are present in the general population and that they are able to induce beta-cell damage in susceptible individuals. In vitro studies have shown that enteroviruses infect beta-cells easily and induce functional impairment and cell death (Roivainen, Rasilainen et al. 2000).

Taken together, accumulated data support the hypothesis that a diabetogenic enterovirus infection is the likely trigger of beta-cell autoimmunity. This is supported by the observed

temporal variation in the appearance of the first diabetes-associated autoantibodies in young children, the profile of which resembles the temporal profile of enterovirus infections in the background population.

Some other viruses, such as encephalomyocarditis virus, act directly by replicating in and destroying pancreatic beta-cells. A single amino acid substitution in the virus, presumably by altering its binding to beta-cells, determines whether or not diabetes develops. The Kilham rat virus, on the other hand, produces diabetes not by infecting beta-cells but by altering the immunoregulatory network of the host (Herr, Dudbridge et al. 2000). Still other viruses are thought to initiate or accelerate the autoimmune response through molecular mimicry (Copeman, Cucca et al. 1995) or by releasing sequestrated autoantigens from damaged beta-cells. In a transgenic autoimmune model (Serreze, Prochazka et al. 1994), the administration of infectious lymphocytic choriomeningitis virus (LCM) to transgenic animals expressing LCM viral proteins in their beta-cells results in diabetes, but the same LCM virus does not produce diabetes in non-transgenic animals.

A recent study has also found evidence of Coxsackie virus infection in beta-cells in three out of six pancreases of patients with recent-onset Type 1 (DM). Coxsackie viruses are known to induce interferon alpha secretion by beta-cells and this could initiate the sequence of events that culminates in their autoimmune destruction.

In summary of this part, Type 1 (DM) may be triggered by an environmental culprit at any age, although a majority of the processes appear to start early in childhood. Viruses have been the leading candidates. In animal experiments viruses have been shown to produce diabetes as well. In humans, case reports and sero-epidemiologic studies (Herr, Dudbridge et al. 2000) suggest that viruses, particularly enteroviruses, may play a role, but most likely as a cofactor, in individuals who already have suffered some autoimmune beta-cell loss. However, for the vast majority of the cases of Type 1 (DM) in humans, a viral cause has not been established.

The identification of exogenous factors triggering and driving beta-cell destruction offers potential means for intervention aimed at the prevention of Type 1 (DM). Therefore, it is important to pursue studies on the role of environmental factors in the pathogenesis of this disease. Environmental modification is likely to offer the most powerful strategy for effective prevention of Type 1 (DM), since such an approach can target the whole population or at least that proportion of the population carrying increased genetic disease susceptibility and would therefore prevent both sporadic and familial type 1 diabetes if successful.

5. Therapeutic interventions

Before the isolation of insulin in the 1920s, most patients died within a short time after onset of Type 1 (DM). Untreated diabetes leads to ketoacidosis, the accumulation of ketones (products of fat breakdown) and acid in the blood. Continued buildup of these products of disordered carbohydrate and fat metabolism result in nausea and vomiting, and eventually the patient goes into a diabetic coma.

Treatment for diabetes mellitus is aimed at reducing blood glucose concentrations to normal levels. Achieving this is important in promoting well-being and in minimizing the development and progression of the long-term complications of diabetes. Despite the widespread use of exogenous insulin, morbidity and mortality caused by Type 1 (DM) continue to place a significant burden on society, both in terms of human suffering and cost. The care of diabetes on self-management is based on the patient's clinical status and his/her ability to participate in self-care. Insulin replacement therapy is the mainstay for patients with Type 1 (DM) while diet and lifestyle modifications are also crucial for the treatment and management of this disease.

Diabetics who are unable to produce insulin in their bodies receive regular injections of the insulin, which are often customized according to their individual and variable requirements. Beef or pork insulin, made from the pancreatic extracts of cattle or pigs, can be used to treat humans with diabetes. However, in the United States, beef and pork forms of insulin are no longer manufactured, having been discontinued in favor of human insulin production. Modern human insulin treatments are based on recombinant DNA technology. Human insulin may be given as a form that is identical to the natural form found in the body, which acts quickly but transiently, or as a form that has been biochemically modified so as to prolong its action for up to 24 hours. The optimal regimen of insulin administration is one that most closely mimics the normal pattern of insulin secretion, which is a constant low level of insulin secretion plus a pulse of secretion after each meal. This can be achieved by administration of a long-acting insulin preparation once daily plus administration of a rapid-acting insulin preparation with or just before each meal. Patients also have the option of using an insulin pump, which allows them to control variations in the rate of insulin administration. A satisfactory compromise for some patients is twice-daily administration of mixtures of intermediate-acting and short-acting insulin. Patients taking insulin also may need to vary food intake from meal to meal, according to their level of activity; as exercise frequency and intensity increase, less insulin and more food intake may be necessary.

There are also several classes of oral drugs used to control blood glucose levels, including sulfonylureas, biguanides, and thiazolidinediones. Sulfonylureas, such as glipizide and glimepiride, are considered hypoglycemic agents because they stimulate the release of insulin from beta-cells in the pancreas, thus reducing blood glucose levels (Pernet, Trimble et al. 1985). The most common side effect associated with sulfonylureas is hypoglycemia (abnormally low blood glucose levels), which occurs most often in elderly patients who have impaired liver or kidney function.

Biguanides, of which metformin is the primary member, are considered antihyperglycemic agents because they work by decreasing the production of glucose in the liver and by increasing the action of insulin on muscle and adipose tissues (Spaans, Kleefstra et al. 2011). A potentially fatal side effect of metformin is the accumulation of lactic acid in blood and tissues, often causing vague symptoms such as nausea and weakness.

Thiazolidinediones, such as rosiglitazone and pioglitazone, act by reducing insulin resistance of muscle and adipose cells and by increasing glucose transport into these tissues

(Garg 2011). These agents can cause edema (fluid accumulation in tissues), liver toxicity, and adverse cardiovascular events in certain patients. Furthermore, oral hypoglycemic agents lower mean blood glucose concentrations by only about 50–80 mg per 100 ml (2.8–4.4 mmol/l), and sensitivity to these drugs tends to decrease with time(Garg 2011).

There are several other agents that can be highly effective in the treatment of diabetes. Pramlintide is an injectable synthetic hormone (based on the human hormone amylin) that regulates blood glucose levels by slowing the absorption of food in the stomach and by inhibiting glucagon, which normally stimulates liver glucose production(Riddle and Drucker 2006). Exenatide is an injectable antihyperglycemic drug that works similarly to incretins, or gastrointestinal hormones, such as gastric inhibitory polypeptide, that stimulate insulin release from the pancreas. Exenatide has a longer duration of action than incretins produced by the body because it is less susceptible to degradation by an enzyme called dipeptidyl peptidase-4 (DPP-4)(Sena, Nunes et al. 2008). A drug called sitagliptin specifically inhibits DPP-4, thereby increasing levels of naturally produced incretins. Side effects associated with these drugs are often mild, although pramlintide can cause profound hypoglycemia in patients with Type 1 (DM).

All patients with diabetes mellitus, particularly those taking insulin, should measure blood glucose concentrations periodically at home, especially when they have symptoms of hypoglycemia. Diet and lifestyle strategies also are required to reduce weight, improve glycaemic control and reduce the risk of cardiovascular complications, which account for 70% to 80% of deaths among those with diabetes.

Research into other areas of insulin therapy includes pancreas transplantation, beta-cell transplantation, and generation of beta-cells from existing exocrine cells in the pancreas. Patients with Type I (DM) have been treated by transplantation of the pancreas or of the islets of Langerhans. However, limited quantities of pancreatic tissue are available for transplantation, prolonged immunosuppressive therapy is needed, and there is a high likelihood that the transplanted tissue be rejected even when the patient is receiving immunosuppressive therapy. Attempts to improve the outcome of transplantation and to develop mechanical islets are ongoing.

Whole pancreas or islet transplantation is another treatment continues to develop and reduced the need for insulin, achieve better glucose stability, and reduce problems with hypoglycemia. The transplantation of vascularized pancreas, from a deceased donor, developed in the 1960s and usually performed concurrently with renal transplantation, can cure Type 1 (DM), as shown by results in more than 15,000 such transplants over about 30 years. Transplantation of isolated pancreatic islets, instead of the whole organ, however, offers an attractive alternative that minimizes surgery and its complications. Although islet transplantation initially met with only modest success, recent changes in patient selection criteria, number and treatment of islets transplanted, and better immunosuppressive regimens dramatically improved the results. The development of clinical islet transplantation was driven by an unmet medical need within the diabetes mellitus patient population and was preceded by the introduction of transplantation of whole, vascularized

pancreas. Despite this promise, organ/islet availability remains an important limitation to this technology. A solution to the problem of limited materials for transplantation may be in the use of stem/progenitor cells.

The presence of beta-cells in patients with long-standing Type 1 (DM), despite ongoing autoimmunity, implies that new formation of beta-cells may be occurring. Although an ambitious aim currently targeted regeneration of such beta-cells offers another strategy to prevent Type 1 (DM). Regeneration of beta-cells is therefore an area of major active investigation, with recent studies reporting differentiation of pancreatic and nonpancreatic progenitors as well as replication of existing islet beta-cells. In this regard some studies have shown that pre-existing beta-cells, rather than pluripotent stem cells, are the main source of new beta-cells during adult life and after pancreatectomy in mice.

6. Cellular and molecular strategy for inhibition of the initiation and progression of beta-cell destruction

Mononuclear cell infiltration into the islets of the pancreas (insulitis) is characteristic of autoimmune diabetes. T lymphocytes are the predominant subpopulation seen in insulitis, and are involved in the autoimmune process. Insulin-producing beta-cells are thought to be destroyed by cytotoxic T cells, cytokines or nitric oxide, and beta-cell death occurs, at least partly, via apoptosis. Beta-cell death induced by inflammatory cytokines can be inhibited by forced expression of Bcl-2 (*B-cell lymphoma 2,* thought to implicate in cell growth and survival) in those cells, suggesting its potential as a tool for gene therapy (Iwahashi, Itoh et al. 1998). The Fas/Fas-ligand system plays a critical role in inducing insulitis and overt diabetes in (NOD) mice as well. Beta-cells are destroyed by apoptosis through Fas-Fas ligand and TNF-TNF receptor interactions and by granzymes and perforin released from cytotoxic effector T cells. Therefore, the activated macrophages and T cells, and cytokines secreted from these immunocytes, act synergistically to destroy beta-cells, resulting in the development of Type 1 (DM). Preventive strategies might be developed by focusing on these molecules involved in beta-cell destruction.

The feasibility of gene therapy in NOD mice by *ex vivo* genetic manipulation of normal hematopoietic stem cells (HSCs) with proinsulin II followed by transfer to recipient mice has been examined as an approach to treat T1D recently (10). The incidence and degree of insulitis was significantly reduced in recipient, and thus this molecular chimerism can potentially protect from destructive insulitis in an antigen-specific manner (10).

In some studies the anti–T cell strategies were also examined to inhibit insulitis. Early studies of cyclosporin in the 1980s provided a proof of principal for the usefulness of immunomodulators in the treatment of Type 1 (DM); the adverse effects of cyclosporin, however, were incompatible with their widespread use. More sophisticated anti–T-cell strategies have been developed more recently. In the hOKT3γ1(Ala-Ala) trial, a humanized, modified anti-CD3 monoclonal antibody, analysis of peripheral blood samples demonstrated an increase in the CD8/CD4 ratio and in particular an increase in CD4+CD25+

regulatory T cells (36). Most studies of regulatory T cells have focused on a subset of naturally occurring CD4+ cells that have the capacity to control self-reactive T cells, and their depletion results in autoimmunity. Strategies that target the action of regulatory T cells *in vivo* offer one of the most attractive options for therapy in Type 1 (DM). The establishment of screening techniques for detecting prediabetic patients is also necessary to allow successful intervention.

Immunosuppressive drugs and anti-T cell antibodies have shown varying degrees of success in suppression of beta-cell autoimmunity in NOD mice. However, these strategies require repeated drug administration and may cause nonspecific harmful effects such as interference with normal immune system functions. A new therapeutic approach for type 1 diabetes is based on prevention of beta-cell loss through vaccine restoration of normal immune system function. Traditionally, vaccination refers to prevention of an infectious disease by exposing the immune system to a weakened or dead infectious agent. Alternatively, "inverse vaccination" (the inhibition of an immune response) arrests autoimmunity through manipulation of the innate and adaptive arms of the immune system (Steinman 2010). In type 1 diabetes, vaccination with β-cell autoantigens was shown to induce a partial state of immunological tolerance in NOD mice (Peakman and von Herrath 2010). Beta-cell self-antigens can induce tolerance through three possible mechanisms: (1) induction of T cell deletion/anergy, (2) induction of anti-inflammatory T helper 2 (Th2) cells, and (3) stimulation of regulatory T cell proliferation (Maldonado and von Andrian 2010). Insulin, GAD and some heat shock proteins are considered to be the first pancreatic autoantigens detected early during diabetes onset in both humans and NOD mice. However, the primary beta-cell antigen responsible for triggering autoimmunity in Type 1 (DM) remains under dispute. Thus, several pancreatic autoantigens have been selected for development into type 1 diabetes vaccines. These results suggest that multi-component vaccine strategies are promising for prevention and reversal of diabetes autoimmunity in humans, although some antigens determined to be most immunogenic, and not successful in trials.

We are still some way from developing a pill to prevent Type 1 (DM), but all the divergent strands of ongoing research, from epidemiology to molecular biology, immunology to clinical trials, appear to be converging to provide clear perspectives on the therapeutic interventions that are most likely to be successful. Two strategies are open to physicians who have patients with Type 1 (DM): the first is to prevent initiation of autoimmunity; the second is to reverse the effects of ongoing autoimmunity coupled with beta-cell regeneration. Although highly ambitious, the prevention of Type 1 (DM) could be possible by identifying and eliminating environmental risk factors. The next line of defense would be to re-educate the immune system through exposure to beta-cell antigens with the use of oral or nasal tolerance strategies. The observation that insulin may be the primary autoantigen provides support for therapies using insulin to induce tolerance. The potential to re-educate the immune system, or to divert it using regulatory T cells, and the rapidly expanding field of islet beta-cell differentiation give hope that improved strategies to manage this chronic disease are on the horizon.

7. Concluding remarks

Type 1 (DM), formerly known as juvenile diabetes is a complex disease caused by multiple environmental and genetic risk factors. It is a T cell-mediated disease characterized by the destruction of the endocrine insulin-producing beta-cells of the pancreatic islets, resulting in plasma glucose dysregulation, persistent hyperglycemia and long-term complications. Evidence for a constant global increase of incidents is worrisome. There is a major international challenge for optimal intervention and prevention strategies. Thus, a better understanding of the events going on in the autoimmune processes and understanding the relative contribution of genetics and environmental factors is necessary for the ultimate prevention/treatment.

Research is ongoing to discover the exact cause of Type 1 (DM), which remains unknown. Eepidemiological studies support the notion that viral infections play a causative role in Type 1 (DM). Indeed, there is a strong association between certain HLA and non-HLA alleles or combinations of alleles that predispose to development of Type 1 (DM). HLA typing is one means in screening to identify individuals at high risk as well.

Based on experimental results from studies using NOD mice and BB rats over the past 3 decades, the possible interactions between beta-cell autoantigens and immunocytes such as macrophages, dendritic cells, T cells, and their secretory products in connection with MHC class I and II molecules has shown in this autoimmune disease. The animal models may not encompass all aspects of the pathogenic mechanisms involved in autoimmune diabetes in humans; nevertheless, it may provide helpful information with respect to the synergistic destruction of beta-cells by immunocytes and their cytokines and a basis for the formation of new hypotheses for further investigation.

Although rarer than type 2, Type 1 (DM) is more severe, and constitutes the fourth or fifth leading cause of death worldwide. There is currently no cure or preventative measure for Type 1 (DM). Patients are dependent for the rest of their lives on regular injections of insulin to control their blood sugar levels. Combined with some conservative lifestyle choices, insulin lets people manage their diabetes, but the control of blood sugar is never perfect. In the long term, tolerogenic, antigen-specific and beta-cell-specific regenerative agents could provide a promising platform for the development of disease-modifying therapies. Thus, combination therapies could be most effective in delivering the long-sought cure.

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8. References

Akerblom, H. K. and M. Knip (1998). "Putative environmental factors in Type 1 diabetes." *Diabetes/metabolism reviews* 14(1): 31-67.

Atkinson, M. A. and N. K. Maclaren (1994). "The pathogenesis of insulin-dependent diabetes mellitus." *The New England journal of medicine* 331(21): 1428-1436.

Bakker, S. F., M. E. Tushuizen, et al. (2012). "Type 1 diabetes and celiac disease in adults: glycemic control and diabetic complications." *Acta diabetologica*.

Barnett, A. H., C. Eff, et al. (1981). "Diabetes in identical twins. A study of 200 pairs." *Diabetologia* 20(2): 87-93.

Bearse, M. A., Jr., Y. Han, et al. (2004). "Local multifocal oscillatory potential abnormalities in diabetes and early diabetic retinopathy." *Investigative ophthalmology & visual science* 45(9): 3259-3265.

Bergerot, I., N. Fabien, et al. (1994). "Oral administration of human insulin to NOD mice generates CD4+ T cells that suppress adoptive transfer of diabetes." *Journal of autoimmunity* 7(5): 655-663.

Betterle, C., F. Zanette, et al. (1984). "Clinical and subclinical organ-specific autoimmune manifestations in type 1 (insulin-dependent) diabetic patients and their first-degree relatives." *Diabetologia* 26(6): 431-436.

Cecil, R. L. (1909). "A Study of the Pathological Anatomy of the Pancreas in Ninety Cases of Diabetes Mellitus." *The Journal of experimental medicine* 11(2): 266-290.

Copeman, J. B., F. Cucca, et al. (1995). "Linkage disequilibrium mapping of a type 1 diabetes susceptibility gene (IDDM7) to chromosome 2q31-q33." *Nature genetics* 9(1): 80-85.

Dardenne, M., F. Lepault, et al. (1989). "Acceleration of the onset of diabetes in NOD mice by thymectomy at weaning." *European journal of immunology* 19(5): 889-895.

Field, L. L. and R. G. McArthur (1987). "The genetics of susceptibility to insulin-dependent diabetes mellitus--possible new markers." *Clinical and investigative medicine. Medecine clinique et experimentale* 10(5): 437-443.

Foulis, A. K., C. N. Liddle, et al. (1986). "The histopathology of the pancreas in type 1 (insulin-dependent) diabetes mellitus: a 25-year review of deaths in patients under 20 years of age in the United Kingdom." *Diabetologia* 29(5): 267-274.

French, M. B., J. Allison, et al. (1997). "Transgenic expression of mouse proinsulin II prevents diabetes in nonobese diabetic mice." *Diabetes* 46(1): 34-39.

Gale, E. A. (2002). "The rise of childhood type 1 diabetes in the 20th century." *Diabetes* 51(12): 3353-3361.

Garg, V. (2011). "Noninsulin pharmacological management of type 1 diabetes mellitus." *Indian journal of endocrinology and metabolism* 15(Suppl 1): S5-S11.

Genuth, S., K. G. Alberti, et al. (2003). "Follow-up report on the diagnosis of diabetes mellitus." *Diabetes care* 26(11): 3160-3167.

Gepts, W. (1965). "Pathologic anatomy of the pancreas in juvenile diabetes mellitus." *Diabetes* 14(10): 619-633.

Harrison, L. C., M. Dempsey-Collier, et al. (1996). "Aerosol insulin induces regulatory CD8 gamma delta T cells that prevent murine insulin-dependent diabetes." *The Journal of experimental medicine* 184(6): 2167-2174.

Herr, M., F. Dudbridge, et al. (2000). "Evaluation of fine mapping strategies for a multifactorial disease locus: systematic linkage and association analysis of IDDM1 in the HLA region on chromosome 6p21." *Human molecular genetics* 9(9): 1291-1301.

Huang, C., Y. Kim, et al. (2002). "Cellular basis of diabetic nephropathy: II. The transforming growth factor-beta system and diabetic nephropathy lesions in type 1 diabetes." *Diabetes* 51(12): 3577-3581.

Huber, A., F. Menconi, et al. (2008). "Joint genetic susceptibility to type 1 diabetes and autoimmune thyroiditis: from epidemiology to mechanisms." *Endocrine reviews* 29(6): 697-725.

Hutchings, P. and A. Cooke (1998). "Protection from insulin dependent diabetes mellitus afforded by insulin antigens in incomplete Freund's adjuvant depends on route of administration." *Journal of autoimmunity* 11(2): 127-130.

In't Veld, P. (2011). "Insulitis in human type 1 diabetes: The quest for an elusive lesion." *Islets* 3(4): 131-138.

Iwahashi, H., N. Itoh, et al. (1998). "Molecular mechanisms of pancreatic beta-cell destruction in autoimmune diabetes: potential targets for preventive therapy." *Cytokines, cellular & molecular therapy* 4(1): 45-51.

Karlsen, A. E., W. A. Hagopian, et al. (1991). "Cloning and primary structure of a human islet isoform of glutamic acid decarboxylase from chromosome 10." *Proceedings of the National Academy of Sciences of the United States of America* 88(19): 8337-8341.

Katavetin, P. (2009). "Renal and retinal effects of enalapril and losartan in type 1 diabetes." *The New England journal of medicine* 361(14): 1410-1411; author reply 1411.

Kent, S. C., Y. Chen, et al. (2005). "Expanded T cells from pancreatic lymph nodes of type 1 diabetic subjects recognize an insulin epitope." *Nature* 435(7039): 224-228.

Lahiri, M. K., P. J. Kannankeril, et al. (2008). "Assessment of autonomic function in cardiovascular disease: physiological basis and prognostic implications." *Journal of the American College of Cardiology* 51(18): 1725-1733.

Lecompte, P. M. (1958). "Insulitis in early juvenile diabetes." *A.M.A. archives of pathology* 66(4): 450-457.

Maldonado, R. A. and U. H. von Andrian (2010). "How tolerogenic dendritic cells induce regulatory T cells." *Advances in immunology* 108: 111-165.

Maser, R. E. and M. J. Lenhard (2005). "Cardiovascular autonomic neuropathy due to diabetes mellitus: clinical manifestations, consequences, and treatment." *The Journal of clinical endocrinology and metabolism* 90(10): 5896-5903.

Moltchanova, E., A. Penttinen, et al. (2005). "A hierarchical Bayesian birth cohort analysis from incomplete registry data: evaluating the trends in the age of onset of insulin-dependent diabetes mellitus (T1DM)." *Statistics in medicine* 24(19): 2989-3004.

Nagler, W. and H. Taylor (1963). "Diabetic coma with acute inflammation of Islets of Langerhans." *JAMA : the journal of the American Medical Association* 184: 723-725.

Nakayama, M., N. Abiru, et al. (2005). "Prime role for an insulin epitope in the development of type 1 diabetes in NOD mice." *Nature* 435(7039): 220-223.

Noble, J. A., A. M. Valdes, et al. (2010). "HLA class I and genetic susceptibility to type 1 diabetes: results from the Type 1 Diabetes Genetics Consortium." *Diabetes* 59(11): 2972-2979.

Owerbach, D. and K. H. Gabbay (1996). "The search for IDDM susceptibility genes: the next generation." *Diabetes* 45(5): 544-551.

Peakman, M. and M. von Herrath (2010). "Antigen-specific immunotherapy for type 1 diabetes: maximizing the potential." *Diabetes* 59(9): 2087-2093.

Pernet, A., E. R. Trimble, et al. (1985). "Sulfonylureas in insulin-dependent (type I) diabetes: evidence for an extrapancreatic effect in vivo." *The Journal of clinical endocrinology and metabolism* 61(2): 247-251.

Pharoah, P. D., A. M. Dunning, et al. (2004). "Association studies for finding cancer-susceptibility genetic variants." *Nature reviews. Cancer* 4(11): 850-860.

Pociot, F. and M. F. McDermott (2002). "Genetics of type 1 diabetes mellitus." *Genes and immunity* 3(5): 235-249.

Pugliese, A., D. Brown, et al. (2001). "Self-antigen-presenting cells expressing diabetes-associated autoantigens exist in both thymus and peripheral lymphoid organs." *The Journal of clinical investigation* 107(5): 555-564.

Rasche, S., R. Y. Busick, et al. (2009). "GAD65-Specific Cytotoxic T Lymphocytes Mediate Beta-Cell Death and Loss of Function." *The review of diabetic studies : RDS* 6(1): 43-53.

Resic-Lindehammer, S., K. Larsson, et al. (2008). "Temporal trends of HLA genotype frequencies of type 1 diabetes patients in Sweden from 1986 to 2005 suggest altered risk." *Acta diabetologica* 45(4): 231-235.

Riddle, M. C. and D. J. Drucker (2006). "Emerging therapies mimicking the effects of amylin and glucagon-like peptide 1." *Diabetes care* 29(2): 435-449.

Roivainen, M., S. Rasilainen, et al. (2000). "Mechanisms of coxsackievirus-induced damage to human pancreatic beta-cells." *The Journal of clinical endocrinology and metabolism* 85(1): 432-440.

Saely, C. H., S. Aczel, et al. (2004). "Cardiovascular complications in Type 2 diabetes mellitus depend on the coronary angiographic state rather than on the diabetic state." *Diabetologia* 47(1): 145-146.

Sakaguchi, S., R. Setoguchi, et al. (2006). "Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in self-tolerance and autoimmune disease." *Current topics in microbiology and immunology* 305: 51-66.

Seki, M., T. Tanaka, et al. (2004). "Involvement of brain-derived neurotrophic factor in early retinal neuropathy of streptozotocin-induced diabetes in rats: therapeutic potential of brain-derived neurotrophic factor for dopaminergic amacrine cells." *Diabetes* 53(9): 2412-2419.

Sena, C. M., E. Nunes, et al. (2008). "Supplementation of coenzyme Q10 and alpha-tocopherol lowers glycated hemoglobin level and lipid peroxidation in pancreas of diabetic rats." *Nutrition research* 28(2): 113-121.

Serreze, D. V., M. Prochazka, et al. (1994). "Use of recombinant congenic and congenic strains of NOD mice to identify a new insulin-dependent diabetes resistance gene." *The Journal of experimental medicine* 180(4): 1553-1558.

Spaans, E. A., N. Kleefstra, et al. (2011). "[Metformin in adolescents and adults with type 1 diabetes mellitus: not evidence-based]." *Nederlands tijdschrift voor geneeskunde* 155(39): A3166.

Steinman, L. (2010). "Inverse vaccination, the opposite of Jenner's concept, for therapy of autoimmunity." *Journal of internal medicine* 267(5): 441-451.

Svensson, M., J. W. Eriksson, et al. (2004). "Early glycemic control, age at onset, and development of microvascular complications in childhood-onset type 1 diabetes: a population-based study in northern Sweden." *Diabetes care* 27(4): 955-962.

Takase, H., C. R. Yu, et al. (2005). "Thymic expression of peripheral tissue antigens in humans: a remarkable variability among individuals." *International immunology* 17(8): 1131-1140.

Toyoda, H. and B. Formby (1998). "Contribution of T cells to the development of autoimmune diabetes in the NOD mouse model." *BioEssays : news and reviews in molecular, cellular and developmental biology* 20(9): 750-757.

Tritt, M., E. Sgouroudis, et al. (2008). "Functional waning of naturally occurring CD4+ regulatory T-cells contributes to the onset of autoimmune diabetes." *Diabetes* 57(1): 113-123.

Viskari, H., J. Ludvigsson, et al. (2005). "Relationship between the incidence of type 1 diabetes and maternal enterovirus antibodies: time trends and geographical variation." *Diabetologia* 48(7): 1280-1287.

Viskari, H. R., P. Koskela, et al. (2000). "Can enterovirus infections explain the increasing incidence of type 1 diabetes?" *Diabetes care* 23(3): 414-416.

Wajchenberg, B. L. (2007). "beta-cell failure in diabetes and preservation by clinical treatment." *Endocrine reviews* 28(2): 187-218.

Wallace, C., G. E. Reiber, et al. (2002). "Incidence of falls, risk factors for falls, and fall-related fractures in individuals with diabetes and a prior foot ulcer." *Diabetes care* 25(11): 1983-1986.

Wan, X., C. Yang, et al. (2010). "BOOST: A fast approach to detecting gene-gene interactions in genome-wide case-control studies." *American journal of human genetics* 87(3): 325-340.

Willcox, A., S. J. Richardson, et al. (2009). "Analysis of islet inflammation in human type 1 diabetes." *Clinical and experimental immunology* 155(2): 173-181.

Yoon, J. W. and H. S. Jun (2005). "Autoimmune destruction of pancreatic beta cells." *American journal of therapeutics* 12(6): 580-591.

Ziegler, D., C. G. Sohr, et al. (2004). "Oxidative stress and antioxidant defense in relation to the severity of diabetic polyneuropathy and cardiovascular autonomic neuropathy." *Diabetes care* 27(9): 2178-2183.

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