

Understanding the Immunology of Type 1 Diabetes— An Overview of Current Knowledge and Perspectives for the Future

Anil Piya, MD¹ and Aaron W Michels, MD²

1. Pediatric Endocrinology Fellow; 2. Assistant Professor of Pediatrics and Medicine, Barbara Davis Center for Childhood Diabetes, University of Colorado Denver, Aurora, Colorado, US

Abstract

Type 1 diabetes is a chronic autoimmune disease resulting from the immune destruction of insulin-producing β -cells in pancreatic islets. It is now a predictable disease in humans with the measurement of islet autoantibodies. Despite the ability to assess disease risk, there is no cure for type 1 diabetes and treatment requires lifelong insulin administration. Individuals with type 1 diabetes are at risk of long-term complications of the disease and the development of concomitant autoimmune disorders. Our understanding of the immunology of diabetes has increased greatly over the last decade at a basic science level, with translation to type 1 diabetes patients. Therapies are emerging to prevent beta cell destruction in these patients. This article centers around our current understanding of the immunology of type 1 diabetes, with a focus on immune intervention for the prevention and ultimate cure of the disease.

Keywords

Type 1 diabetes, autoimmunity, immunology, immune therapies, autoantibodies, T cells, antigen presentation

Disclosure: The authors have no conflict of interests to declare.

Acknowledgments: Aaron W Michels, MD, receives research support from the National Institutes of Health (DK09599 and AI050864), Juvenile Diabetes Research Foundation, and Children's Diabetes Foundation.

Received: June 15, 2012 **Accepted:** June 28, 2012 **Citation:** *US Endocrinology*, 2012;8(1):12–6 DOI: 10.17925/USE.2012.08.01.12

Correspondence: Aaron W Michels, MD, Assistant Professor of Pediatrics and Medicine, Barbara Davis Center for Childhood Diabetes, University of Colorado Denver, 1775 Aurora Court, MS A140, Aurora, CO 80045, US. E: Aaron.Michels@ucdenver.edu

Type 1 diabetes is classified into two types by the American Diabetes Association; type 1A diabetes is the immune-mediated form and type 1B the non-immune mediated form of the disease, both leading to β -cell destruction and absolute insulin deficiency. It is estimated that approximately 1.5 million people in the US have type 1A diabetes. The incidence of type 1 diabetes is increasing worldwide at a rate of 3–5 % each year. Strikingly, it has doubled in each of the last two decades, children less than five years of age being the most commonly affected group.^{1,2} Recently, it has been reported that if the present trends continue, the number of patients younger than five years of age will have risen by 70 % by the year 2020.³ The increasing incidence of type 1 diabetes is unlikely due to genes, as the increase has occurred over a relatively short period of time. Environmental causes have been hypothesized to increase the incidence of diabetes.^{4,5} A number of associations are reported between an environmental stimulus and diabetes incidence, including age of gluten exposure, introduction of infant formulas to the diet and type of these formulas, change in gut microbial flora, vitamin D deficiency, viral infections, and others.^{6–11} Alternatively, an unknown protective element in the environment may have been removed 20–30 years ago. There are a number of large prospective studies under way to identify environmental determinants of type 1 diabetes, including The environmental determinants of diabetes in

the young (TEDDY) study¹² and the MIDIA (Norwegian acronym for 'environmental triggers of type 1 diabetes') study in Norway.¹³

Genetics

Type 1A diabetes is a polygenic disorder and much is known about the genetics associated with it. Approximately 1/300 individuals from the general population develop type 1 diabetes while 1/20 siblings of patients with type 1 diabetes develop the disorder.^{14–16} It was previously thought that the concordance rate for monozygotic twins with type 1 diabetes was relatively low (<50 %); however, following a cohort of monozygotic twins for longer than 50 years, the concordance rate for type 1 diabetes development is 66 %. A recent analysis of these long-term twin data indicates that there is no age at which an initially discordant monozygotic twin is no longer at risk, with some developing type 1 diabetes in the fourth and fifth decades of life.¹⁷

The major genetic determinant of type 1 diabetes is conferred by genes in the human leukocyte antigen (HLA) complex, which is divided into three regions: class I, II, and III. Alleles of the class II genes, DQ and DR (and to a lesser extent DP), are the most important determinants of type 1 diabetes. These major histocompatibility complex (MHC) class II molecules are expressed on antigen-presenting cells (macrophages,

dendritic cells, and B cells) and present antigens to CD4+ T lymphocytes. DQ2 and DQ8 alleles are strongly associated with type 1 diabetes and more than 90 % of people with type 1A diabetes possess one or both of these genes, compared with 40 % of the US population in general.¹⁸

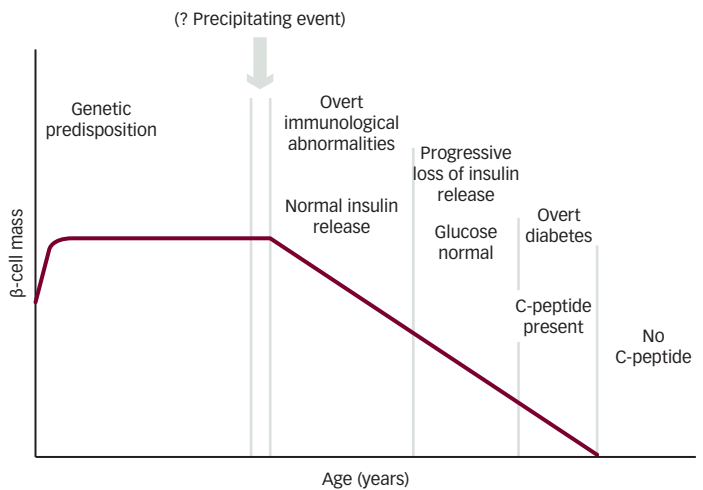
In addition to HLA genes, many other genetic loci contributing to diabetes risk have been implicated through genome-wide association studies (GWASs).¹⁹ These studies involve analyzing thousands of single nucleotide polymorphisms from large populations to find alleles associated with a particular disease. The largest of these studies was completed by the Type 1 Diabetes Genetics Consortium (T1DGC).^{20, 21} The T1DGC is an international collaboration established to create a large repository of DNA samples (>14,000) to identify genetic loci that contribute to type 1 diabetes risk. Of all the type 1 diabetes-associated genes, the HLA alleles DQ2 and DQ8 remain the strongest, with odds ratios (ORs) >11 for specific DR/DQ haplotypes.²²

Pathogenesis

Type 1 diabetes is a T cell-mediated autoimmune disease resulting from the specific destruction of pancreatic β -cells.²³ In a genetically susceptible individual, the development of diabetes occurs in stages (see *Figure 1*).²⁴ The presence of antibodies directed against proteins in β -cells (termed islet cell autoantibodies) is the first indication of the development of diabetes. There are currently four autoantibodies used to predict the development of type 1A diabetes: antibodies against glutamic acid decarboxylase (GAD65), the tyrosine phosphatase-like protein ICA512 (also termed IA-2), insulin, and the recently identified zinc T8 transporter (ZnT8).^{25,26} These immunologic markers precede any abnormalities in glucose homeostasis. Following autoantibody development, there is progressive loss of insulin release as the autoimmune response progresses. During later stages, patients progressively develop subclinical hyperglycemia, which can be initially detected through an oral glucose tolerance test. In the final stages of development, decreased C-peptide levels cause patients to present with overt signs of diabetes resulting from hyperglycemia.

Autoimmunity results from the body's immune system targeting self-proteins, termed autoantigens. Much of our understanding about the underlying immunology of type 1A diabetes comes from the study of animal models. The non-obese diabetic (NOD) mouse model develops spontaneous insulinitis, sialitis, and thyroiditis. Similar to humans, NOD mice spontaneously develop autoimmune diabetes with insulin autoantibodies and furthermore develop concomitant autoimmune disorders. NOD mice have genes within the MHC that influence antigen presentation to T lymphocytes, resulting in the development of autoimmunity. In the NOD mouse, insulin is a primary autoantigen, with the amino acids 9-23 of the insulin B chain recognized by 'diabetogenic' T lymphocytes.^{27,28} During disease progression, activated T cells invade the pancreas, cause inflammation, and destroy β -cells with resultant insulin deficiency and hyperglycemia. T lymphocytes signal B cells to produce autoantibodies, with NOD mice producing insulin autoantibodies. Once β -cell destruction starts, other antigens become targets for the immune response in a phenomenon termed epitope spreading. In the NOD mouse, another β -cell-specific protein, islet glucose-related phosphatase, is targeted after insulin.²⁹ Recent work demonstrated that certain T lymphocytes, regulatory T cells, aid in

Figure 1: Hypothetical Stages of Development and Loss of β -cells in an Individual Progressing to Type 1A Diabetes



Source: reproduced with permission from Eisenbarth, 2010.⁴

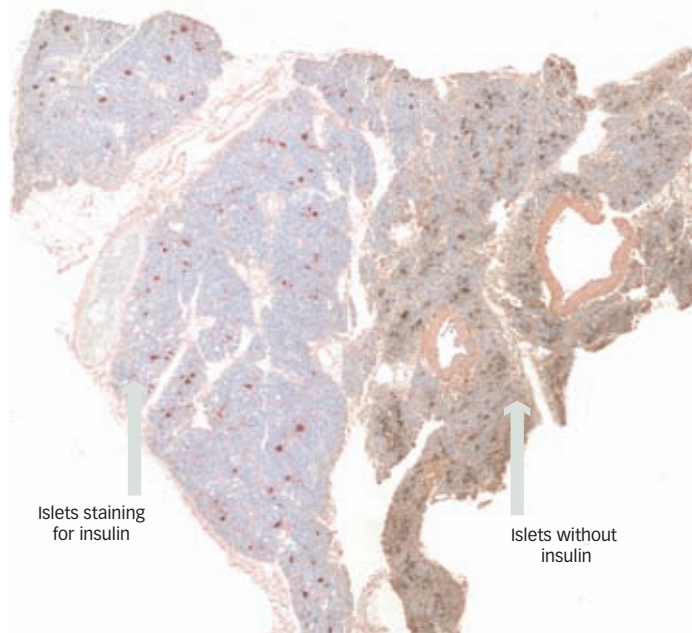
preventing the autoimmune destruction of self-tissues.³⁰ NOD diabetes can be prevented by increasing the number of regulatory T cells present in mice.³¹ Potentially, shifting the balance of harmful (effector T cells) and helpful (regulatory T cells) T lymphocytes will aid in controlling autoimmune β -cell destruction. Trials are under way that are treating type 1 diabetes individuals with autologous expanded regulatory T cells.^{32,33}

Prediction

Type 1A diabetes is now a predictable disease in humans with the measurement of islet autoantibodies. The four islet autoantibodies (GAD65, IA-2, insulin, and ZnT8) are now all commercially available. Although type 1 diabetes is a T cell-mediated disorder, detecting and measuring autoreactive T cells in the peripheral blood has proven to be difficult. T cells targeting specific proteins are at very low frequencies in the periphery, estimated to be in the range of 1/50,000 to 1/100,000 peripheral blood mononuclear cells.³⁴ Antibody assays are robust and have good sensitivity and excellent specificity. Newer assays are being developed using electrochemiluminescence (ECL), as opposed to radioactive fluid phase assays (radioimmunoassays), which further increases specificity.³⁵ Use of ECL also provides a platform for potentially multiplexing all four of the islet autoantibodies into a single assay.

The number of islet autoantibodies correlates to the risk of developing type 1 diabetes. With two or more islet autoantibodies, the risk of developing type 1 diabetes over the ensuing ten years is 70 %.^{36,37} Longer follow-up results in a higher percentage of individuals progressing to type 1 diabetes. Type 1 Diabetes TrialNet, sponsored by the National Institutes of Health, has a screening program in place across the US. Its natural history studies screen individuals who have a first-degree relative with type 1 diabetes for islet autoantibodies on a yearly basis. Islet autoantibodies can develop at any age, necessitating repeat measurements. Those individuals with two or more antibodies are screened with oral glucose tolerance tests at six-month intervals. Screening leads to an earlier diagnosis of type 1 diabetes and fewer individuals presenting with diabetic ketoacidosis.

Figure 2: Histology Section from a Type 1 Diabetic Pancreatic Organ Donor Showing Lobular Destruction of Insulin-producing β -cells in Islets



On the left, numerous islets with β -cells stain for insulin while the islets on the right lack β -cells and insulin staining. Histology section from the Juvenile Diabetes Research Foundation (JDRF) Network for Pancreatic Organ Donors with Diabetes (nPOD) (www.jdrfnpod.org, pancreas 6038).

It is desirable not only to assess type 1 diabetes risk, but also to predict the age of diabetes onset. Analyzing data from the Diabetes autoimmunity study in the young (DAISY)³⁸ indicates that the age at first antibody detection and mean insulin autoantibody level were significant predictors of age of diabetes onset. Interestingly, it was only the insulin autoantibody and not GAD or IA-2 levels that significantly predicted diabetes onset. Taking into account these two variables (age at first detected islet autoantibody and mean insulin autoantibody level), equations have been derived to calculate the predicted age of diabetes onset.³⁷ These equations need to be validated in a prospective manner.

Pancreatic Pathology

The presence of islet autoantibodies indicates that there is ongoing autoimmune destruction of pancreatic β -cells. There is much interest in understanding the pancreatic pathology of type 1 diabetes. The pancreas is a retroperitoneal organ and is very difficult to assess with biopsies, as its primary function is the secretion of enzymes to digest protein and fat. Until recently, pancreas histology from type 1 diabetes patients was limited. The Juvenile Diabetes Research Foundation (JDRF) started the Network for Pancreatic Organ Donors with Diabetes (nPOD) to obtain pancreata and other lymph organs from organ donors with longstanding type 1 diabetes, new-onset type 1 diabetes, and those with multiple islet autoantibodies but without overt hyperglycemia.³⁹ Remarkably, pancreata from longstanding type 1 diabetes patients indicate that most patients retain some islet β -cells (approximately 1–2 %) while some patients even with type 1A diabetes have significant β -cell mass.⁴⁰ Figure 2 depicts a pancreas section obtained through the nPOD program from an individual with longstanding type 1 diabetes. There is lobular destruction of β -cells in that some islets have no β -cells (they are termed

pseudoatrophic islets) and, in the same slide, there are islets with insulin staining.^{41–43} This is reminiscent of vitiligo, another autoimmune condition, in which there is patchy destruction of melanocytes in the skin.

An area of active research is the development of imaging modalities to define β -cell mass and the degree of insulinitis.^{44,45} This is an especially difficult undertaking, as islets only comprise about 1 % of the total pancreatic mass. There has been successful imaging of islets in animal models; however, it is still unclear if the imaging technologies can translate to humans.^{46,47} A recent study in humans used magnetic resonance imaging-magnetic nanoparticles (MRI-MNP) to visualize pancreata of individuals recently diagnosed with type 1 diabetes and age-matched controls. It was determined that overall pancreatic volume is decreased in type 1 diabetes individuals, presumably due to atrophy of acinar cells from lack of trophic factors produced by β -cells.⁴⁸

Immune Therapies in Type 1 Diabetes

Treatment of type 1 diabetes requires lifelong exogenous insulin administration to control the resultant hyperglycemia. Despite treatment with insulin therapy, long-term complications—including nephropathy, retinopathy, neuropathy, and cardiovascular disease—can result. While the progress to complete insulin dependence occurs quickly after clinical onset, initially after diagnosis the pancreas is able to produce a significant amount of insulin.⁴⁹ The Diabetes control and complications trial (DCCT) found that 20 % of patients studied, who were within five years of diagnosis, had remaining endogenous insulin production as measured by C-peptide levels;⁵⁰ within the first five years after diagnosis, immunologic intervention can potentially save β -cell function and reduce reliance on insulin administration. Even partial β -cell function is beneficial, as patients who maintain endogenous insulin production have better metabolic control than those who rely solely on exogenous insulin⁵¹ and improved metabolic control reduces the long-term complications from diabetes.⁵² Therapies that halt β -cell destruction would result in continued endogenous insulin production, greatly improving the metabolic control and reducing the prevalence of complications in type 1 diabetes. Over the last two decades, therapies aimed at stopping the autoimmune destruction of β -cells have been investigated.^{23,53–55} At the current time, there are no therapies approved by the US Food and Drug Administration to block the autoimmune process in type 1 diabetes. In the last year, large Phase II and III clinical trials using immune altering therapies in newly diagnosed type 1 diabetes individuals have been completed and will be reviewed below.

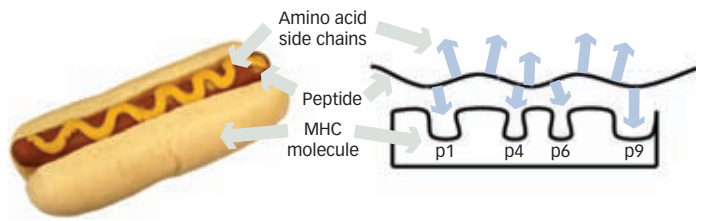
Immunotherapies in type 1 diabetes consist of immune suppressive agents and antigen-specific therapies, with newer classes of agents (anti-inflammatories, small molecules, and regulatory T cells) under current evaluation. The most well studied immune suppressive agent is a monoclonal antibody to the CD3 protein on T lymphocytes (anti-CD3 monoclonal antibody).^{56–58} The initial trial performed by Herold and colleagues treated newly diagnosed type 1 diabetes patients (within six months of diagnosis) and led to preserved C-peptide levels in a subset of treated patients up to five years following a single two-week treatment with the antibody.⁵⁹ Despite the preserved C-peptide levels, 12 months following the therapy, the loss of C-peptide production paralleled that seen in the control group. Clinically, glycosylated hemoglobin (HbA_{1c}) and insulin use improved and there were no long-term adverse effects.

Side effects during the study infusions were mild and included cytokine-release symptoms (rash, fever, headache, and myalgias). T lymphocytes were depleted for one month in the peripheral blood following daily intravenous treatment for two weeks before returning to normal levels in circulation. Recently, trials have been completed using repeat doses (Protégé trial)⁶⁰ and use further from diabetes onset (Delay trial, ClinicalTrials.gov identifier NCT00378508). In the large Phase III Protégé trial, the anti-CD3 monoclonal antibody did not meet the primary endpoints of a reduction in HbA_{1c} and less insulin/kg body weight in treated versus control subjects.⁶⁰ Post hoc analysis revealed that the drug, teplizumab, had more effect the earlier it was administered after diagnosis (within six weeks) and in children. Again, the safety and tolerability of the drug was good. Currently, teplizumab is being used in a diabetes prevention study. Relatives or type 1 diabetes patients with two or more islet autoantibodies and impaired glucose tolerance after an oral glucose challenge are being treated to prevent or delay the onset of disease (the study is sponsored through the TrialNet organization).⁶¹

Another therapy, abatacept (CTLA4-Ig), was used to block T cell activation in newly diagnosed type 1 diabetes patients. This fusion antibody blocks co-stimulation of T cells by binding to a protein (CD80/86) on antigen-presenting cells.⁶² In this randomized, double-blind, placebo controlled trial, type 1 diabetes patients within three months of diagnosis received monthly infusions for 24 months. C-peptide loss was slowed in the treated patients compared with controls over a two-year period. However, similar to the anti-CD3 monoclonal antibody effects on C-peptide preservation, after six months the loss of C-peptide paralleled that seen in the control group. It was estimated that the lag time in delaying the loss of C-peptide from treatment compared with control was 9.6 months—i.e., with treatment, the ‘honeymoon’ period was extended by 9.6 months.⁶³

Besides anti-CD3 monoclonal antibodies and abatacept, antigen-specific therapies have also been used in type 1 diabetes. The mechanism of antigen-based therapies is to administer an autoantigen to induce a favorable immune response.⁵⁴ In both animal models and humans, antigen-specific therapy results in regulatory T cells and anti-inflammatory cytokines such as interleukin-10.⁶⁴⁻⁶⁶ A potential benefit of antigen therapies over immune suppressant agents is that antigen therapy is site-specific. Regulatory T cells only act where islet antigens are present, and for type 1 diabetes this limits their effect to the pancreatic lymph nodes and islets themselves.^{67,68} The most well studied antigen-specific therapy in type 1 diabetes is the GAD-alum vaccine.⁶⁹ Recently, two large randomized, double-blind, placebo controlled trials were completed using the GAD-alum vaccine in new-onset type 1 diabetes patients. Unfortunately, there was no difference between treatment and control groups in regard to preservation of C-peptide.^{70,71} Despite this outcome, the therapy had an excellent safety profile and the protective immunologic effects of antigen therapy could be detected in the peripheral blood of treated patients.⁷² This suggests that antigen-specific therapy may have a role in immune modulation but is unable to arrest the autoimmune process once overt hyperglycemia is present.

Figure 3: Model of a Peptide Binding to an MHC Class II Molecule Comparable to a Hot Dog in a Bun



The major histocompatibility complex (MHC) molecule is the bun, the peptide is the hot dog, and the mustard represents the amino acid side chains of the peptide that point upwards. There are pockets (p1, p4, p6, and p9) in the MHC molecule that hold the peptide in place. The T cell receptor is represented by each individual eating a hot dog. In a typical bite, that individual (the T cell receptor) consumes some bun (the MHC molecule), some hot dog (the peptide), and some mustard (the amino acid side chains), analogous to a T cell interacting with a peptide bound to an MHC class II molecule.

Directions for the Future

Our knowledge regarding the immunology of type 1 diabetes has increased greatly over the last decade. There has been translation of work done in animal models into human clinical trials. The initial trials at inducing tolerance (stopping the autoimmune destruction of β -cells) have had limited success. We are able to delay the loss of endogenous C-peptide production for approximately one year with safe therapies. Moving forward, a combination therapy approach may provide an avenue to induce tolerance. Studies in preclinical animal models demonstrate synergy with combined therapies. For example, anti-CD3 monoclonal antibodies paired with intranasal insulin are able to reverse diabetes better than either single agent alone in NOD mice.⁷³ Recent recommendations have been made for developing combination immunotherapies in type 1 diabetes, such as administering an antigen-specific therapy under the umbrella of an immune suppressive agent (anti-CD3).⁷⁴ Combined therapies provide the benefit of synergy with the potential to lower doses, which will lessen the side effects from long-term immune suppression.

Ultimately, we believe more specific therapies with new molecular targets are needed to prevent and cure type 1 diabetes. One novel approach is to target the anti-insulin trimolecular complex.⁷⁵ The trimolecular complex consists of MHC class II molecules (DQ2 and DQ8, present in 90 % of all type 1 diabetes patients), self-peptide, and a T cell receptor (see Figure 3). We have identified small ‘drug-like’ molecules targeted to the pockets along the high-risk MHC class II molecules that present self-peptides to T lymphocytes to block autoreactive T cell activation.⁷⁶ Humans have three MHC class II molecules (DP, DQ, and DR) and blocking one has the potential to inhibit autoimmune responses while leaving intact normal immune responses through the other MHC class II molecules. Understanding how peptides bind MHC class II molecules and how T cell receptors interact with these complexes is crucial to our understanding of the immune pathogenesis of type 1 diabetes.⁷⁷⁻⁷⁹ With this knowledge will come the ability to design safe and specific therapies to inhibit these interactions, hopefully leading to the prevention and cure of type 1 diabetes. ■

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