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 β-cell destruction in these patients. This article centres 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

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 β-cell destruction in these patients. This article centres 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.

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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. 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.

The major genetic determinant of type 1 diabetes is conferred by genes in the human leucocyte 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.
In addition to HLA genes, many other genetic loci contributing to diabetes risk have been implicated through genome-wide association studies (GWASs). These studies involve analysing 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). 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.

### Pathogenesis

Type 1 diabetes is a T cell-mediated autoimmune disease resulting from the specific destruction of pancreatic $\beta$-cells. In a genetically susceptible individual, the development of diabetes occurs in stages (see Figure 1). The presence of antibodies directed against proteins in $\beta$-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). 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 hyperglycaemia, 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 hyperglycaemia.

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 insulitis, 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 recognised by “diabetogenic” T lymphocytes. During disease progression, activated T cells invade the pancreas, cause inflammation, and destroy $\beta$-cells with resultant insulin tolerance and hyperglycaemia. T lymphocytes signal $\beta$ cells to produce autoantibodies, with NOD mice producing insulin autoantibodies. Once $\beta$-cell destruction starts, other antigens become targets for the immune response in a phenomenon termed epitope spreading. In the NOD mouse, another $\beta$-cell-specific protein, islet glucose-related phosphatase, is targeted after insulin. Recent work demonstrated that certain T lymphocytes, regulatory T cells, aid in preventing the autoimmune destruction of self-tissues. NOD diabetes can be prevented by increasing the number of regulatory T cells present in mice. Potentially, shifting the balance of harmful (effector T cells) and helpful (regulatory T cells) T lymphocytes will aid in controlling autoimmune $\beta$-cell destruction. Trials are under way that are treating type 1 diabetes individuals with autologous expanded regulatory T cells.

### 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. 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. 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%. 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 programme in place across the US. Its natural history studies screen individuals who have a first-degree relative with type 1 diabetes for type 1A diabetes 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.

It is desirable not only to assess type 1 diabetes risk, but also to predict the age of diabetes onset. Analysing data from the Diabetes autoimmunity study in the young (DAISY) 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
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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 patients was limited. The Juvenile Diabetes Research Foundation (JDRF) Network for Pancreatic Organ Donors with Diabetes (nPOD) (www.jdrfnpod.org pancreas 43M).

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)

predicted age of diabetes onset. These equations need to be validated in a prospective manner.

Immune Therapies in Type 1 Diabetes

Treatment of type 1 diabetes requires lifelong exogenous insulin administration to control the resultant hyperglycaemia. 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. 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; 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 insulin and improved metabolic control reduces the long-term complications from diabetes. 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. 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). 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. 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 haemoglobin (HbA1c) and insulin use improved of C-peptide production paralleled that seen in the control group. There was no reduction in C-peptide production in the treated group. 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 insulitis. 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. A recent study in humans used magnetic resonance imaging-magnetic nanoparticles (MRI-MNP) to visualise 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.

Recent studies in both animal models and humans have identified that some islets have no β-cells or insulin staining. β-cells in Islets staining for insulin Islets without insulin

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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 organisation).71

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 CTA (CD80/86) on antigen-presenting cells.72 In this randomised, 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.73

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 favourable immune response.74 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 therapy over immune suppressant agents is that immune suppressant agents have a role in peripheral blood of treated patients.75 This suggests that antigen-specific therapy may have a role in immune modulation but is unable to arrest the autoimmune process once overt hyperglycaemia is present.

**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.76 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).77 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.78 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.79 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.80–82

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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