The autoimmune basis of Type 1 diabetes

The concept of Type 1 diabetes (T1DM, also called juvenile onset and insulin-dependent diabetes) as an autoimmune disease became well established in the mid-1970s with the identification of islet cell antibodies (ICA) in 1974 (1), HLA (Human Leukocyte Antigen) associations for genetic susceptibility to the disease (2) and the earlier description of insulitis (3).

Autopsy studies identified a lymphocyte rich inflammatory infiltrate in the islets of people with T1DM that was termed insulitis (3,4). Insulitis has also been observed in animal models of autoimmune diabetes. The infiltrate consists of CD8 T lymphocytes, CD4 T cells and macrophages (5,6). There is an associated increase in expression of class 1 Major Histocompatibility Complex (MHC) molecules and other inflammatory markers of possible pathological significance including adhesion molecules, interferon-α and Fas (CD95) (7). Insulitis is direct evidence for the immunopathogenesis of the disease. Insulitis was also found in pancreas from normal individuals donated to their identical twins with diabetes when Type 1 diabetes recurred in recipients (8).

CD4+ and CD8+ T cells and macrophages play critical roles in the pathogenesis of T1DM and at least in animal models all are required for efficient progression of β-cell destruction and diabetes. CD8+ cytotoxic T cells appear to be the most directly damaging cell type. β-cell death occurs by apoptosis and probably by several molecular pathways. These include those targeted by the contents of the cytolytic granules of CD8+ T cells including perforin and granzymes, cell death receptors such as Fas and pro-inflammatory cytokines including tumour necrosis factor, interleukin 1 and interferon-γ (9).

Islet cell antibodies – probes for the natural history of TIDM

Islet cell antibodies (ICA) measured by indirect immunofluorescence in the sera of subjects with Type 1 diabetes (1) have become established as markers of the β-cell autoimmune response but there is good evidence that autoantibodies are not directly involved in β-cell destruction. Identifying the molecular targets of ICA has been a major priority. The ICA reaction is now believed to be due to autoantibodies against the proteins, glutamic acid decarboxylase (GAD) and IA-2. Insulin autoantibodies (IAA) are also found prior to insulin therapy. Of these, insulin is the only one that is β-cell specific. Almost all (>95%) patients with T1DM have one of these autoantibodies and almost no (<1%) normal subjects have all three.

Patients with stiff-man syndrome, a rare neurological disorder, were found to have autoantibodies to a 64 kDa protein in brain that was identified as GAD. These patients also had ICA and 30% developed diabetes. These observations led to the identification of GAD as a molecular target of ICA. GAD is a rate-limiting enzyme in the biosynthesis of γ-aminobutyric acid (GABA). The function of GABA in islets is not fully understood. IA-2 and its reactivity with autoantibodies were discovered by screening β-cell cDNA expression libraries with patient serum.

The discovery and application of autoantibody testing have dramatically changed how the natural history of T1DM is viewed. It is now recognised that acute metabolic decompensation is the final stage in a relatively slow disease process that erodes the reserve of insulin secretion capacity in the pancreas. This was revealed by a landmark sequential study of islet cell antibodies over 15-year period in monzygotic twins and triplets. It was found that the natural history of Type 1 diabetes is characterised by a long preclinical period when immunological markers of diabetes are present but glucose is normal (10).

Autoantibody testing has enabled identification of people at risk of developing T1DM particularly among first-degree relatives, who are at about ten-fold risk of developing diabetes. In the Melbourne prediabetes study, 2.6% of over 3000 first-degree relatives had elevated ICA levels, 1.3% elevated IAA levels and 0.3% had both (11). Predictors of future T1DM in first degree relatives include titre of ICA, presence of IAA, young age and low insulin response to intravenous glucose. Screening of patients clinically diagnosed with Type 2 diabetes has identified a significant sub-group (10% in the United Kingdom prediabetes study) with evidence of β-cell autoimmunity (12). They appear to have a slowly progressive form of Type 1 diabetes that has been termed latent autoimmune diabetes in adulthood, which may be almost as common as classical T1DM.

Genetics of T1DM

The concordance of T1DM diabetes in monozygotic twins is 30-40%, compared with 5% in siblings which indicates substantial genetic and non-genetic influences. The most clearly established genetic locus associated with Type 1 diabetes is the MHC, in humans also known as the HLA locus, found on the short arm of chromosome 6 (13). The MHC or HLA region accounts for at least 35% and up to 50% of familial clustering in Type 1 diabetes and is therefore the major susceptibility locus currently identified (14). Other genes are also being explored. Of these the best established as a risk allele is the insulin gene where promoter polymorphisms may affect level of insulin expression. How this affects T1DM is incompletely understood.

MHC class II proteins function as peptide binding proteins that present peptides as antigen to CD4 positive T cells. Polymorphisms within MHC molecules affect which antigenic peptides are bound and presented. Pancreatic β-cells do not express class II MHC proteins and therefore presentation to CD4+ T cells of autoantigens derived from β-cells is likely to occur on professional antigen presenting cells such as dendritic cells.
• **Type 1 Diabetes**

DQ alleles, that encode one of the three MCH class II molecules are most closely associated with diabetes. The HLA region tends to be inherited en bloc so that particular class I and class II alleles are closely linked, e.g. DQ and DR alleles are tightly linked. HLA-DR3, HLA-DR4 or both class I and class II alleles are closely linked, e.g. DQ and

Epidemiology

The current prevalence of Type 1 diabetes in western nations is estimated to be 0.2-0.5%. There is a 60-fold difference between Finland, the country with the highest rate, and Japan, which has the lowest recorded rate of diabetes worldwide. Data from 37 studies in 27 countries from 1960-1996 showed that the incidence of Type 1 diabetes is increasing world wide at a rate of approximately 3-4 % per year with the age group 5-10 years having the largest proportional increase. It is proposed that changes in diet or childhood infections leading to altered development of the immune system may be involved.

Treatment of T1DM

T1DM was a rapidly fatal illness prior to the discovery and mass production of insulin in 1922 (see article by Lance Macaulay in this Showcase on Research). The aims of treatment in Type 1 diabetes are to control glucose to near normal levels, limit hypoglycaemia, improve quality of life and prevent secondary complications. Patients have to learn to vary insulin doses according to food intake, physical activity and in times of illness. Multiple insulin analogues with varying pharmacokinetics are used in an attempt to emulate physiological insulin levels at meal times and when fasting. Programmable continuous insulin infusion pumps, which are now quite compact, have offered an alternative to multiple injections via pen or syringe systems (15). Glucose monitoring requires four or more blood samples over 24 hours usually obtained from a finger prick. Non-invasive glucose monitors are available but multiple blood tests are required to calibrate them during the course of a day limiting their usefulness currently. Overall, despite advances in technology, therapy is inadequate to achieve the treatment aims for many patients.

Replacement of insulin-producing tissue by transplantation is the intervention closest to a cure for T1DM at present. Pancreas transplant with renal transplant is available for patients with end stage renal failure and T1DM. For those patients able to undergo a major operation, it offers good glycemic control and has a one-year graft survival approaching 90%. The pancreatic duct, draining pancreatic digestive enzymes, is connected to the bladder or the intestine. It involves a six-month recovery period from surgery and a significant complication rate. Mortality of up to 5% at one year has been reported in this group of patients. Islet transplantation on the other hand is a relatively non-invasive procedure with most recipients able to leave hospital within 24 hours.

Until recently one-year insulin-independence rates for islet transplantation have been less than 20%. Autologous transplantation in patients having undergone pancreatico-pancreatectomy for chronic pancreatitis has been more successful (16). In 2000, allogeneic pancreatic islets were transplanted successfully into seven diabetic patients with life threatening hypoglycemic episodes, all of who remained insulin independent at one year follow-up (17). This significant achievement by the Edmonton group sparked new hope for treatment of T1DM. The major factors contributing to previous high failure rates were inadequate functional islet mass and graft failure due to toxicity of drugs, rejection and autoimmunity. The Edmonton group addressed all of these in their protocol. Islet isolation techniques have dramatically improved with an average of 380,000 islet equivalents (IE) obtained from each donated pancreas.

In brief, the islet isolation process involves removing the donor pancreas and perfusing it with a purified collagenase. The tissue is digested at 37°C and physically dissociated. The digestate is then centrifuged on a Ficoll density gradient to separate islets from exocrine tissue (18). Finally, the islet preparation is transfused into the portal vein of patients via a catheter inserted under radiological guidance. Transplantation of 12,000 IE/kg on average was required to achieve freedom from insulin therefore multiple donors were required and recipients were generally less than 70 kg. Even though almost a million islets are transfused into patients, metabolic testing shows that functional insulin reserves are approximately a fifth to a tenth that of normal subjects, implying that a high percentage of the islets are destroyed. Non-specific platelet activation is a possible cause of immediate graft non-function.

Conventional immunosuppressive regimens for organ transplant include glucocorticoids such as prednisolone and cyclosporine or tacrolimus which are diabetogenic due to adverse affects on islet function. A glucocorticoid-free immunosuppression regimen was achieved in the Edmonton Protocol by using a combination of low dose tacrolimus, rapamycin and induction with an interleukin-2 receptor monoclonal antibody (Daclizumab).

The recent unpublished results indicate that 85% of 34 recipients were insulin independent at one year. Side effects of the drugs were minor but included high cholesterol and mouth ulcers (both consistent with rapamycin). The procedure was safe with two cases of partial portal vein thrombosis and five hepatic bleeds, one requiring surgery. The two main problems with islet transplantation are risks and complications of the drug therapy and an inadequate supply of donor organs. Already, alternate immunosuppressive protocols are being trialled.

Infliximab, a chimeric anti-tumour necrosis factor-α monoclonal antibody has become part of the induction protocol (19). Other regimens are being tested in trials conducted through the International Tolerance Network, a worldwide collaboration, supported by the National Institutes of Health and the Juvenile Diabetes Research Foundation.

A sufficient supply of islets will probably depend on differentiation of β-cells from adult and embryonic stem cells or xenotransplantation, all which are still experimental and some way off clinical application. In xenotransplantation the theoretical risk of transmitting porcine endogenous retroviruses remains a problem as does identifying a drug regimen that will block xenorejection. While amazing progress has been made on stem cells in recent times, this remains early work with much to do before clinical application is realistic (20).
**Type 1 Diabetes**

References