Review

Immunology of type 1 diabetes

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Introduction

The incidence of type 1 diabetes in the UK is 20 per 100,000 and increasing, particularly in the under-5-years age group.¹ It comes with the burden of daily insulin injection and blood testing, as well as both short- and long-term complications, and this can include premature death. The standardized mortality ratio for type 1 diabetes has been estimated as 4-fold for females and 2.7-fold for males in the UK.² Even with tight glucose control, there is a significant risk of neuropathy, retinopathy and nephropathy, as well as a 3-fold increase in the risk of severe hypoglycaemia.³

Understanding the pathology of type 1 diabetes may help improve management. Type 1 diabetes is characterized by an absolute loss of insulin secretion, and results from an autoimmune process that destroys insulin-producing β cells within the pancreatic islet. This review will focus on the immunology of type 1 diabetes, and how this understanding may influence the clinical management, and development of new treatments for this disease.

The immune nature of type 1 diabetes

There are over one million islets in a healthy adult pancreas. They make up 1% of the total pancreatic volume, weigh about 1 g, and contain about 1 mg of insulin.⁴ Histological analysis of the pancreas from patients with type 1 diabetes shows immunological activity not present in a healthy or a type 2 diabetic pancreas.⁵ This activity is limited to insulin-containing islets, includes infiltration by activated lymphocytes, antibodies and components of the complement system. These histological findings are consistent with type 1 diabetes being an immune-mediated disease (Figure 1).

Further evidence comes from studies showing that drugs that suppress the immune response can modulate type 1 diabetes. Drugs such as cyclosporin and azathioprine slow progression of β cell destruction, and have been used in trials of type 1 diabetes prevention.⁶,⁷

The importance of genetic factors in the aetiology of type 1 diabetes is demonstrated by concordance rates of 5–10% in dizygotic twins and up to 27% in monozygotic twins.⁸ Human leukocyte antigen (HLA) genes contribute up to 40% of risk, and are the single major contributor. These genes encode cell surface proteins that are required for interaction with cells of the immune system, and are involved in immune recognition and killing. Distinct loci within the HLA region determine risk, though the HLA Class II region appears to be most influential. In particular, in Caucasians, HLA types DR3-DQA 0501-DQB1 0201 and DR4-DQA1
0301-DQB1 0302 appear associated most strongly with risk, and DQB1 0602 with protection from diabetes.9

Type 1 diabetes is characterized by the presence of antibody (humoral) and T-cell (cellular) responses to islet proteins (antigens) (Table 1). Immune responses to these antigens predate the clinical onset of diabetes, giving further support for an immune aetiology to type 1 diabetes (Table 2).

The natural history of type 1 diabetes

The identification of islet auto-antigens and the development of robust antibody assays has allowed us to explore the period of ‘prediabetes’. Strikingly, many subjects will have antibodies to candidate auto-antigens long before they develop disease. This may be as early as in utero, with subsequent development of type 1 diabetes more than a decade later.15

Antibody screening of first-degree relatives of type 1 diabetes patients suggests that antibodies to insulin are the first to appear.16–18 GAD65 and IA-2 antibodies may follow, but in no particular sequence. Within two years of age, over 10% of first-degree relative infants appear to have at least one islet antibody, suggesting that the autoimmune process is initiated early. The presence of islet immunity however, does not necessarily imply loss of β cell function. Abnormalities in intravenous glucose tolerance testing are often only detectable late in the disease process, despite the presence of antibodies for several years beforehand.19 Furthermore, subjects may persist with abnormal β cell function for some years without developing type 1 diabetes. This suggests that the preclinical period of type 1 diabetes lasts much longer than classically thought, and allows for interventions that can save residual β cell mass and delay or even prevent type 1 diabetes.

Following diagnosis and treatment with insulin, a few patients exhibit the ‘honeymoon’ phenomenon whereby they become insulin-independent for up to a year.20 The mechanism underlying this may relate to correction of a ‘glucotoxic’ effect of hyperglycaemia on β cell function. The β cells surviving at the time of diagnosis continue to be attacked by the immune system, and gradually disappear over a 5-year period.

Association of type 1 diabetes with other autoimmune diseases

Patients and relatives with type 1 diabetes are at increased risk of other immune mediated diseases. These most commonly include thyroid disease, coeliac disease, autoimmune gastritis and Addison’s disease.

Up to 25% of patients with type 1 diabetes will have evidence of thyroid disease (the commonest autoimmune disease associated with type 1 diabetes);21 three times more common than in

Table 1 The major β cell autoantigens in type 1 diabetes

<table>
<thead>
<tr>
<th>Islet cell antigen (ICA)10</th>
<th>The first islet ‘autoantigen’ to be described. Now known to be a complex of autoantigens. Antibodies to ICA are present in 90% of type 1 diabetes patients at the time of diagnosis.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin and pro-insulin11,12</td>
<td>Antibodies to insulin and pro-insulin, the biochemical precursor to insulin, are present at diagnosis in 23% and 34% of type 1 diabetes patients, respectively.</td>
</tr>
<tr>
<td>Glutamic acid decarboxylase (GAD)13</td>
<td>A constituent of the ICA antigen complex. Present in the 65kDa form in the human islet. Also present in the central nervous system. GAD antibodies are present in 73% of type 1 diabetes patients at diagnosis.</td>
</tr>
<tr>
<td>Protein tyrosine phosphatase (IA-2)14</td>
<td>A transmembrane protein from the insulin secretory granule. Also present in central nervous tissue. IA-2 antibodies are present in 75% of type 1 diabetes patients at diagnosis.</td>
</tr>
</tbody>
</table>
Table 2  Evidence for an immune aetiology to type 1 diabetes

1. Pancreatic β cells contain immune cell infiltrates.
2. Immunosuppressive drugs reduce disease incidence.
3. β cell immunity predates disease onset.
4. HLA genes associate with disease risk or protection.
5. Established autoimmune diseases cluster with type 1 diabetes.

the general population. Most of these type 1 diabetes patients will have subclinical disease, although about 5% will be clinically hypothyroid. Undiagnosed hypothyroidism can affect glycaemic and lipid control in patients with type 1 diabetes, making a good case for regular routine screening for this condition. Annual screening with a single TSH measurement is a quick and reliable indicator of thyroid function. The measurement of thyroid antibodies in newly diagnosed type 1 diabetes patients will help delineate those at highest risk of developing thyroid disease, but is insufficient screening for clinical hypothyroidism because it will miss a proportion that develop thyroid-antibody-negative hypothyroidism.

Coeliac disease is a gluten-sensitive enteropathy, prevalent at up to 1% of the Caucasian population, and treated by strict avoidance of gluten-containing products.22,23 The prevalence of coeliac disease is increased at 7% in subjects with type 1 diabetes, but not other types of diabetes.24 Many patients with coeliac disease do not describe the symptoms of tiredness, diarrhoea, steatorrhoea or weight loss traditionally associated with this disease, and up to 40% can be asymptomatic. Hence it is important to screen actively for this disease, particularly in type 1 diabetes patients, where it can contribute to poor diabetes control and in particular to unexplained hypoglycaemia. Correct adherence to a gluten-free diet is associated with lower insulin requirements and higher weight for age measures.25 The risks of type 1 diabetes and coeliac disease being present together are higher at a younger age of onset and in the female sex.26 Screening is through measurement of serum anti-endomysial and anti-transglutaminase antibodies, with confirmatory biopsy of the duodenum through upper gastro-intestinal endoscopy. Some studies suggest that antibody levels can fluctuate and that screening should be conducted at regular intervals, particularly where clinical suspicion exists.27

The gastric sodium-potassium ATPase appears to be the major autoantigen in autoimmune gastritis.28 The disease is associated with achlorhydria and iron deficiency anaemia. Parietal cell antibodies are a marker of pernicious anaemia, and are present in up to 20% of patients with type 1 diabetes.29 This is three times commoner than in the general population. The increased frequency of pernicious anaemia30 and iron-deficiency anaemia31 in the type 1 diabetes population necessitates regular screening for these conditions, at least with a full blood count.

The earliest descriptions of islet cell antibody were in patients with Addison’s disease,10 and the prevalence of islet cell antibody has been reported to be as high as 6% in patients with Addison’s disease.32 Conversely, the prevalence of antibodies to 21 hydroxylase, a marker of autoimmune Addison’s disease, in those with type 1 diabetes is about 2%.33 Most type 1 diabetes patients with adrenal antibodies will not have adrenal failure, and in the absence of clinical suspicion or of a strong family history, there is little evidence as yet that type 1 diabetes patients should be routinely screened for this condition.34

Neufeld and Blizzard originally noted that where multiple autoimmune endocrinopathies are present, they tend to segregate in definable groups.35,36 These are termed autoimmune polyglandular syndromes (APS) and two broad groups were originally defined. Type 1 diabetes can occur in all of these, but is most frequent in APS 2 (Table 3). APS 2 (Schmidt’s Syndrome) is the commonest of the syndromes and is characterized by the presence of Addison’s disease with type 1 diabetes and/or autoimmune thyroiditis. APS 1 is characterized by the presence of at least two of Addison’s disease, hypoparathyroidism and recurrent mucocutaneous candidiasis.

These associations help guide further investigation of type 1 diabetes patients noted to have co-existing autoimmune conditions. It also allows for an investigation and understanding of the underlying pathogenesis of autoimmune diseases, as has been illustrated by the study of APS 1.37

Islet immunity in patients presenting with symptoms of type 2 diabetes

A subset of adults who present with type 2 diabetes have detectable ICA and GAD antibodies. In the UK Prospective Diabetes Study (UKPDS), 6% and 10% of subjects with presumed type 2 diabetes had ICA and GAD antibodies, respectively.38 These patients are considered to have a slowly progressive form of type 1 diabetes that has been termed latent autoimmune diabetes in adults (LADA). The diagnosis of LADA is based on a gradual onset of diabetes in adult life (generally considered to be
30 years or older) and the presence of islet autoimmunity, most commonly to GAD.

LADA patients appear to have lower residual β cell function than those with type 2 diabetes. This is reflected in lower fasting and glucose-stimulated C peptide and insulin levels. For these reasons they do not respond as well to treatment with diet or oral hypoglycaemic agents, and progress more rapidly to insulin dependency. After 6 years of follow-up in the UKPDS, 94% of patients with ICA and 84% with GAD antibodies required insulin, compared with 14% of antibody-negative patients. The positive predictive value of GAD antibodies for future insulin requirement is between 84–95%, and is a better predictor of insulin requirement than clinical judgment is.

A similar pattern appears in patients with gestational diabetes. Those who proceed to insulin treatment soon after delivery appear to have both a genotype and antibody profile (including GAD antibodies) similar to that of adult onset type 1 diabetes.

The prevalence of recognized type 2 diabetes in the UK is approximately 3%. If up to 10% of these are GAD-antibody-positive, the prevalence of LADA is at least half that of classical type 1 diabetes. The recognition of LADA is important, because it identifies subjects who are at the slower progressing end of the type 1 diabetes disease spectrum, but who nevertheless are insulin-deficient and will require lifelong insulin replacement, and allows earlier treatment of this insulin deficiency.

**Absence of islet autoimmunity in patients presenting with type 1 diabetes**

The absence of circulating islet antibodies in patients presenting with the classical symptoms of type 1 diabetes raises the possibility of other forms of diabetes, including type 2 diabetes. The hyperglycaemia characterizing type 2 diabetes results from a defect in peripheral tissue insulin sensitivity as well as β cell loss. The latter is through apoptosis rather than autoimmune attack, and can result in weight loss and osmotic symptoms very similar to that of type 1 diabetes. These patients will require treatment with insulin.

Cases of severe insulin resistance can present with hyperglycaemia and ketosis, and sometimes diabetic ketoacidosis, suggestive of absolute insulin deficiency. This has been termed ‘Flatbush’ diabetes and is thought to result from ‘gluco-toxic’ effects of hyperglycaemia on β cell function. These patients will have a significant β cell mass but poor insulin secretion. Following initial treatment with insulin and reversal of gluco-toxicity, patients can often be safely switched to oral hypoglycaemic medication. It is important to make the distinction between this condition and type 1 diabetes, because patients with ‘Flatbush’ diabetes are better treated with insulin-sensitizing medication rather than insulin, which can result in weight gain and a further increase in insulin resistance.

Rarer causes of islet-antibody-negative diabetes that can present with symptoms of type 1 diabetes include maturity-onset diabetes of the young (MODY), and in particular those involving mutations of the hepatocyte nuclear factor 1α, 1β or 4α genes. However, these patients are not insulin-dependent, have no evidence of islet autoimmunity, and will have a strong family history of diabetes extending over three generations. It is important to make the distinction, because some patients with MODY can be safely treated with sulphonylurea drugs.

In 1997 the American Diabetes Association proposed that type 1 diabetes be subcategorized according to the presence (Type 1A) or absence...
Subjects with histological evidence of autoimmune islet destruction can however be serologically negative for the commonly measured islet antibodies. It is possible that other islet autoantigens are preferentially targeted in these cases, and that they still have an autoimmune basis to their type 1 diabetes. It would also be worth considering the other causes of islet-antibody-negative diabetes mentioned above before labelling a patient with type 1B diabetes.

New strategies for prevention and treatment of type 1 diabetes

(1) Interventions to prevent the development of islet immunity

The ideal intervention would prevent the development of islet immunity (i.e. islet antibodies). Such an intervention could be targeted to all healthy subjects, but this exposes those who may never develop type 1 diabetes to potential adverse effects of treatment. Identifying subjects at risk of developing type 1 diabetes, before they develop antibodies, can at present rely only on genetic analysis. Current strategies for genetic screening would identify only half of paediatric diabetic subjects, and in addition would target a proportion of the healthy population who may never develop diabetes. Therefore only therapies with a low risk of adverse effects can be used. To date, only the approach of avoiding putative environmental triggers has been used for the primary prevention of Type 1 diabetes.

Cows milk

The suggestion that cow’s milk proteins may contribute to the development of Type 1 diabetes arose from observations that prolonged breast feeding mildly protects against development of type 1 diabetes and that cow’s milk modulates diabetes development in animal models of autoimmune diabetes. Nonetheless, retrospective analyses of infant feeding patterns have not shown any relationship with the development of islet autoimmunity. Formal trials of cow’s mild avoidance in subjects genetically at risk of type 1 diabetes are in progress, and results are awaited with interest.

Viruses

At least 40% of children with congenital rubella who are born to mothers infected early in their pregnancy will develop islet antibodies, and about half of these will develop type 1 diabetes. Vaccination of the female population has almost eliminated this risk. Rubella is the only virus that has been convincingly associated with type 1 diabetes. Enteroviruses such as coxsackie and echoviruses have previously been implicated but more recent studies show no association, and it is possible that these viruses infect pancreatic exocrine tissue in preference to the islets. Cytomegalovirus and rotavirus contain peptide sequence similarities with islet autoantigens that could potentially trigger islet immunity following infection. In particular, episodes of rotavirus infection in children have been associated with increases in islet immunity in children at risk of type 1 diabetes. This has yet to be taken forward to prevention trials of type 1 diabetes.

Gluten

Coeliac disease is a gluten-sensitive enteropathy, and occurs more frequently in patients with type 1 diabetes. Coeliac disease has been proposed to drive islet autoimmunity, and certainly the avoidance of dietary gluten in animal models prevents development of type 1 diabetes. In humans, however, gluten avoidance over a one-year period in children with islet antibodies, but without type 1 diabetes, does not reduce islet antibody number or the incidence of disease.

(2) Intervention after the onset of islet autoimmunity

The autoimmune process leading to type 1 diabetes is long, and even when the disease is diagnosed, up to 30% of β cell mass can remain. Arresting the autoimmune process before or even at diagnosis with type 1 diabetes may allow the residual β cells to contribute to long-term glucose control. They may even allow beta cell regeneration and complete avoidance of hyperglycaemia. Several large studies in this group of subjects have recently been concluded, and some show promising results.

OKT3

Blocking the CD3 T cell receptor has immunosuppressive effects. Humanized and modified monoclonal antibodies to CD3 prevent type 1 diabetes when administered to animal models, and have been used in humans to prevent renal allograft rejection. On this basis, a modified monoclonal antibody to CD3, hOKT3y1, was administered to patients recently diagnosed with type 1 diabetes in a randomized and double-blind fashion. OKT3 treatment was associated with a significant maintenance of improvement in insulin production,
lowering or glycated haemoglobin and insulin doses. The benefits were maintained for over a year. Treatment was associated with an incidence of mild fever, anaemia and urticarial rash, all of which resolved within a month of treatment. The mechanism of action may involve the generation of a population of cells with immunoregulatory properties. Further follow-up of these patients is underway.

**Nicotinamide**

Nicotinamide is a soluble B group vitamin. Studies in several animal models of type 1 diabetes have demonstrated a β-cell-protective effect, probably mediated through inhibition of the intranuclear repair enzyme poly-ADP-ribose polymerase. The European Nicotinamide Diabetes Intervention Trial (ENDIT), a randomized double-blind placebo-controlled trial of nicotinamide in 552 first-degree relatives with elevated ICA and no diabetes has recently been reported. It showed no difference in diabetes incidence or of first phase insulin secretion across treatment groups over the five-year trial period. Though it showed no serious adverse effects of treatment, it concluded that nicotinamide treatment at the dose used was ineffective at preventing type 1 diabetes.

**Insulin**

Insulin administration to pre-diabetic animal models of type 1 diabetes has been shown to delay or even prevent the onset of type 1 diabetes, and small pilot studies suggested this may also work in pre-diabetic human subjects. Two mechanisms have been proposed. Firstly, supplementing pancreatic insulin production may allow the β cell to ‘rest’. This proposes that β cells are less immunogenic when resting than when active. Secondly, insulin administration through ‘tolerogenic’ routes such as nasal or gut mucosa will down-regulate the subsequent immune response to this protein. Since insulin is a major autoantigen in type 1 diabetes, down-regulating the immune response to it will also reduce immunity against the islet.

The Diabetes Prevention Trial 1 (DPT1) was a large clinical trial based on insulin prophylaxis. First-degree relatives of type 1 diabetes patients with a >50% forecasted risk of developing type 1 diabetes were randomized and treated with either placebo or a combination of subcutaneous and intravenous insulin for a mean of 3.7 years. There was no difference in incidence of diabetes across treatment groups. Intranasal insulin has also been used in subjects at risk of developing type 1 diabetes in a randomized crossover fashion. This also did not retard loss of β cell function, although it did show encouraging changes in the immune response towards insulin. Further trials would be necessary to investigate this further.

**Cyclosporin and azathioprine**

Two major placebo-controlled trials in the 1980s showed cyclosporin has the potential to slow the progression of β-cell destruction in newly diagnosed subjects. The benefits of cyclosporin were however transient, and lost within 2–4 years of diagnosis. Other than immunosuppression, the concern with the use of cyclosporin is dose-related nephrotoxicity, and therefore serum levels of cyclosporin need to be monitored carefully. For these reasons and due to lack of efficacy over the long term, cyclosporin has not been taken forward to routine use in type 1 diabetes prevention.

Azathioprine is a cytotoxic agent that is commonly used as an immunosuppressant. It has been shown to improve residual insulin secretion in newly diagnosed patients either on its own, or in combination with prednisolone. Once again the potential for side-effects, including myelosuppression and hepatotoxicity, has limited the further use of this agent.

Small studies of immunosuppression with methotrexate and antibodies to CD4 T-cell markers have not shown any benefit. Trials with dacluzimab and mycophenolate mofetil, both of which inhibit lymphocyte proliferation, are ongoing.

**Heat shock protein**

The administration of islet auto-antigens through tolerogenic routes can down-regulate the subsequent immune response to them, and the trials of nasal and oral insulin described previously use this phenomenon. The 60kDa form of heat shock protein is an islet auto-antigen, and the administration of a peptide of this protein has a β-cell-protective effect in animal studies. On this basis, a modified form of this peptide has been administered to newly diagnosed subjects with type 1 diabetes in a small randomized trial. Encouragingly, treatment appeared to be associated with an attenuation of β cell loss, although further follow-up results and trials are awaited.

**GAD**

LADA (see above) is characterized by the presence of antibodies to GAD. A specific dose of a GAD-based vaccine administered in a tolerogenic form has recently been reported to improve β cell function in these patients. Further trial results and analysis are awaited.
(3) Intervention in established type 1 diabetes

Patients with established type 1 diabetes have no residual β cells, and require full replacement of insulin-secreting capacity. Combined pancreas and renal transplant is available for patients with end-stage renal failure and type 1 diabetes. It offers those patients able to undergo a major operation good glycaemic control, and has a 4-year survival approaching 90%. However it involves a 6-month recovery period from surgery, and a significant complication rate. Islet transplantation on the other hand is a relatively non-invasive procedure, with most recipients able to leave hospital within 24 h. In 2000, Shapiro et al. from the Edmonton Islet Transplant Unit published success in seven consecutive subjects transplanted successfully with allogeneic pancreatic islets, all of whom remained insulin-independent at 1 year follow-up. One-year graft survival rates were <10% prior to this.

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 were dramatically improved, and islets from multiple donors were used. The islet preparation was transfused into the portal vein of patients via a catheter inserted under radiological guidance. Even though almost a million islets are transfused into patients, metabolic testing shows that functional insulin reserves are a fifth to a tenth that of normal subjects, implying that a high percentage of the islets is destroyed. Conventional immunosuppressive regimens for organ transplant include glucocorticoids, which are diabetogenic. A glucocorticoid-free immunosuppression regimen was achieved in the Edmonton protocol by using a combination of low dose tacrolimus, rapamycin and induction with an IL-2 receptor monoclonal antibody (daclizumab). More recent results indicate that 80% of 34 recipients were insulin-independent at one year.

Side effects of the drugs included a rise in blood pressure, hyperlipidaemia and most commonly, mouth ulcers. The procedure was safe, with two cases of partial portal vein thrombosis and five hepatic bleeds, one requiring surgery. Portal pressure appears to rise with successive transfusions, the long-term consequences of which are unknown.

The main problems with islet transplantation are the complications of immunosuppressive drug therapy and an inadequate supply of donor organs. Already, alternate immunosuppressive protocols are being trialled, and recently infliximab, a chimeric anti-tumour-necrosis-factor-α monoclonal antibody, has become part of the induction protocol. A sufficient supply of islets will probably eventually depend on differentiation of β cells from adult and embryonic stem cells, or xenotransplantation, all of which are still experimental and some way off clinical application.

Conclusion

This is an exciting time for the therapy of type 1 diabetes. Several large studies have been conducted for diabetes prevention, some with promising results. These studies also demonstrate unequivocally that appropriate trial subjects can be identified and used in large multinational settings for such purposes. Guidelines for trial design in type 1 diabetes have been proposed, and other trials are in progress. In the meantime, our greater understanding of the immunology of type 1 diabetes has helped us appreciate the association with other autoimmune diseases, and to fit it into the spectrum of conditions that result in diabetes. Clinically implemented, this understanding can help improve the quality of life and prognosis of patients with type 1 diabetes.

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