ABSTRACT – Autoimmune diseases affect 10% or more of the UK population. In organ-specific autoimmune diseases a particular tissue is targeted by the aggressive immune response. Type 1 diabetes is due to destruction of insulin-secreting islet cells. Both genetic and environmental factors cause type 1 diabetes and environmental events can operate very early, even in utero. The early induction of diabetes-associated autoantibodies and the long pre-diabetic period means that autoimmune diabetes in children can be predicted by detecting these autoantibodies. The purpose of prediction is prevention and initial studies suggest that it is possible to modify the disease process. However, the impact of such therapy on the disease is as yet extremely modest.

KEY WORDS: autoantibodies, autoimmunity, diabetes, immunomodulation, prediction, prevention, twins

Autoimmune diseases affect 10% or more of the UK population. In organ-specific autoimmune diseases, a particular tissue is targeted by an aggressive immune response which can damage and even destroy it. One such disease, type 1 diabetes, is due to destruction of the insulin-secreting beta-cells in the islets of Langerhans of the pancreas.1 Type 1 diabetes has its peak incidence in adolescence, and is the second commonest chronic childhood disease after asthma, though the disease is probably more prevalent in adults than in children. At the clinical onset of type 1 diabetes, about 80% of islets contain no insulin-secreting islet cells and the islets may be infiltrated with mononuclear cells, predominantly macrophages and CD8+ T lymphocytes. This infiltrate is, directly or indirectly, probably the major cause of the beta-cell destruction.

Understanding the induction of autoimmunity has enabled us not only to predict diabetes but also to initiate efforts to alter the disease process with the aim of preventing clinical disease. Lessons learned from autoimmune diabetes could be relevant to other autoimmune diseases.

Limited genetic causation in type 1 diabetes

Type 1 diabetes is genetically determined, as shown by family, twin and genetic studies. The frequency of type 1 diabetes is greater in siblings of diabetic patients (eg in the UK 6% by age 30) than in the general population (0.4% by age 30).2 Familial clustering could be due to shared genetic or environmental factors. To distinguish between them, twin studies have been employed. Concordance rates for autoimmune diseases are higher in identical than in non-identical twins. This is consistent with a genetic influence on these diseases.3 The most important genes implicated in the genetic susceptibility to type 1 diabetes are in the histocompatibility (HLA) region of chromosome 6.2 There is substantial disease heterogeneity, hence age-related genetic factors influence the risk of type 1 diabetes, as well as the presence of diabetes-associated autoantibodies, the rate of progression to clinical diabetes, and the severity of reduced insulin secretory capacity. Not only is the age-related incidence of type 1 diabetes lower in adults than in children, the range of incidence across European countries is also reduced. Furthermore, there is a male excess in disease incidence which becomes evident during puberty and is most striking in the age group 25 to 29 years. Non-diabetic identical twins of probands diagnosed with type 1 diabetes under 25 years of age had, in one study, a 38% probability of developing diabetes compared with only 6% for twins of probands diagnosed later (Table 1).4–6 Such a remarkably low twin concordance rate for adult-onset type 1 diabetes, lower than that for influenza, implies that the genetic impact in adult-onset diabetes is limited, and certainly lower than that in childhood-onset disease.4 The limited genetic impact on type 1 diabetes introduces the possibility that non-genetic effects, particularly environmental ones, are relevant.

An early environmental event as cause of type 1 diabetes

We speculated that an early environmental event could induce autoimmune diabetes based on the
known epidemiological features of the disease, including a rapid decline in disease incidence both in childhood and in identical twins of patients with type 1 diabetes. Subsequent observations suggest that critical disease-inducing environmental events can indeed operate very early, even in utero, though this may only be true for childhood-onset disease and not necessarily for adult-onset autoimmune diabetes.

Some maternally related events associated with a greater disease risk in children but not in adults are shown in Table 2. Children of diabetic mothers are less likely to develop type 1 diabetes than children of diabetic fathers and the risk in children of diabetic mothers is less than the expected risk based on their HLA status. The mean life-table risk of diabetes in offspring of diabetic mothers and fathers in one study was 1.3% and 6.1% respectively. This low disease risk is confined to offspring of diabetic mothers and fathers in one study was 1.3% and 6.1% respectively. This low disease risk is confined to offspring of diabetic mothers and fathers in one study was 1.3% and 6.1% respectively.

The first born has the highest risk of diabetes, the risk falling with maternal age at birth, while the effect of paternal age is smaller. The risk of offspring developing diabetes increases with maternal age at birth, while the effect of paternal age is smaller. The first born has the highest risk of diabetes, the risk falling thereafter by 15% per child born. Blood group incompatibility between mother and child may also predispose to diabetes, though the cause remains obscure.

### Induction of autoimmunity leading to diabetes

If the critical event which induces the destructive immune process operates in early childhood, it follows that diabetes-associated immune changes which reflect that process may also be detected at an early age (Table 2). Timing of the onset of autoimmunity is a prerequisite for unmasking triggers in the pathogenesis of this disease. At birth, children of diabetic mothers often have islet cell autoantibodies (ICA), insulin autoantibodies (IAA) and glutamic acid decarboxylase (GAD) autoantibodies. But these autoantibodies can also be found in the maternal serum and are probably placentally transferred to the child, since autoantibody specificities are similar in mother and cord blood and are not detected in the infants of mothers without such autoantibodies. Passively acquired maternal autoantibodies disappear after birth as expected, but can subsequently be replaced by the infant’s own autoantibodies. In one study, 3 out of 58 infants of diabetic mothers developed ICA, IAA and GAD de novo by two years of age and only then were autoantibodies associated with diabetes risk. The cumulative risk of type 1 diabetes in 1,353 offspring of diabetic parents was 18% at age five years but the risk was 50% in those with more than one diabetes-associated autoantibody. Intriguingly, passively acquired maternal autoantibodies may protect children from later autoimmune diabetes.

Whilst cord blood autoantibodies are mainly transplacentally acquired, diabetes-associated autoantibodies can appear at a very young age. For example, 85% of New Zealand schoolchildren who seroconverted to ICA did so before five years of age. Of 137 children with ICA from a prospective Finnish study of 4,590 consecutive newborns with the disease-risk HLA-DQB1, IAA and GAD autoantibodies usually appeared before ICA while insulinoma-associated-2 (IA-2) autoantibodies usually appeared afterwards. Strikingly, 95% of seroconversions to IAA, GAD, or IA-2 autoantibodies occurred in a cluster (~12 to +8 months) around the time of ICA seroconversion. Children at high genetic risk seroconverted steadily at approximately twice the rate of those at moderate risk. Thus, induction and activation of diabetes-associated autoantibodies is not confined to early childhood and seroconversion may be detected up to at least 10 years of age.

Despite the limited genetic risk implied by twin studies, patients with adult-onset autoimmune diabetes show HLA genetic susceptibility irrespective of whether they present with insulin or non-insulin requiring diabetes. The latter patients, mistakenly diagnosed first with type 2 diabetes, have autoimmune non-insulin requiring diabetes designated latent autoimmune diabetes of adults (LADA). This form of auto-

### Table 2. Potential environmental agents that could induce autoimmune diabetes in children.

<table>
<thead>
<tr>
<th>General factors</th>
<th>Special factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hygiene</td>
<td>Toxins</td>
</tr>
<tr>
<td>Parasites</td>
<td>Cow’s milk (through early exposure)</td>
</tr>
<tr>
<td>Co-existent infections (TB or malaria)</td>
<td>Viruses (eg enteroviruses)</td>
</tr>
</tbody>
</table>

### Table 1. Concordance for type 1 diabetes in identical twins according to age at clinical onset in the index twin. Note the substantially lower concordance rates in the older-onset twins consistent with a marked non-genetically determined effect causing diabetes in them.

<table>
<thead>
<tr>
<th>Young onset (years)</th>
<th>Older onset (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK/USA (Ref 4)</td>
<td>38% (&gt;25)</td>
</tr>
<tr>
<td>USA (Ref 5)</td>
<td>44% (&gt;15)</td>
</tr>
<tr>
<td>Finland (Ref 6)</td>
<td>50% (&gt;10)</td>
</tr>
<tr>
<td></td>
<td>6% (&gt;25)</td>
</tr>
<tr>
<td></td>
<td>13% (&gt;15)</td>
</tr>
<tr>
<td></td>
<td>23% (&gt;10)</td>
</tr>
</tbody>
</table>

### Key Points

Autoimmunity is due to the interaction of genes and the environment

Type 1 diabetes in children is due to an early environmental event

Type 1 diabetes of adult-onset is predominantly environmentally determined

Presence of autoantibodies predicts autoimmune disease

Autoimmune diseases can be modified by immunomodulation
immune diabetes affects about 10% of recently diagnosed non-insulin requiring European adults, implying that it is more prevalent than childhood type 1 diabetes and is now the subject of a major European Union project, Action LADA, designed to characterise it. About 90% of LADA patients progress to insulin dependence within six years, so that, rates of progression to insulin dependence apart, it is difficult to distinguish between adult-onset type 1 diabetes and LADA.\textsuperscript{11-13} Despite all we know about the induction and activation of the autoimmune response in childhood-onset disease, we know next to nothing about induction events in adult-onset autoimmune diabetes.

Taken together, these observations suggest that non-genetic activation of the diabetes-associated immune process, possibly by viruses or dietary factors, can occur in early childhood. However, seroconversion is not confined to early childhood; so, by implication, activation of the diabetes-associated immune response cannot solely occur in early childhood in all cases of autoimmune diabetes.

Prediction of autoimmune diabetes

Niels Bohr warned, ‘Never predict, especially about the future’, but it is the predictable pattern of diseases, both in their natural history and their response to therapy, which has been the cornerstone of modern medicine. The early induction of diabetes-associated autoantibodies and the long pre-diabetic period suggested the possibility that autoimmune diabetes could be predicted. Indeed, autoantibodies, which appear in the peripheral blood long before clinical symptoms, are more reliable predictive markers than the presence of high-risk genes, not only in diabetes but also in a substantial number of other autoimmune diseases.\textsuperscript{12,13}

If an autoantibody is used to predict a disease, then three criteria must be fulfilled:\textsuperscript{13}

• Every non-diseased subject with the autoantibody will eventually develop the disease (high disease positive predictive value).
• Every non-diseased subject with the autoantibody will develop the associated disease and not any other disease (high disease specificity).
• Every subject who developed the disease will have that particular autoantibody (high disease sensitivity).

The higher the positive predictive value the greater is the population risk of developing the disease (disease-risk). The feasibility of screening for autoantibodies as predictors of disease has been convincingly demonstrated over the last few years in the case of type 1 diabetes.\textsuperscript{12,13} International workshops have demonstrated the validity of assays, in terms of consistency and accuracy, for certain antigen-specific autoantibodies.\textsuperscript{14} Using these assays, the positive predictive value for diabetes increases for one, two or three autoantibodies from approximately 10% to 50% and 80% respectively within five years and even higher thereafter.\textsuperscript{13}

As before, there is the caveat that our ability to predict autoimmune diabetes in childhood-onset cases has yet to be demonstrated in adult-onset individuals. If the immune process associated with the development of type 1 diabetes is sometimes initiated later in life, then population screening will have to be performed at different ages to detect induction of diabetes-associated autoantibodies in the pre-diabetic period.\textsuperscript{15} Indeed, as autoantibodies to different antigens appear sequentially, and the predictive value of an autoantibody combination varies with age, disease-risk based on autoantibody combinations will require repeated screening with different combinations.\textsuperscript{13} Screening strategies will therefore need to be flexible.

Prevention of autoimmune diabetes

The aim of disease prediction is disease prevention. Type 1 diabetes could be prevented by avoiding those environmental factors which cause the disease process (primary prevention); or by modulating the destructive process before the onset of clinical diabetes (secondary prevention); or by trying to cure the disease process at the time of diagnosis (tertiary prevention).

Primary prevention

A primary prevention strategy for type 1 diabetes requires that critical environmental factors such as diet or viruses are recognised and removed, or their effect negated, whilst remembering that infections could be protective (Table 3).\textsuperscript{15} Thus, diabetes could theoretically be prevented by vaccination against enterovirus infections or by postponing the introduction of cow’s milk beyond four months of age.\textsuperscript{15} Alternatively, maintaining breast-feeding beyond three months post partum could limit the risk of disease, since studies suggest that cessation of breast feeding before that time is associated with a greater risk of diabetes. The Trial to Reduce the Incidence of diabetes in the Genetically at Risk (TRIGR) is under way and will test the hypothesis that late introduction of cow’s milk protein prevents type 1 diabetes.\textsuperscript{15} This multinational study plans to enrol 2,400 genetically high-risk babies (identified by HLA alleles) who will be randomised to formula feed containing either cow’s milk or casein hydrolysate after breastfeeding up to the age of nine months. If, however, environmental factors causing diabetes can operate later, then these factors might be different and could induce a different type of destructive immune process. In that case, primary and secondary prevention strategies might also differ from those which are used for childhood-onset diabetes.\textsuperscript{15,16}

Table 3. Autoantibodies as predictors of type 1 diabetes.

<table>
<thead>
<tr>
<th>Autoantibodies:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• can appear at an early age, even around the time of birth</td>
</tr>
<tr>
<td>• can precede the clinical onset of diabetes by some years</td>
</tr>
<tr>
<td>• are more predictive when they recognise certain autoantigens</td>
</tr>
<tr>
<td>• have a positive predictive value that increases for number of autoantibodies</td>
</tr>
</tbody>
</table>

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**Secondary prevention**

Secondary prevention (ie after disease induction but before clinical diabetes develops) could prevent autoimmune diabetes by:

- protecting insulin-secreting cells
- resting insulin secreting cells
- immune modulation including antigen-based strategies.

The field of study has been hindered by the extensive use of an animal model, the non-obese diabetic (NOD) mouse, which can be cured of diabetes in many different ways that offer little of value for modifying human autoimmune diabetes. A number of studies suggested that nicotinamide could prevent diabetes onset both in NOD mice and in man. Nicotinamide may operate through promoting DNA repair. A large study in New Zealand suggested possible benefit, but subsequent studies set up to assess whether nicotinamide could prevent progression to type 1 diabetes in high disease-risk children of individuals with type 1 diabetes (DENIS and ENDIT) failed to show any benefit.17

In an alternative approach, again successful in the NOD mouse, early and aggressive therapy with insulin therapy before the onset of clinical diabetes was used to rest the insulin-secreting beta cell, making it less prone to immune attack. As a result a trial was mounted to determine whether insulin therapy could delay or prevent diabetes in non-diabetic relatives of patients with diabetes (DPT-1); it had no beneficial effect.16 Alternatively, insulin might modulate the aggressive immune response if that response is targeting insulin as an antigen. Again, in both biobreeding (BB) rats and NOD mice such insulin therapy delayed the development of diabetes and of insulitis. But a study of oral insulin in at-risk children based on such hypothetical immunomodulation also failed to identify a positive benefit (unpublished data). Such trial failures have been disappointing but highlight the problem of relying too heavily on an inconsistent animal model. Further, given the differences between childhood-onset and adult-onset autoimmune diabetes, therapy to modify the disease process in each case could also differ; for example, antigen-specific therapy might involve insulin-related compounds in children, while in adults GAD or IA-2 related strategies could be more relevant. Future protocols may benefit from incorporating the patient’s age at diagnosis into the study design.

**Tertiary prevention**

Tertiary prevention (in recently diagnosed patients with diabetes) has the ethical advantage that a more aggressive therapy can be considered as the patient already has the disease, but the disadvantage that it may be too late to offer therapy since much of the insulin secretory capacity is already lost. Two approaches have been employed:

- general immunosuppression
- immune modulation including antigen-based strategies.

The first immunosuppression study was started in 1976 using high dose steroids, azathioprine, anti-lymphocyte globulin and plasmapheresis, over a one-month period in men newly diagnosed with type 1 diabetes.18 No controls were considered necessary since a cure would have been sufficient and only a cure could have justified such aggressive therapy. No cure was obtained. Subsequently, the goal of immune therapy shifted from a cure to an effect, as physicians became more modest in their ambitions, seeking improved C-peptide levels, reduction in hyperglycaemia or a fall in glycated haemoglobin (an index of blood glucose control). These endpoints may well prove beneficial but are some way off the initial grandiose target of a cure. The immunosuppressant cyclosporin, studied in large randomised multinational trials, could modify the disease process: at two years after the diagnosis patients in the treatment arm had more C-peptide, indicating some preservation of islet beta-cell function.19

Another interesting approach has been the use of anti-CD3 antibodies. In animal models of diabetes, these antibodies can reverse established disease. A small study found some effect of anti-CD3 antibodies in preserving C-peptide response to a mixed-meal challenge in patients with newly diagnosed type 1 diabetes.20 The mode of action is unclear but there is some depletion of T cells which is incomplete and transient. Most likely anti-CD3 antibodies operate, at least in NOD mice, through immunomodulation causing tolerance induction by modifying CD4+CD25+ cells, known as regulatory cells.

A more targeted approach uses immune modulation with antigen-based strategies. Heat shock protein (hsp60) is a stress protein that could be a major target antigen in several inflammatory diseases, including type 1 diabetes and rheumatoid arthritis.21 T cells that recognise hsp60 derived from pre-diabetic NOD spleens can adoptively transfer insulitis and hyperglycaemia to young pre-diabetic NOD mice, thus demonstrating that the autoimmune response to hsp60 is not an epiphenomenon, but plays a role in the pathogenesis of diabetes in NOD mice.21 The diabeticogenic T cells recognise a hsp60 epitope corresponding to positions 437-460, called p277. A more stable modified peptide called DiaPep277 proved to be a potent inducer of protection from the development of diabetes in the mouse models. One preliminary study in man suggests that DiaPep277 injections can preserve endogenous insulin production in patients recently diagnosed with type 1 diabetes, though it will again be important to determine whether this effect is age-related and consistent.21

Finally, a preliminary study of autoimmune non-insulin requiring diabetes patients with GAD antibodies found that a tolerance induction approach using alum formulated whole GAD (Diamyd™) had a significant effect on the C-peptide responses at one dose, but not at other doses, consistent with modulation of the aggressive disease process (RDG Leslie et al, unpublished observations). Further, an increase in CD4+CD25+/CD4+CD25- T cell ratio, a ratio which reflects the proportion of CD4+CD25+ T cells which include regulatory T cells, was positively correlated with the change in both fasting and stimulated C-peptide levels over the initial 24 weeks of the study. These effects are promising but must be considered in the context of the small clinical effect which suggests that the
therapeutic effect, if confirmed, would need to be enhanced and, perhaps, introduced at an earlier stage.

Conclusion

In summary, although many issues remain unresolved, screening of populations for susceptibility to certain autoimmune diseases is now feasible. Apart from the studies on type 1 diabetes, the value of autoantibodies in most other autoimmune diseases, both as disease predictors and for disease classification, has not been fully explored. It is likely that, in the future, risk assessment will use mathematical models that incorporate the number and character of autoantibodies together with genetic markers. High throughput methods should make it possible to rapidly screen for dozens of autoantibodies at low cost, and screening for autoantibodies may become a routine part of a medical examination. The practical value of screening healthy populations to detect individuals at high risk for a particular autoimmune disease will be enhanced once preventative measures and safe therapy become available. Those therapies are not yet available. We will have to turn our attention to environmental factors that induce autoimmune diabetes at different ages, as well as the impact such events have on the dynamic stability of our physiology. Initial studies suggest that although we can modify the disease process even when it has reached the stage of clinical diabetes, the impact of such therapy, if any, on the disease is extremely modest. We are still a long way from preventing autoimmune diabetes.

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Trial acronyms

DENIS = Deutsche Nicotinamide Intervention Study
ENDIT = European Nicotinamide Diabetes Intervention Trial
TRIGR = Trial to Reduce the Incidence of diabetes in the Genetically at Risk

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