Autoimmunity to Glutamic Acid Decarboxylase in Insulin-Dependent Diabetes

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Most individuals produce autoantibodies and autoreactive T lymphocytes but only about 5% of any population develop an autoimmune disease. Organ specific autoimmune diseases, including insulin-dependent diabetes mellitus (IDDM), tend to target functional elements such as enzymes. A 64 kD protein is a major islet antigen associated with IDDM and at least part of the antigen complex has been identified as glutamic acid decarboxylase (GAD) which exists as multiple isoforms and both forms are recognized by diabetes-associated antibodies. About 80% or more of newly diagnosed patients with IDDM have autoantibodies to intact 64 kD and its trypsin-released fragments. These antibodies could be important predictors of subsequent disease in non-diabetic individuals. Strategies for disease prevention will involve identification of high risk individuals and their treatment with immunomodulation which alters the immune response to critical antigens such as GAD.

Nature of Autoimmunity

Essential elements of the immune system, such as the T cell receptor and immunoglobulins, are likely to be derived from early recognition molecules and their corresponding genes. Since the earliest recognition molecules were probably involved in recognition of self, the earliest antibodies were probably autoantibodies. Autoimmunity should not be considered as inevitably deleterious. On the contrary, it is an essential and beneficial mechanism allowing the clearance of self-antigen debris from the circulation and the production of a complex network of immune regulation called the idiotypic network. In short, autoimmunity is important to the fitness of the organism.

Most individuals produce autoantibodies and autoreactive T lymphocytes. However, only about 5% of any population develop an autoimmune disease. Control mechanisms must therefore operate to control the development of autoimmune diseases. These control mechanisms remove cytotoxic immune cells in various ways including: clonal deletion, clonal anergy and limiting antigen accessibility to the immune system. Antigen accessibility is limited by antigen being sequestered in privileged sites such as the brain, by antigen not being processed for presentation to the immune system or by autoreactive T lymphocytes circulating in an inert state. Breakdown in these control mechanisms could lead to disease.
Diseases associated with autoimmune phenomena tend to distribute themselves within a spectrum of organ specific diseases, such as insulin-dependent diabetes (IDDM) and non-organ specific diseases such as systemic lupus erythematosus. There may be clustering of diseases at either end of this spectrum; thus, IDDM is more common in patients with thyroiditis or adrenalitis.

**Diabetes as an Autoimmune Disease**

At diagnosis of IDDM, both cellular and humoral immune changes occur in peripheral blood and about 80% of islets contain no insulin-secreting B cells but are heavily infiltrated with lymphocytes. There is no evidence that exocrine pancreatic cells or other islet cells are involved in this destructive process. The destruction could result from either an exogenous agent, e.g. a virus, or an endogenous agent e.g. the immune system. The rapid destruction of apparently normal B cells when transplanted from a non-diabetic twin to their diabetic co-twin indicates that the destructive process must be outside the islet, B cell specific and retain its cytotoxic memory. The immune system is the most likely candidate for such an extra-islet cell effect.

Autoimmune diseases, including IDDM, tend to have a genetic basis, particularly through genes in the HLA DR and DQ region on chromosome 6, though these diseases are not due to an abnormal general but rather susceptibility conferred by certain normal genes. Genetic influences are important in IDDM but are not crucial, since only about 36% of identical twins and about 10% of non-identical twins of diabetic patients develop the disease; a heritability of 52%. As most identical twin pairs are discordant for IDDM non-genetic, probably environmental, factors must be important.

**Targets of Autoimmunity**

In distinguishing between self and non-self the organism's most efficient approach is to identify features (antigens) conserved within a species and not features unique to a subtype of that species. Therefore, conserved elements, both structural and functional, are obvious targets for immunity (Table 1). Organ specific diseases tend to target functional elements such as enzymes, e.g. thyroid peroxidase (Hashimoto's thyroiditis) whilst non-organ specific diseases target structural elements, e.g. histones and DNA (systemic lupus erythematosus). The 'luxury function' of all endocrine cells is to recognize a signal (glucose for the B cell) and produce and secrete a hormone (insulin and insulin precursors). Whilst IDDM patients can have antibodies which interfere with B cell glucose transport, hormone secretion systems may be more important antigen targets and include enzymes (carboxypeptidase H and glutamic acid decarboxylase) and insulin and proinsulin.

**Islet cell antibodies**

These antibodies are detected by indirect immunofluorescence in about 80% of newly diagnosed patients with IDDM and only 0.8-4% of the normal community. The antibodies are IgG, polyclonal and usually restricted to the IgG1 isotype. They recognize antigens on islet B cells but also other non-B islet cells. The nature of the antigen remains unclear but in polyendocrine patients it appears to be the enzyme glutamic acid decarboxylase (GAD) and in patients with IDDM it may be, in part, a glycolipid.

**Insulin autoantibodies**

Insulin autoantibodies (IAA) can be detected using a radiobinding assay or an enzyme-linked immunosorbent assay (ELISA). Antibodies detected by the radiobinding assay are more closely associated with IDDM than IAA detected by ELISA. IAA are not specific for IDDM and can also be found in other autoimmune diseases, in drug-induced autoimmunity and following viral infections. Newly diagnosed diabetic patients have IAA but the frequency of IAA, unlike ICA, varies inversely with age being found in up to 100% of diabetic children aged less than 5 years but as little as 4% of adult diabetics.

**Autoantibodies to a 64 kD Islet Protein**

The 64 kD antigen was originally detected as a major radiolabelled protein immunoprecipitated from detergent extracts of metabolically labelled islets by circulating autoantibodies in the sera of IDDM patients. Antibodies to 64 kD were also detected in two animal models of diabetes – the BB rat and the NOD mouse. The protein is typically
resolved as two components of Mr-64 000 and 67 000 by SDS polyacrylamide gel electrophoresis and may therefore represent a complex of more than one polypeptide chain. At least part of the antigen complex has more recently been identified as glutamic acid decarboxylase (GAD).7

Location and specificity

GAD is the enzyme responsible for the synthesis of the neurotransmitter, gamma-amino butyric acid (GABA) in the GABA-ergic neurons of the central nervous system. In addition to the central nervous system and pancreatic B cells, GAD and GABA have also been detected in several other tissues including kidney, liver, adrenal gland, ovary and testis. Within the pancreatic islet, GAD and GABA expression have been shown to be restricted to the B cells where GABA synthesis and secretion may be involved with paracrine regulation of glucagon and somatostatin secretion.8

There is evidence that GAD exists as multiple isoforms. In mammalian brain two forms of GAD (GAD 65 kD and GAD 67 kD) have been distinguished on the basis of antigenicity and intraneuronal distribution, and three others on their chemical characteristics.9 Both GAD 65 kD and GAD 67 kD have been detected in pancreatic islets and both forms are recognized by diabetes-associated antibodies. The identities of isoforms of GAD expressed by other non-neuronal tissues have yet to be resolved but appear to be antigenically distinct and lack determinants recognized by diabetes-associated antibodies.10

Both GAD 65 kD and GAD 67 kD derive from a single separate gene localized to chromosome 10 and chromosome 2 respectively.11 The genes code for polypeptides of 585 and 594 amino acids respectively and there is considerable sequence homology (65%) between the two genes and also between these genes and their counterparts in rats, cats and the fruit fly. These extensive sequence homologies suggest that GAD structure has been subject to intense selective pressure during phylogeny.

Clinical studies of GAD antibodies

Using the immunoprecipitation technique about 80% of newly diagnosed patients with IDDM have 64 kD autoantibodies. Antibodies to 64 kD persist for up to 3 years after the diagnosis of IDDM despite declining insulin secretion and a decreasing frequency of ICA.12 Evidence that the 64 kD antigen is GAD is derived from studies of a rare and severe neurological disease called stiff-man syndrome. Most patients with this syndrome have autoantibodies to GAD.13 These patients have an increased incidence of IDDM and those patients with IDDM and stiff-man syndrome not only had GAD antibodies but also ICA. The evidence is that ICA in these patients is predominantly, maybe entirely, composed of antibodies to GAD. Patients with polyendocrine diseases including IDDM also tend to have serum ICA which persists for many years and, in them, the predominant antigen appears to be GAD. These observations suggest that GAD is a major target antigen in IDDM and that antibodies to it, together with antibodies to other antigens, to a variable degree, comprise ICA as detected by immunofluorescence.

Antibodies to trypsin-derived fragments of 64 kD

Tryptic proteolysis of purified 64 kD antigen reveals several major fragments which are themselves antigenic including fragments, of molecular weights 50 kD, 40 kD and 37 kD.14 It was proposed that such trypsin digestion might either reveal hidden or distinct epitopes, enabling further analysis of any heterogeneity in the immune responses to the 64 kD antigen. Comparison of antibodies immunoprecipitating the 50 kD trypsin-solubilized polypeptide with 64 kD antibodies and GAD antibodies demonstrated a significant association between these specificities; whereas, no significant association was found between 64 kD or GAD antibodies and antibodies to either the 40 kD or 37 kD trypsin-solubilized fragments. A strong positive correlation was observed between antibodies to the 40 kD and 37 kD tryptic fragments. These observations were interpreted as showing two distinct antibody specificities associated with the 64 kD antigen, one specificity associated with the 50 kD fragment which retains GAD activity, and another specificity associated with the 37 kD and 40 kD fragments which does not have GAD activity. These 37 kD and 40 kD antigens might be part of GAD or separate polypeptides completed with GAD in the 64 kD antigen.

Approximately 93% of patients recently diagnosed with IDDM have at least one of these two distinct antibody specificities; that is 81% had antibodies to the 50 kD fragment and 78% had antibodies to the 37 kD or 40 kD fragment but only 7% did not have antibodies to either antibody specificities.14 These antibodies are rare in controls. Thus, screening for antibodies to epitopes on tryptic polypeptides of the 64 kD antigen may be of even greater predictive value for development of
IDDM than analysis of antibodies reactive with the intact 64 kD antigen alone.

**Antibodies and Prognosis**

All the antibodies described above can be detected in non-diabetic relatives of patients with IDDM and may have predictive value. The risk of developing IDDM has been assessed by actuarial analysis in prospective studies. The positive predictive value of an antibody has been calculated as the chance that an individual with it develops IDDM. The positive predictive value for ICA varies from 90% to 3% in twins and siblings of patients with IDDM respectively, depending on the titre of the antibody. Prospective studies of IAA in non-diabetic siblings using a radioligand assay showed a positive predictive value for IAA of between 6% and 25%. These observations suggest that IAA in itself is of little predictive value.5

Antibodies to the 64 kD antigen were detected in samples obtained from 34 of 42 patients tested up to 91 months before the onset of IDDM.15,16 These observations suggested that antibodies to 64 kD islet antigen might be an early marker of B cell autoimmunity. On the other hand, 64 kD antibodies have been detected in individuals who have remained non-diabetic for up to 6 years without any deterioration in their B cell function17 suggesting that they are unlikely to develop IDDM. Thus, the predictive value of 64 kD antibodies may be limited. In addition, the assays are costly and laborious and do not lend themselves readily to population screening.

Preliminary studies have been performed to assess the potential of the various antibodies we have described above as predictors of diabetes. Two cohorts of non-diabetic subjects at risk of IDDM have been studied; a group of non-diabetic identical twins with IDDM18 and a group of non-diabetic patients with polyendocrine disease who had ICA (unpublished observations). Both groups were followed prospectively during which time a fraction of them developed IDDM. Sera from these subjects were tested for antibodies to GAD using an enzyme activity assay as well as antibodies to cryptic fragments of the 64 kD antigen. Antibodies to GAD, 50 kD and 37 kD components were detected at high frequencies in both twins and polyendocrine patients who subsequently developed diabetes but in low frequency in patients who remain non-diabetic (Table 2). Whilst a number of twins and polyendocrine patients who remain non-diabetic had antibodies to GAD and 50 kD antibodies, only one twin had 37 kD antibodies. Thus distinct antibodies to the 64 kD antigen confer different risks for IDDM, the highest risk being associated with the 37 kD antigen.

It should be noted that all the antibodies we have discussed, i.e. ICA, IAA and GAD, 64 kD, 37 kD and 50 kD antibodies have been detected in individuals who are considered unlikely now to develop diabetes. In no study has every individual with these antibodies developed IDDM. Thus the immune process associated with IDDM does not inevitably lead to the disease.19

**Antibodies do not Destroy Islet B Cells**

If autoantibodies did cause IDDM they should fulfil the following criteria which, to date, they do not: (1) specificity for the B cell and in particular its membrane; (2) destructive potential; (3) presence in all patients with IDDM and in all individuals who subsequently develop IDDM; (4) ability to transfer disease from IDDM patients to healthy individuals or animal models. Autoantibodies could therefore be beneficial scavengers of antigen debris or result from a T cell dependent cytotoxic response.

**Implications for Disease Prevention**

It is envisaged that some of the antigens identified by antibodies play an essential role in the aetiology of IDDM. These antibodies could be important predictors of subsequent disease. However, the disease process is probably mediated by autoreactive T lymphocytes and preliminary studies suggest that peripheral blood lymphocytes from patients with IDDM are reactive to GAD.20 The value of identifying antibodies associated with IDDM is twofold: firstly, as predictive markers for IDDM, and secondly, to identify critical disease-associated antigens. Strategies for disease prevention will involve identification of high risk individuals and their treatment with immunomodulation targeted at altering the adverse immune response to critical antigens. Investigation

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Table 2

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<th>Frequency of antibodies in 58 non-diabetic identical twins of diabetic patients who later developed IDDM (n = 12) or remain non-diabetic (n = 46). 37/40 kD antibodies had the highest positive predictive value</th>
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<td>Prediabetic twins (%)</td>
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of humoral autoimmunity in IDDM will therefore continue to play a vital role in elucidating and ultimately preventing this devastating disease. The identification of GAD as one of the major target antigens in IDDM has been an important step towards this goal.

References