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Review Articles

Islet Cell Antibodies – Theoretical and Practical Implications

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Type 1 (insulin-dependent) diabetes in man is associated with a major loss of pancreatic B cells. This seems to be remarkably cell-specific since other endocrine cell populations of the islets of Langerhans survive even in long-standing disease [1].

The destructive process and the initiating mechanisms are unknown. The results from several studies demonstrate that antibodies reactive with pancreatic islet cells (Table 1) are often present at the time of diagnosis of Type 1 diabetes.

The demonstration of circulating islet cell antibodies was preceded by observations of lymphocytic infiltration of the endocrine pancreas (insulitis) in newly diagnosed juvenile diabetic patients [2] and by evidence of cellular hypersensitivity against islet antigens [3].

There may be a link between these immunopathological phenomena and the observation that Type 1 diabetes is associated with certain HLA-DR determinants, in particular HLA-DR3 and HLA-DR4 [4, 5]. More than 90% of caucasoid children with Type 1 diabetes carry one or both of these specificities [5]. The genetic loci coding for these antigens, which are expressed mainly on B lymphocytes and macrophages, reside in the major histocompatibility complex on the human 6 chromosome. The association between HLA-DR specificities and Type 1 diabetes argues that the HLA-DR locus may be in linkage disequilibrium with some other unknown genetic factor predisposing to Type 1 diabetes. The major histocompatibility complex contains genes coding for cell surface proteins involved in a variety of immunological phenomena including allograft rejection, initiation of antibody formation and certain cell-cell interactions between subsets of immunocompetent cells [6, 7]. In addition, proteins belonging to the complement system are also coded for by genes in the HLA region. The role of the major histocompatibility complex in regulating the immune response may be relevant to an understanding of those events which result in the formation of islet cell antibodies. At present, islet cell antibodies can be detected in several assay systems which we have listed in Table 1.

Islet Cell (Cytoplasmic) Antibodies

Several studies have examined the prevalence of islet cell antibodies detected in an indirect immunofluorescence assay on pancreatic sections from blood group 0 donors [8, 9]. This limiting pre-requisite is due to a non-specific exocrine reaction on pancreatic sections from donors of other blood groups [8]. The fluorescent reaction involves a common antigen found in the cytoplasm of all the endocrine cells in the islets. Bound antibodies can also be revealed by an immunofluorescent complement-fixation test (Table 1) or by immunocytochemical staining with peroxidase-

Table 1. Assay systems for islet cell antibodies

Islet cell antibody assay	Method of detection
Islet cell (cytoplasmic) antibodies	Immunofluorescence on tissue sections
Complement-fixing islet cell antibodies	Immunofluorescence on tissue sections
Islet cell surface antibodies	Immunofluorescence or ^{125}I-Staphylococcus protein A on dispersed islet cells
Complement-dependent cytotoxic islet cell antibodies	^{51}Cr-release or vital staining
Antibody-dependent cellular cytotoxic antibodies	^{51}Cr-release from islet cells
Immunoprecipitating islet cell antibodies	Gel electrophoresis of precipitated islet cell antigens

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conjugated antibodies (Marner, personal communication).

The character of the islet cell antigen, whether a protein, lipid or carbohydrate remains to be determined. Adsorption of serum to subcellular fractions of islet cells should make it possible to determine whether the antigen is located, e. g. in the mitochondrial, microsomal or secretion granule fraction. Such information would be helpful in our attempts to understand the pathogenetic role of the islet cell cytoplasmic antibodies.

The islet cell cytoplasmic antibodies are usually found at the onset of Type 1 diabetes [8, 9] and their prevalence decreases with increasing duration of Type 1 diabetes except when the disease is associated with other autoimmune endocrinopathies [8, 9]. The value of islet cell cytoplasmic antibodies as a 'marker' of ongoing B cell destruction in Type 1 diabetes is not known. It is not clear to us how this will ever be achieved unless the antibodies are determined quantitatively. It will be necessary to standardize the assay, report positive serum samples in titres and define assay reproducibility and precision.

Islet Cell Surface Antibodies

In a hypothetical model involving recognition of the B cells by the immune system, whether cellular or humoral, target molecules or antigens are likely to reside in the plasma membrane, facing the external environment. Several studies have examined the prevalence of islet cell surface antibodies in Type 1 diabetes (Table 1) using fixed or living islet cell suspensions of human or rodent origin in an indirect immunofluorescence test. The prevalence of islet cell surface antibodies seems to parallel that of cytoplasmic antibodies [10] although the two assay systems do not always yield concordant results [11]. Again we believe that it will be necessary to determine the antibodies quantitatively. The availability of defined suspensions of endocrine islet cells [12, 13], which in time will be replaced by specific endocrine cell lines [14] will allow surface-bound antibodies to be determined both objectively and quantitatively, e. g. with ^{125}I-labelled protein A [12].

The fact that living islet cells bind antibodies to their surface makes it possible to examine their effects, if any, on cell function. Islet cell surface antibodies were found to inhibit the biosynthesis of insulin [15] while insulin secretion was little affected. It remains to be shown whether the antigen recognized by islet cell (surface) antibodies is a protein, perhaps a membrane receptor for agents regulating B cell function.

Cytotoxic Antibodies

Two other immunological phenomena involving antibodies bound to living cells (Table 1) are of possible importance in destruction of the islet cells in Type 1 diabetes.

Firstly, cell surface-bound antibodies are known to be recognized by components of the complement system. Recognition of IgM or IgG molecules on the cell surface will allow the complement cascade reaction to occur resulting in a proteolytic attack on the cell membrane. The lesions lead to an altered membrane permeability and eventually to cell death. Fresh serum [16] and sera from Type 1 diabetic patients, or immunoglobulins prepared from such sera, appear capable of mediating a complement-dependent cytotoxic reaction against a variety of islet cell preparations [17, 18]. The assay systems, especially when employing radioisotope release, are objective (Table 1) and should prove useful in the prospective analysis of the presence of islet cell antibodies, e. g. in children with previous viral infections.

The pathogenetic importance of the complement-dependent cytotoxic reaction remains unclear. Autoimmune reactions involving blood cells [19] and B cells differ in that the B cells are normally separated from the blood stream by capillaries. Therefore, both antibodies and macro-molecules of the complement system must transverse this barrier. An immunoglobulin fraction from a patient with islet cell surface antibody did not block insulin release from an isolated mouse pancreas perfused with complement [20].

Secondly, cell surface antibodies may be the target of so-called natural killer cells (K cells). The resulting reaction is known as antibody-dependent cellular cytotoxicity (Table 1). The K cell can be found among the circulating monocytes [21] and is thought to possess cell surface receptors which bind the constant portion of the antibody molecule. The K cell acts either as an armed cell with antibodies attached, or attacks cells with an antibody bound to the surface. There is evidence that Type 1 diabetic patients have increased levels of low affinity E-rosette forming cells [22], a blood cell population shown to contain the major proportion of circulating K cells.

A xenogenic rat islet cell surface antiserum has been found to induce an antibody-dependent cellular cytotoxic reaction against rat islet cells [20]. Further studies are needed to show if the antibody-dependent cellular cytotoxicity is merely a phenomenon in vitro, or whether information relative to the pathogenesis of Type 1 diabetes can be derived from this system. In patients with systemic lupus erythematosus, antibodies to T-lymphocytes can mediate an antibody-

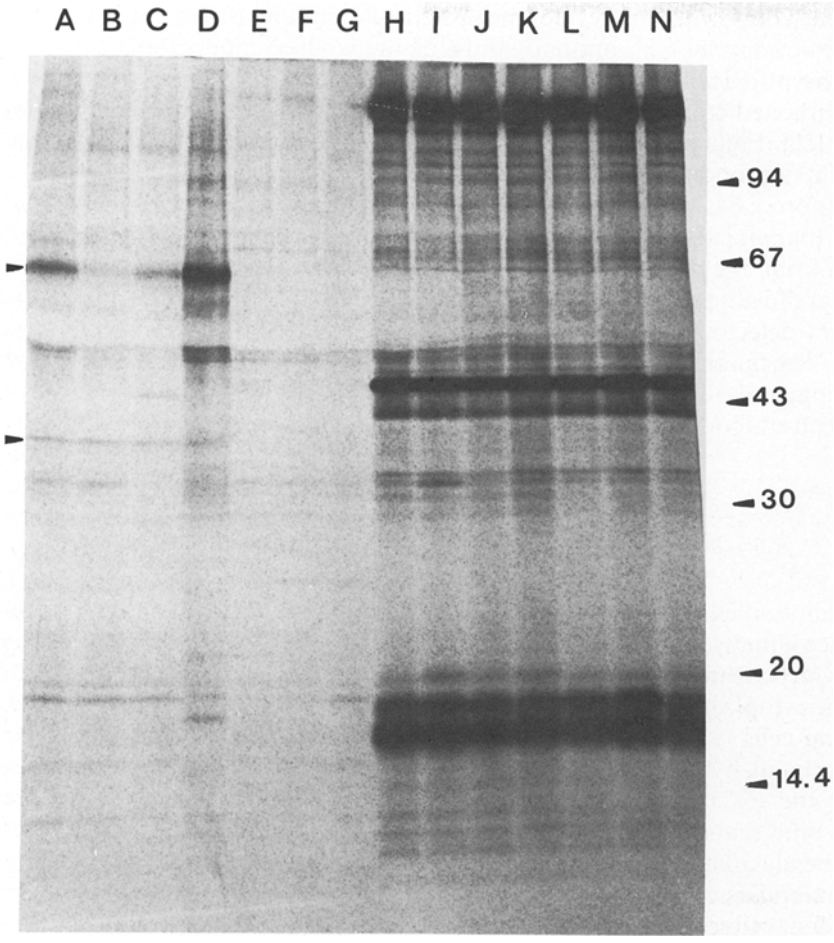

Fig. 1. Immunoprecipitation of human islet cell and human peripheral lymphocyte proteins with diabetic and normal serum.

Islets of Langerhans were isolated from the pancreas of a cadaver kidney donor being HLA-A1, 25; B8, 35 C4 and DR3/3 positive. The islets were labelled biosynthetically for 16 h with ^{35}S-methionine. The islet cells were solubilized in NP-40 and the lysate treated with normal human serum and immunoprecipitated with heat-killed formalin-fixed *Staphylococcus aureus* bacteria. The resulting supernatant was used for immunoprecipitation with diabetic and normal sera. Human peripheral lymphocytes were labelled with ^{35}S-methionine for 8 h and treated identically.

Immunoprecipitates were solubilized and subjected to electrophoresis on a 10% polyacrylamide gel in the presence of sodium dodecyl sulphate. The Figure shows an autoradiogram of the dried gel.

Lanes *A–G:* Immunoprecipitation of human islet cell proteins. Lanes *H–N:* Immunoprecipitation of human peripheral lymphocyte proteins. Lanes *A, B, C, D,* and *H, I, J, K:* Sera from four newly diagnosed Type 1 diabetic children, all HLA-DR 3/4 positive. Lanes *E* and *L:* Sera from an adult patient with insulin dependent diabetes for approximately one year. Lanes *F, G* and *M, N:* Serum from two healthy individuals.

Arrows to the right indicate the position of molecular weight ($\times 10^{-3}$) markers. Arrows to the left indicate the proteins immunoprecipitated specifically from human islet cells by diabetic sera.

dependent cellular cytotoxic reaction against human T-lymphocytes [23].

Immuno-Precipitating Antibodies

Islet cell antibodies can now be assayed by several different procedures (Table 1). It is open to doubt, however, whether there is any point in searching for further immune correlates in Type 1 diabetes until a possible B cell specific target, perhaps a host antigen induced by a preceding viral infection, has been identified. Degeneration of tissues and cells apparently attracts the immune system, but the remarkable cell specificity of this attention needs to be explained. A certain HLA-DR type seems to be necessary as well as a precipitating event possibly involving a virus or another environmental factor. In children with mumps the prevalence of islet cell cytoplasmic antibodies is high, but the number of children developing Type 1 diabetes is very low [24].

Why do islet cell antibodies not react only with 'diabetic' B cells and what is the antigenic stimulus? We have recently demonstrated that biosynthetically labelled islet cell antigens can be precipitated from detergent extracts of pancreatic islets [20]. Figure 1 shows that diabetic sera may contain antibodies against at least two proteins (mol. wt. approx. 64,000 and 38,000 daltons) present in normal human islets. In contrast, normal sera and the serum from one patient with diabetes for more than one year did not precipitate these two proteins, nor were they detected in lysates of labelled human lymphocytes. The relationship between these immuno-precipitating antibodies and the different assay systems for islet cell antibodies needs to be investigated.

Theoretical and Practical Implications

We believe that islet cell cytoplasmic antibodies are unlikely to be of pathogenetic importance if they solely react with intracellular components which are inaccessible in the living cell. In addition, the cytoplasmic antibodies react with all islet endocrine cells [8, 9]. Islet cell surface antibodies, on the other hand, by definition bind to component(s) facing the exterior. Whether all endocrine cells are able to bind diabetic islet cell surface antibodies remains to be elucidated.

The presence of islet cell surface antibodies capable of mediating a complement-dependent cytotoxic reaction does not necessarily imply that these antibodies are actively 'diabetogenic' since they have been found in a large proportion of first degree relatives of diabetic probands [18]. The various approaches to islet cell antibody determination and the different characters of the tissue preparations may be taken as an argument that islet cell antibodies are directed against several different antigens. The possible presence of B cell specific antigens which may be recognized in autoimmune diabetes is currently being studied with xenogenic [12] as well as monoclonal [25] antibodies. Such antibodies have proved useful in the biochemical analysis of transplantation antigens expressed on the B cells [26].

An important achievement has now been reported by Eisenbarth et al. [27] who fused human myeloma cells with lymphocytes from a diabetic child. One hybridoma culture, B6, produced an IgM antibody which bound to the cytoplasm of islet cells in sections of human pancreas. These experiments are important since they demonstrate that B lymphocytes carrying islet cell antibody-producing cells can circulate in the blood of a patient with Type 1 diabetes. Furthermore, the production of human monoclonal autoantibodies offers a number of practical implications such as the development of radioimmunoassays for the autoantibody utilizing the labelled monoclonal autoantibody as a tracer. In addition, it may be possible to produce a form of immune intervention with antibodies raised against the variable regions of the monoclonal autoantibody (anti-idiotypic antibodies). Passive transfer of autoantibodies to immunosuppressed mice could be used to test the hypothesis that islet cell antibodies are diabetogenic.

In conclusion, we do not think that the many cross-sectional clinical studies of islet cell antibodies allow any judgement to be made as to whether islet cell antibodies are causal or just an association or consequence of cell damage. A confusing factor is the lack of defined workshop sera and tissue preparations. Even if the assay systems are made quantitative, it will be difficult to draw any conclusions about the meaning of islet cell antibodies, while we do not know the antigen.

The advent of human monoclonal antibody production should provide means by which not only the diabetic antibody in itself can be characterized, but also the antigen(s) with which these antibodies react. Since diabetic sera immunoprecipitate some human islet cell proteins, we believe that it should be possible to extract the messenger RNA for these proteins and determine their structure by molecular cloning. This approach should eventually allow these islet cell antigens to be made by polypeptide synthesis [28]. We think it is important to establish the role these proteins play in B cell destruction and to develop a radioimmunoassay for circulating islet antibodies.

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