# Autoantibodies to Islet Cells in Diabetes Mellitus

ANTOINE KALDANY

**SUMMARY**

**Numerous reports have confirmed the presence of islet cell antibodies in diabetic patients. These are found mostly in newly diagnosed insulin-dependent diabetic patients and in patients who have autoimmune polyendocrine disorders. Antibodies to beta cells, somatostatin, and glucagon-producing cells have been described as well. All these antibodies give strictly intracytoplasmic staining. It is therefore difficult to understand their role in the pathogenesis of pancreatic damage. The presence of another antibody is thus postulated. DIABETES *28*:102–105, February 1979.**

Numerous reports have confirmed the presence of islet cell antibodies (ICA) in some diabetic patients. More interestingly, those diabetics with the HLA–B8 antigens seem to have a much higher prevalence of ICAs.[9] While other anti-endocrine pancreas antibodies have been reported, the so-called ICAs have generated a sustained and widespread interest. Through a review of the data published to date, an attempt will be made to assess and elucidate the nature and significance of these antibodies in regard to their potential role, if any, in the pathogenesis of diabetes mellitus (DM).

## BACKGROUND INFORMATION

**Human immunoglobulins and immunofluorescence.** Although quite heterogenous (sedimentation constants of 7S and 19S), the immunoglobulins share slow electrophoretic mobility, producing a wide (polyclonal) $\gamma$-globulin spike on serum electrophoresis. Human Igs are divided into five principal classes on the basis of chemical and isotypic properties: IgG, IgA, IgM, IgD, and IgE. These molecules are antigenic and induce the production of specific humoral responses with antibody production when injected in laboratory animals (goat, rabbit, etc.). Animal immunoglobulins can also be raised against human complement component.

The fluorescent properties of certain organic residues that can be attached to antibody molecules provide the basis for the immunofluorescent technique. This method is widely used for rapid identification and localization of cellular antigens and antibodies. In the direct staining reaction, the antibodies labeled with fluorescent isothiocyanates are specific for the antigen of interest. In the indirect reaction, the labeled antibodies are specific for the antibodies of another species (e.g., goat anti-rabbit immunoglobulin, rabbit anti-human immunoglobulin, etc.).

In 1966 Nakane et al. introduced the application of enzymes as labels in immunohistocytochemistry.[1] Since then the peroxidase-labeled antibody methods (so-called immunoperoxidase technique) has helped localize antigens at both the light and the electron microscopic levels. Recent improvements of this methodology (conjugation of peroxidase to Igs, fixation on antigens, etc.) enhance intracellular and tissue localization of antigens.[2]

**Mechanisms of immunopathogenic reactions.** In vivo, tissue damage can be caused by one or more of the following immune reactions according to Gell and Coombs[3]: (a) type I, involving preformed IgE on most cells and basophilic polymorphonuclear cells and mediated by various monoamines; (b) type II, cytolytic reactions involving (fixation of) IgG or IgM class antibodies on cell membranes with either complement-mediated damage, or involvement of "killer" lymphocytes (Fc receptor-bearing cells); (c) type III, initiated by immune complexes, with participation of neutrophils, which release their proteolytic enzymes; and (d) type IV, tissue damage due to chemically active mediators (lymphokines) released by specifically reactive (precommitted) lymphocytes.

## CIRCULATING ICAs IN DM

**Technical aspects.** The presence of ICAs is detected by indirect immunofluorescence. Sera collected from various subjects are serially diluted and then incubated over thin

From the Joslin Clinic and E. P. Joslin Research Laboratory, Divisions of the Joslin Diabetes Foundation; the Departments of Medicine, New England Deaconess and Peter Bent Brigham Hospitals; and Harvard Medical School, Boston, Massachusetts.

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frozen sections of fresh human pancreatic, adrenal, thyroid, and gastric tissue; rat and guinea pig pancreatic tissue; and rat liver and kidney tissue. The human tissues are harvested from individuals of blood group O.[4] The tissue sections are then stained with fluorescein-labeled anti-human immunoglobulins and anti-human complement preparations. Tissue sections are then examined under ultraviolet light.

**Circulating ICAs in insulin-dependent diabetes (IDD).** The first reports of circulating ICAs linked their presence to coexisting polyendocrine autoimmune disorders:[5,6] 18 of 276 patients tested in the first two series had circulating ICAs. All 18 had circulating autoantibodies to one or more other organs (adrenal, thyroid, gastric mucosa, intrinsic factor). All 18 had polyendocrine disorders. Fifteen of the 18 patients had IDD (83%).[5,6] In a more recent study, 36 of 522 nondiabetic patients with proven AID (6.9%) had circulating ICAs. This differs significantly from the very low prevalence of ICAs in the general population (0.5%).[7] A long term follow-up of these patients with repeated glucose tolerance tests (GTT) revealed that 14 patients (38.9%) developed glucose intolerance.[7] Thus, the presence of circulating ICAs in patients with polyendocrine autoimmune disorders is a marker for asymptomatic prediabetes in this patient population.

However, the detection of circulating ICAs does not imply that a given patient had autoimmune disease, as circulating ICAs are detectable in uncomplicated IDD.

All available series show a surprisingly high prevalence of ICAs in recently diagnosed IDD. Nearly one half of recently diagnosed IDD children tested by Lendrum et al.[8] had circulating ICAs. In contrast, thyrogastric antibodies were detected in only 0.06% of the juveniles; and these were found in older children only.

Three groups have recently completed extensive and representative screening of large populations.[9–11] Their combined results are shown in Table 1; the overall prevalence of ICAs in IDD is 29.2%. Whether or not circulating ICAs are detected in IDD depends largely on the duration of symptoms.[9–11] Lendrum et al. found ICAs in 85% of IDD immediately after the onset of diabetes.[10] ICAs became less frequent as the duration of symptoms increased.[9,10] A similar phenomenon was reported by Irvine et al.: 60% of patients with IDD had circulating ICAs during the first year, only 20% had ICAs at 2–5 yr; and only 5% at 1–20 yr.[9] No correlation can be found between ICAs and patients' ages at the time of testing when the duration of diabetes is taken into account.[9]

In most patients with IDD, circulating ICA titers tend to decrease gradually and possibly disappear. In a sizable percentage of cases, however, ICA titers remain stable for several years.[9–11,13] Furthermore, ICA can be detected in nondiabetic patients: (a) The overall incidence of ICA in the population at large varies from 0.9% to 1.7%; (b) 31 of 522 patients (6%) with documented autoimmune disorders have circulating ICAs;[7] and (c) ICAs can be detected in relatives of patients with IDD[7–12] as well as in playmates of children with newly diagnosed IDD.[12]

In an effort to interpret this array of facts, Bottazzo and Doniach proposed a classification of IDD into two categories (A and B):[13] type A, uncomplicated IDD, characterized by absence of autoimmune polyendocrine disorders, mostly in juveniles (age < 30 yr) with a very high incidence of ICA at onset (85%) that tends to decrease and disappear gradually; and type B, IDD with autoimmune disease and very high frequency of autoantibodies, where ICA levels can be very high years before onset of diabetes and remain stable for years.

TABLE 1
ICAs in diabetic patients and nondiabetic controls

| Patients tested (no.) | ICA (+) | ICA (+) (%) |
|---|---|---|
| IDD: 1848 | 540 | 29.2 |
| IID: 851 | 54 | 6.3 |
| ND: 1716 | 22 | 1.3 |

Combined data from Irvine et al.;[9] Lendrum et al.,[10] and Del Prete et al.[11]

IDD, insulin-dependent diabetic; IID, insulin-independent diabetic; ND, nondiabetic.

To confirm this proposed classification, one has to show that ICAs appear at, or right after, the onset of the disease in uncomplicated (type A) IDD. This is quite difficult to ascertain and can be accomplished only via a pursuit of a systemic and inclusive screening of large populations and families with multiple cases of IDD without polyendocrine autoimmune disorders.

**Circulating ICA in insulin-independent diabetes (IID).** The reported prevalence of ICAs in IID varies from 8% to as low as 6%.[7,9,14] Of the 851 patients with IID reported to date, 54 have documented circulating ICAs (mean percent ICAs = 6.3) (Table 1).

Irvine et al. found circulating ICAs in the sera of 20 patients with IID treated with oral hypoglycemic agents.[14] On long term follow-up (>4 yr), only two patients (10%) remained stable while 13 patients (65%) required insulin and five patients (25%) required a combination of oral hypoglycemic agents at maximal doses. In contrast, only 14 of 150 (8.8%) patients lacking ICAs required insulin over a mean period of 50 months. It seems as if IID with circulating ICAs is an earlier stage of a disease process, culminating in ICA-positive IDD.

In fact, a greater prevalence of IDD is found in family members of patients with IID and circulating ICAs.[7,9] Further, in an extended survey of patients with AID, Irvine et al. identified and followed 36 such patients with circulating ICAs.[7] Fourteen of these patients (38.9%) had an abnormal GTT. Four of the 36 patients (11%) developed IDD.

Although all of these series are relatively recent, with much still to be learned from longer follow-up, it seems that circulating ICAs can serve as markers for future insulin dependence in some cases of IDD and latent diabetes. A problem that remains to be settled is whether ICAs are responsible for future beta cell failure, or whether they are simply a result of an ongoing damage.

## ICA AND HLA ANTIGENS

Several reports have confirmed the association between some HLA antigens and IDD. HLA–B8, Dw3, and, more recently, Dw4 are increased in Caucasian populations.[15–18] On the other hand, HLA–B8 and Dw3 are increased in autoimmune disorders such as Addison's disease, Graves' disease, and hypogonadotrophic hypogonadism.[15] As one

TABLE 2
ICAs and HLA-B8 in diabetes

| Patients tested | No. of patients | HLA-B8 frequency (%) |
|---|---|---|
| Normal controls | 300 | 28 |
| ICA (−) diabetics | 20 | 35 |
| ICA (+) diabetics | 99 | 61 |
| ICA (+) diabetics (at 1 yr) | 56 | 55 |
| ICA (+) diabetics (at >5 yrs) | 35 | 71 |

Data from Morris et al.[24]

would expect, there is a strong association between HLA-B8 and ICAs (Table 2). This correlation becomes stronger if ICAs persist for more than 5 yr after the onset of DM.[9,11,13] No data is yet available for Dw3 or Dw4 and ICA.

## OTHER ANTIBODIES TO ENDOCRINE PANCREAS

**Antibody reacting to human insulinoma cells.** Maclaren et al. incubated sera from 39 IDD patients with cultured human insulinoma cells.[20] These cells had been maintained in cell cultures since 1959. Positive immunofluorescence with presence of IgM and IgG was given by 34 out of 39 IDD sera. In contrast, only two of 15 IID and one of 30 nondiabetic controls reacted with these cell cultures.

The presence of anti-insulinoma cells antibodies in IDD sera does not seem to be related with insulin therapy; in six IDD patients, sera were obtained prior to any insulin therapy. The relationship between positive insulinoma cells fluorescence and circulating ICAs is unclear, since these 39 IDD sera were not tested for so-called ICAs.

**Autoantibodies to human glucagon and somatostatin-producing cells.** In 1976 Bottazzo and Lendrum reported the presence of specific granular intracellular fluorescence reaction against either glucagon or somatostatin-producing cells.[21] Out of 1279 sera tested, 13 (1.01%) gave specific alpha cell fluorescence and four (0.31%) gave specific somatostatin cell fluorescence. This specificity was confirmed by double staining techniques; the antibodies were of either IgG or IgM class. These autoantibodies are clearly distinct from the so-called ICA; in some instances ICAs were identified as IgG, while anti-glucagon cell antibody reacted as an IgM. The absence of plasma membrane staining indicates that circulating antibodies fail to react with plasma membrane determinants.

The exact significance of these antibodies is unclear, as they were found in diabetic patients as well as in normal controls. The reported cases are too few in number to permit speculation on their eventual role in the pathogenesis of any endocrine disorder.

**Autoantibodies to beta cells.** Pretreating diabetic sera with peroxidase, Sorensen et al.[22] obtained indirect IgG fluorescence staining of beta cells only. Once again the staining was strictly intracytoplasmic. The significance of such antibodies is uncertain because sera from 12 patients with cystic fibrosis and 19 nondiabetic controls gave a similar fluorescence of beta cells only.

## SUMMARY AND DISCUSSION OF PATHOGENESIS

The presence of antipancreatic autoantibodies seems to correlate with progressive destruction of islet cells and increased insulin deficiency.

**Circulating ICAs and immunogenetic factors.** Some important differences between patients with polyendocrine autoimmune disorders and those without evidence of autoimmune disorders have been outlined in this review. HLA-B8 correlates strongly with autoimmune disease IDD and circulating ICAs (Table 2).[24] One would then speculate that the major histocompatibility complex gene products play a role in disease susceptibility and development of autoimmunity.[16] Although suggestive, the data are only preliminary.

**Nature of circulating ICAs.** Circulating ICAs are distinct entities: the acinar cells never stain, and the immunoperoxidase technique, for example, gives a different pattern.

It is impossible to speculate whether or not the presence of ICAs reflects beta cell damage. While circulating ICAs tend to disappear gradually in some IDD, they persist in some others. Without serial measurements of C-peptide levels, no answer can be provided.

**What is the role of circulating ICAs?** An antibody can be either protective or destructive. One could speculate that ICAs protect beta cells against type IV reactions[3] by forming antigen-antibody complexes. This could explain the presence of circulating ICAs in a nondiabetic twin for several years without evidence of carbohydrate intolerance.[23] Conversely, if ICAs are to act as destructive antibodies, they could do so either by promoting K-cell cytotoxicity, or by causing target cell lysis via complement activation. Both mechanisms require interaction with target cell membrane. This is difficult to imagine considering that ICAs, or any of the above-mentioned antibodies, do not produce cell membrane staining. How then could ICAs promote beta cell lysis by leaving the membrane intact?

**If not ICAs—another antibody?** The same difficulties remain if one considers any of the aforementioned antibodies. However, there is always a possibility that another antibody exists. This antibody could well have a very high affinity to beta cells, which explains why its presence has gone undetected. The presence of such antibody will remain speculative until direct immunofluorescence studies are performed on fresh pancreatic tissue sections obtained from juveniles with recent onset IDD. Furthermore, acid elution of this pancreas should retrieve this active antibody and allow both in vitro and in vivo (passive transfer) assessment of its anti-beta cell affinity and cytotoxic activity.

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