

“SEROLOGICAL MARKERS OF ISLET CELL AUTOIMMUNITY”

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Introduction

Islet cell autoantibodies constitute the basic serological markers of islet cell autoimmunity and are important to our understanding of diabetogenesis and potential future therapies. The two main classes of islet cell autoantibodies include:

1. Those directed against currently ill denned cell membrane associated islet autoantigens (ICAb-'CM').
2. Those directed towards the insulin molecule, the polypeptide hormonal secretory product of islet beta cells. (ICAb-INS).

Anti cell membrane islet cell antibodies are commonly detected by immuno-histochemical assays and anti insulin islet cell antibodies are measured by liquid phase radioligand assays.

The anti cell membrane islet cell antibodies are of Ig G class, fix complement and participate in immune cytolysis. They are detectable several years prior to the onset of insulin dependence and disappear from circulation, months to a few years after clinical diagnosis. They are often associated with organ specific autoimmunity (thyroid, gastric, adrenal etc.) and in such subjects with multiple organ specific autoimmunity they persist for a much longer time and at higher titres.

Anticell membrane islet cell antibodies are associated with recurrence of autoimmune insulinitis in pancreatic and islet cell grafts during transplantation. These auto antibodies are possibly more directly relevant to immunopathogenesis of beta cell destruction and insulin dependent diabetes mellitus.

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The anti insulin islet cell antibodies develop spontaneously, also several years prior to onset of insulin dependence and therapy. In studies of newly diagnosed insulin dependent diabetics and pre insulin dependent diabetes mellitus subjects, their levels correlated inversely with age with the highest levels being found in younger children. Anti insulin islet cell antibodies possibly reflect ongoing islet cell injury and the rate of beta cell destruction.

Insulin dependent diabetes mellitus is found in 15% of patient of idiopathic Addison's disease and 7-10% patients of auto immune thyroid disease¹. There is also evidence to suggest that different endocrine disorders of autoimmune character are 4-5 times more prevalent among patients of insulin dependent diabetes mellitus than in the general population. In addition, the prevalence of antithyroid, parietal cell, intrinsic factor or adrenal antibodies is reported to be higher in sera of insulin dependent diabetics than in the control population. Bottazzo² has reported that patients with polyendocrine disorders may develop islet cell antibodies as early as ten years before the onset of clinical diabetes.

In nondiabetic patients Hirata³ first described "the autoimmune insulin syndrome" which is characterized by fasting or reactive hypoglycemia and auto antibodies directed towards the endogenous insulin molecule. In Japan a significant percentage of patients with "autoimmune insulin syndrome" had received methimazole as treatment for thyrotoxicosis.

Blackshear⁴ reported the presence of insulin autoantibodies in an 82 year female who developed systemic lupus erythematosus due to hydralazine and pro-cainamide therapy together with "autoimmune insulin syndrome". Benson⁸ reported the same in two patients being treated with d'pencillamine for rheumatoid arthritis.

Thus, ICAb-'CM' and ICAb-INS constitute two independent and disparate but overlapping serological markers, both useful for clinical assessment of islet cell autoimmunity.

Materials and Methods

Subjects:

The study group was drawn from the outpatients' and patients admitted in the Department of Endocrinology, Metabolism and Diabetes. All-India Institute of Medical Sciences, New Delhi.

There were five study groups:

Group I: (NIDDM group) This consisted of 116 patients of non insulin dependent diabetes mellitus who were on oral hypoglycemic agents or diet therapy. A sub group of those patients also required insulin, but they had received oral hypoglycemic agents for at least one year after the diagnosis of diabetes (secondary failure).

The onset of diabetes mellitus in these patients was at the age of 30-45 years. This group consisted of 76 females and 40 males, the mean age being 47.3 years. The diagnosis of diabetes had been established in these patients by doing an oral glucose tolerance test (W.H.O. criteria).

Group II: (IDDM group) This consisted of 35 subjects with insulin dependent diabetes mellitus who required insulin right from the beginning of the disease and were in the age group 7-45 years. There were 14 males and 21 females in this group, the mean age being 20 years. The diagnosis of diabetes mellitus was made by W.H.O. criteria. All thirty five had already received insulin at the time of the study.

Group III: (Auto immune thyroid disease group) This consisted of 61 proved cases of autoimmune thyroid disease, viz. Graves' disease, idiopathic hypothyroidism, and Hashimoto's thyroiditis. There were 44 females and 17 males in the group, the mean age being 38.8 years. These patients had been investigated from the endocrine clinic of A.I.I.M.S. and serum T3, T4, TSH, thyroid micro-somal antibodies had been done. Fine needle aspiration cytology was also done in patients of Hashimoto's disease. Out of 61 patients, 36 had Graves' disease, 20 had idiopathic hypothyroidism and 5 had Hashimoto's thyroiditis.

Group IV: (Rheumatoid Arthritis group) This consisted of 28 patients, 22 females and 6 males, mean age being 30 years, of proved seropositive rheumatoid arthritis who had been on gold or d-penicillamine therapy for 3 months to 1 year. These patients were referred from the Immunology clinic, A.I.I.M.S. by Dr. R.R. Singh and Prof. A.N. Malaviya.

Group V:

Control group: consisted of 41 healthy blood bank volunteers, of which 40 were males and 1 female. Mean age 35 years.

ASSAY FOR ISLET CELL ANTIBODIES

FITC-protein A method (Srikanta)*

Human Pancreata

The human pancreas were surgical pancreatectomy specimens. Half-centimeter blocks were snap frozen in liquid nitrogen and stored at - 70°C. Thin

cryostat sections (5 microns) were cut onto gelatine dichromate coated slides before use and stored at 70°C.

Human sera

Sera from the various subgroup and controls were stored at -20°C before use.

Second step Reagent

FITC conjugated Protein A- Protein A used in the experiments was prepared from staphylococcus aureus (Cowan strain) using ion exchange and gel filtration chromatography and then labelled with fluorescein isothiocyanate. The final preparation had an immunoglobulin binding capacity of 9.7 mg human Ig G/mg protein and contained 70.5 ug FITC/mg protein (Sigma, ST. Louis, Missouri P-5145).

Indirect Immunofluorescence- Human pancreatic sections were incubated for 30 minutes at room temperature with undiluted human sera. The sections were washed thrice with phosphate buffered saline containing 0.05 sodium azide (PBS) and incubated for 30 minutes at room temperature with FITC - protein A 1:1000 diluted in PBS with 1% bovine serum albumin. The sections were washed thrice again with PBS, the first wash being very fast and the next two washes for 5 min each. After washing, the sections were mounted in PBP with 30% glucose examined under a fluorescent microscope. The relative intensity of islet immuno fluorescence was graded on an arbitrary visual scale of 0-4 based on subjective assessment of differential staining between islet and the background acinar tissue. If islet cell antibodies are present, islet cells are stained apple green, the background islet auto fluorescence is orange red.

Insulin autoantibody assay (ICAb-INS)

Soeldner,⁷

Principle

This is a liquid phase radioligand assay. When radiolabelled (I^{126}) insulin is added to human serum containing anti insulin antibody, the latter binds the former and the bound ligand can be displaced with excess of unlabelled hormone. The amount of antibody present is assessed by determining the extent of binding of the ligand ($^m I$) to the binder (ICAb-INS).

Materials

Labelled and unlabelled insulin

Porcine insulin was procured from Novo (Denmark) and labelling with $^{125} I$ was done by chloramine T method in the radioimmunoassay laboratory of

the Department of Endocrinology, Metabolism and Diabetes AIIMS. Purification of the label was carried out using Sep - Pak CIS cartridge chromatography.

Counter

Radioactivity was counted in a Kontron Analytical MDA 12 channel counter.

Method: A modified method of Palmer described by Soeldner⁷ was followed for the insulin antibody assay.

Results

Table 1

Islet cell autoimmunity: Serological markers Results ICAb-'CM'

	N	+ ve	%
IDDM	35	4	11.4
NIDDM	116	0	0
* ATD	61	2	3.3
**RA	28	0	0
CONTROLS	41	0	0
IDDM <2 YRS	12	3	25
IDDM + ATD	4	2	50
NIDDM + ATD	7	0	0

*ATD=autoimmune thyroid disease **RA = Rheumatoid Arthritis

Table 1. shows the results of ICAb-CM studies in the various study groups.

The anti cell membrane islet cell antibodies (ICAb-'CM') were positive in 11.4% of patients of insulin dependent diabetes mellitus irrespective of duration of the disease. In IDDM patients of less than two yrs. duration of the disease 25% were positive for ICAb-'CM'. In the subgroup of IDDM patients with coexistent autoimmune disease 50% were positive for ICAb-'CM'.

None of the 116 patients of NIDDM or 28 patients of rheumatoid arthritis or the 41 controls were positive for ICAb-'CM'.

Table 2
Islet cell autoimmunity: Serological markers
Results: ICAb-INS(Insulin Rx negative)

	n	+ ve	%	% specific binding
IDDM	0	0	0	0
NIDDM	95	1	1.1	6.9%
ATD	54	2	3.7	2.0 (2.2)
RA	28	0	0	0
CONTROLS	41	0	0	0

ATD=autoimmune thyroid disease RA=rheumatoid arthritis ICAb-INS were taken to the positive when the % specific binding was >mean+3D of the 41 controls.

The mean + 3 SD was 0.3 + 1.5=1.8.

So ICAb-INS was taken to be positive when specific binding was > 1.8%.

Table 2. shows the results of ACAb-INS studies in the various study groups.

All IDDM patients had taken insulin prior to study and this could not be analysed for spontaneous ICAb-INS. In the NIDDM group 1.1% were positive for ICAb-INS. None of 28 patients of rheumatoid arthritis or 41 controls were positive for ICAb-INS.

(There were 4 more patients of NIDDM who were ICAb-INS positive but whose insulin status could not be reconfirmed as they were lost to follow up. For purity of data those four patients have not been included in the analysis).

Table 3
Islet cell autoimmunity: Serological markers
Results: ICAB-INS+ +VE patients: Clinical profile

No.	Age	Sex	Disease	Duration	Rx	Symptoms of Hypo-glycemia	% Specific Binding	HLA
1.	55	F	NIDDM	15 yr	Glibenclamide + diet	— ve	6.9%	A3 A9, B13 B40, CW3, CW4, DR2, DQW1
2.	48	F	Hashimoto's thyroiditis	1 month	Proloid	+ ve	2.0%	A1 A3, B8 B40 BW6 DR3, DRW52
3.	25	M	Graves'	1 yr	Neomercazole	+ ve	2.0%	

Table No. 3. shows the clinical profile of ICAb—INS positive patients.

Patient No. 1 was a uncomplicated case of NIDDM controlled on glyben-claimed for 13 years and is now on diet alone the specific binding was 6.9%. Patients No. 2 was a case of Hashimoto's thyroiditis with hypothyroidism and was on replacement therapy. The third patient was a case of Graves' disease being treated with neomercazole. The ICAb—INS were positive in very low titres in the two cases of autoimmune thyroid disease (specific binding of 2%) both these cases also had clinical symptoms of hypoglycemia.

ICAb—INS following exogenous insulin administration.

In parallel with the above mentioned studies we found that 21/35 (60%) of IDDM patients on unpurified insulin therapy had insulin antibodies above the normal range (specific binding 1.9% to 26.4% mean =• 14.2%).

12/17 (70.6%) of NIDDM patients on exogenous insulin therapy had insulin antibodies above the normal range (specific binding 2—27.8% mean=13.48%) The duration of insulin therapy did not matter and in one case we found that only one injection of plain insulin produced insulin antibodies of specific binding 20%.

Discussion

In this study we have studied anti cell membrane islet cell antibodies (ICAb—'CM') and anti insulin islet cell antibodies (IcAb-INS) in five groups of subjects namely IDDM, NIDDM, autoimmune thyroid disease, rheumatoid arthritis and healthy volunteers.

1. *IDDM Group:* 4/35 (11.4%) patients were positive irrespective of duration of the disease. However IDDM patients less than two years duration, were positive for ICAb—'CM' in 25% cases. The prevalence in this study was lower than that of Lendrum⁸, (39%), Irvine⁹, (22%) and Delprete¹⁰, (16%) Srikanta¹¹, (31%). The main reason appears to be a small sample size of only 35 patients.

Two of the patients positive for ICAb—'CM' were males, in the younger age group with a short duration of diabetes (<1 yr) and without any organ specific autoimmunity. The other two were females in the older age group, with a longer duration of diabetes (> 2 yrs) and with associated with organ specific autoimmune disease in the form of Grave's disease.

The former two meet the criteria for type Ia DM and the latter for type Ib DM described by Bottazzo¹², given in table 4.

Table 4
Heterogeneity in type 1 diabetes

	Type I (a)	Type I (b)
Etiology	? Viral	Related to organ specific autoimmunity
Overall frequency of DM	10%	1%
Insulin dependent sex	Yes M=F	Yes F>M
Age	<30 yrs	Any age
Associated AI disorder	None	Adrenalitis, gastritis thyroiditis etc.
Incidence of other autoantibodies	Low	High
Frequency of ICA	At onset 85% After 1 yr 20% Tends to dissappear	Not known 38% Remains stable
liter of ICA	1/250	1/250
First appearance of ICA	? at time of viral infection	years before onset of DM

(Bottazzo ¹²)

In the subgroup of IDDM with autoimmune thyroid disease group 2/4 (50%) were positive for ICAb—'CM', whereas in the subgroup without autoimmune thyroid disease 2/31 (6.4%) were positive. Irvine* also found ICAb—'CM*' to be prevalent in 38% IDDM patients with organ specific autoimmune disease and only 22% patients without organ specific autoimmune disease. ICAb—INS in our IDDM patients were not evaluated as all our 35 patients had been treated with insulin.

Table 5
Prevalence of ICAb-'CM' in NIDDM

Country	Positive	Percentage	Study	Duration of NIDDM
U.K.	20/179	11.2	Irvine ¹⁸	<3 months
Italy	17/106	16.0	Di Mario ^{1*}	<1 month
U.K.	—	6%	Irvine ^{9b}	2 years
U.K.	11/154	7.2%	Groop ¹⁵	Irrespective of duration
Japan	19/153	3.2	Kobayashi ¹⁶	— do—
India	0/116	0%	Current study	-do—

II NIDDM Group:

'ICAb—'CM': We studied 116 patients of NIDDM and found the prevalence of ICAb—'CM' to be 0/116 (0%) (Refer to table 5). Compared to the prevalence reported from elsewhere, prevalence in the current study is low (or absent) which could be due to several possibilities including.

1. Difference in the NIDDM population sampled.
2. Differences in the selection criteria.
3. Delayed access to medical care in our country, thus autoimmunity associated diabetes may have already progressed to IDDM before initial physician consultation.
4. A relatively greater incidence of type 2 (non-autoimmune related) diabetes mellitus and or a relatively lower incidence of type I(autoimmune DM) in Indians i.e. immunogenetic and environmental differences.
5. Differences in the assay methodology.

There were 7/116 patients of NIDDM with associated autoimmune thyroid disease. None of them were positive for ICAb-'CM', there was no sex preponderance and mean age was 48.9 years as compared to 29.5 years in the IDDM

with autoimmune thyroid disease group and coexistence seems to be coincidental.

NIDDM group (ICAb-INS):

1/95(1.1%) of NIDDM patients were positive for ICAb-INS. There has been no study reported in India or abroad about this phenomenon. The significance of this finding may become clear when we study IV GTT and serum insulin levels in this patient. The HLA typing of this patient shows A3 A9, B13 B40, Cw3 Cw4, DR2, DR-DQW1 loci which shows there was no prevalence of DR3 or DR4 as occurs in IDDM. The reported hyperglycemia hypoglycemia syndrome¹⁵ has some similarities though the patient was not symptomatically hypoglycemic.

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