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A Prospective Analysis of Antibodies Reacting with Pancreatic Islet Cells in Insulin-Dependent Diabetic Children

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Summary. Islet cell cytoplasmic and cell surface antibodies along with other autogenic tissue antibodies were determined prospectively from the day of diagnosis of insulin-dependent diabetes in a group of children and adolescents. Prior to the initiation of insulin therapy 30 out of 33 were antibody-positive, 67% having islet cytoplasmic antibodies and 67% islet cell surface antibodies. Among 74 age- and sex-matched non-diabetic individuals 1% had islet cell cytoplasmic antibodies and 3% had islet cell surface antibodies. A prospective analysis in 17 patients showed a diminishing prevalence of islet cell antibodies with increasing duration of diabetes. Islet cell cytoplasmic or cell surface antibodies were found independently of each other or in combination and with various patterns of persistence. The results indicate a strong association of islet cell antibodies with the onset of insulin-dependent diabetes in childhood and adolescence.

Key words: Insulin-dependent diabetes, islet cell antibodies, islet cell surface antobodies, autoimmunity.

In insulin-dependent diabetes mellitus (IDD) there is a high incidence of circulating antibodies which react with pancreatic islet cells [1, 2, 3, 4, 5, 6]. Cross-sectional analyses indicated that the prevalence of islet cell antibodies is maximal at the time of diagnosis of IDD [1, 3, 5]. In these studies islet cell antibodies were determined by an indirect immunofluorescence test on sections of frozen human pancreas. The assay requires the use of group 0 human pancreas and a positive reaction, primarily due to IgG [7], covers the cytoplasm of all the different endocrine cells in the islets [7, 8]. However, serum from individuals with IDD also contains antibodies binding to the surface of dispersed islet cells [6]. Such antibodies were visualized in an indirect immunofluorescence test after incubation with serum from diabetic children [6]. Since living cells are impermeable to IgG the antigens in the two assay systems may not be the same. We have recently observed that islet cell cytoplasmic and surface antibodies may occur independently of each other in patients with IDD [9].

Little is known, however, about the prevalence and fate of islet cell antibodies determined by the different methods in IDD with onset before the age of twenty. In the present study we have determined prospectively from the onset of IDD both islet cell cytoplasmic and cell surface antibodies as well as other autoantibodies, in a group of children and adolescents.

Materials and Methods

Patients

Serum or plasma was collected from 33 insulin-dependent diabetics, 1–16 years of age, admitted to the Departments of Paediatrics at the University Hospitals of Umeå or Linköbing (Table 1). In all patients blood was drawn on the day of diagnosis before the start of insulin therapy. In 17 of the patients blood samples were obtained at routine clinical visits 1, 3, 6 and 9 months later.

Controls

Serum or plasma was obtained from 74 non-diabetic school children, 4–20 years of age (Table 1) from Umeå, Sweden. None had a family history of IDD among first-degree relatives. Informed consent was obtained from all the diabetic and non-diabetic individuals and their parents.

Islet Cell Cytoplasmic Antibodies

Plasma or serum samples were stored at −20 °C before use in an indirect, immunofluorescent assay [8], with undiluted samples applied to fresh, snap-frozen pancreatic tissue of a donor with

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Table 1. Islet cell and other tissue antibodies in healthy and insulin-dependent diabetic children at the day of diagnosis

	Healthy subjects	Insulin-dependent diabetics
No. of subjects	74	33
Age (years)		
Mean	13	9
Range	4–20	1–16
Female/male ratio	36/38	14/19
Islet cell antibodies		
Cytoplasmic (frozen pancreas sections)	1	22
Surface (living mouse B-cells)	2	22
Cytoplasmic and/or surface	3	30
Antibodies against		
thyroid cells	3	2
gastric-parietal cells	1	2
smooth muscle	3	2
reticulin	1	1
mitochondria	0	0

blood group 0 and fluorescein-isothiocyanate-conjugated rabbit anti-human IgG (Wellcome Laboratories, Buckinghamshire, U K). The results were read by two independent readers with a Leitz Orthoplan Microscope fitted with a mercury vapour lamp and Ploem illuminator with KGq, BG 38, KP 490/X 2, GT 475, TK 510 dichroic mirrors and K 515-filters.

Islet Cell Surface Antibodies

Plasma or serum was heat-inactivated (56 °C, 20 min) and subjected to ammonium sulphate precipitation at 33% saturation. After incubation for 60 min at 4 °C with slow rotation of the tubes, the precipitate was collected at 4 °C by centrifugation at 2200 x g, and dissolved in the original sample volume in a 10 mmol/l Hepes (N-hydroxyethyl piperazine-N-2-ethane-sulfonic acid) buffer, pH 7.4, containing 140 mmol/l NaCl. The resulting solution was dialyzed against 100 to 200 volumes of the same buffer for 24 h at 4 °C and finally centrifuged at 100000 x g for 60 min. The supernatant fluid was stored in aliquots at −20 °C.

Pancreatic islets were prepared from Sprague-Dawley rats or ob/ob mice as described in detail elsewhere (6, 10, 11). The pancreatic islets were dispersed into cell suspensions by mechanical shaking in Swim's S-77 medium (Grand Island Biological Company, New York, USA) containing ethylene-glycol-bis (oxyethylenenitrilo)tetra-acetic acid (EGTA). The dispersed cells were incubated with the serum preparations as described in detail elsewhere [6]. Antibodies binding to the surface of the cells were revealed in an indirect, immunofluorescence test with fluorescein-isothiocyanate conjugated rabbit anti-human IgG (Miles Laboratories, Elkhart, Indiana or DAKO Immunoglobulins, Copenhagen, Denmark). The cells were mounted live on coded slides. The serum samples were run at random and were evaluated independently by two investigators in Umeå and by one in Chicago using a phase contrast microscope equipped with a Zeiss IV FI epifluorescence condenser. A minimum of 100 single and intact cells, as judged by phase-contrast microscopy, were evaluated for the presence of a cell surface immunofluorescence reaction in a 1:1 dilution of fractioned serum.

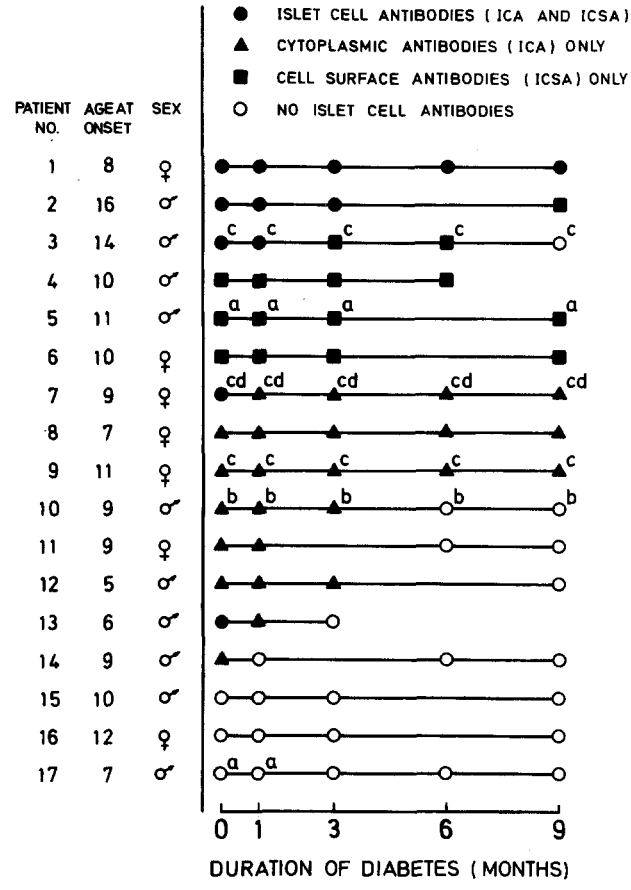

Fig. 1. Prospective analysis of islet cell antibodies in 17 insulin-dependent diabetic children and adolescents. Cytoplasmic islet cell antibodies (ICA) were determined by an indirect immunofluorescence test on sections of frozen human pancreas. Surface islet cell antibodies (ICSA) were determined in an indirect immunofluorescence test on suspensions of live ob/ob mouse and rat islet cells. Other autogenic antibodies: [a]smooth muscle cell antibodies, [b]gastric parietal cell antibodies, [c]anti-nuclear antibodies (the fluorescence analysis was carried out at a sample dilution not diagnostic for anti-nuclear factor), [d]thyroid cell antibodies

The analyses on rat and ob/ob mouse islet cells were carried out independently on coded samples at separate laboratories. Out of 155 samples from diabetic and healthy children 85% of the samples were scored the same, positive or negative, when tested on either mouse or rat islet cells. Only samples scored as antibody positive in both tests were entered as an antibody-positive sample in the final statistical analysis.

In a group of 16 children sufficient serum was available from the day of diagnosis to determine islet cell cytoplasmic and cell surface antibodies on ob/ob mouse islet cells only.

Other Tissue Antibodies

Antibodies to thyroid cytoplasm, gastric parietal cells, smooth muscle, mitochondria, reticulin, as well as anti-nuclear factor were assessed by indirect immunofluorescence test using frozen sections of human thyroid, rat stomach, rat liver or rat kidney. The results were read as in the islet cell cytoplasmic antibody test.

Table 2. Islet cell antibodies in insulin-dependent diabetic children in relation to the duration of the disease

Islet cell antibodies	Duration of diabetes (months)					
	At admission	1	3	6	9	18
Cytoplasmic	11/17	10/17	7/15	3/11	4/15	1/4
Cell surface ob/ob mouse islet cells	11/17	10/16	9/13	4/8	7/16	2/5
Rat islet cells	9/17	6/15	4/13	3/10	4/16	1/5

Results

Prior to the start of insulin therapy 91% of serum samples obtained from insulin-dependent diabetics were positive in the immunofluorescence tests for islet cell antibodies (Table 1). The proportion of discordant scores between islet cell cytoplasmic or surface antibodies was 49% (16 out of 33 patients analyzed). Thus, 67% of the patients were antibody-positive using sections of frozen human pancreas and 67% with suspensions of living ob/ob mouse B-cells.

Only 4% of the sera from 74 non-diabetic individuals were islet antibody-positive with a low frequency of antibodies against other cells or cellular constituents (Table 1). None of the individuals with anti-thyroid or anti-gastric-parietal cell antibodies had islet cell antibodies. Two out of the three non-diabetics with smooth muscle antibodies also had detectable islet cell surface antibodies. The frequency of various tissue antibodies among insulin-dependent diabetic children was similar to that of the healthy controls.

In 17 children and adolescents followed prospectively from the day of diagnosis over 9 months of diabetes, 14 (82%) had islet cell antibodies prior to the start of insulin therapy (Fig. 1). Eight of the 14 antibody-positive patients (57%) remained positive when followed for 6 (1 patient) to 9 (7 patients) months. The results in Figure 1 demonstrate that cytoplasmic and cell surface antibodies may be present independently of each other and that the pattern of persistence of one or both is very variable.

Other tissue antibodies were present in six patient sera (Fig. 1). In all but one sample the tissue antibodies remained positive throughout the nine-month period. In one patient (No. 10) gastric-parietal cell antibodies were present throughout the nine-month period, while sera remained positive for islet cell cytoplasmic antibodies for only three months.

The prevalence of islet cell antibodies determined in the three different assay systems diminished with increasing duration of diabetes (Table 2). Out of 16 patients 4 (25%) had cytoplasmic and 4–7 (25–44%) cell surface antibodies after 9 months of diabetes. The prevalence decreased to 1–2 antibody-positive patients out of 4–5 after 18 months.

Discussion

Our results provide evidence that there is a strong association of islet cell antibodies with the onset of insulin-dependent diabetes in childhood and adolescence. In all patients, representing about 70% of those IDD-patients diagnosed during one year at two Swedish paediatric clinics, care was taken to obtain a serum sample before the insulin treatment was initiated. While the assay of islet cell antibodies using sections of human pancreas confirms previous observations in young IDD patients with sera obtained at, or close to, diagnosis [1, 3, 5], the simultaneous determination of islet cell surface antibodies increased the frequency of antibody-positive sera from 67% of either surface or cytoplasmic alone to more than 90%. In a previous investigation with a mixed group of young and adult IDD-patients [9] with disease duration of less than one month, the use of both methods for antibody-detection increased the number of patients with an immunological marker of the disease from 41–68% to 80%.

When islet cell surface antibodies were analysed by cell surface immunoflourescence on rat or ob/ob mouse islet cells, comparable results were obtained. The assay on ob/ob mouse islet cells tended to give a higher frequency of antibody positive sera (Fig. 2). This may be due to species differences or to the composition of endocrine cells, being 75–80% B-cells in the rat but more than 90% in ob/ob mouse [10, 12, 13]. Since positive sera tend to react with 40–75% of the cells observed in a given sample [6] it was concluded that the islet cell surface antibodies were directed towards B-cells. However, this does not exclude the possibility that antibodies reactive with other endocrine islet cell types are present as well.

The data in Table 1 and Figure 1 suggest that islet cell antibodies may be tissue specific since there was no correlation with other tissue antibodies. In addi-

tion, sera positive to living rat islet cells were little affected by pre-absorption to rat hepatocytes or erythrocytes, or to various rat tissues [6]. Prospective analyses of islet cell antibodies and other tissue antibodies (Fig. 1) suggested that the antibodies may appear and disappear independently of one another. In accordance with previous reports [2, 3, 4, 5] islet cell antibodies were evanescent. Thus, within 9 months of diagnosis they disappeared in 6 out of 13 (46%) patients who were positive on the day of diagnosis (Fig. 1). In one individual, smooth muscle cell antibodies were also temporarily present, whilst other tissue antibodies did not disappear. There is no obvious explanation of why the prevalence of islet cell antibodies depends on the duration of the diabetic condition.

The role of islet cell antibodies in the pathogenesis of IDD is unclear. We conclude from the present investigation that islet cell antibodies at the time of diagnosis of IDD are heterogenous, which may signify the presence of antibodies against different antigens.

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