Islet autoantibodies in cord blood from children who developed Type I (insulin-dependent) diabetes mellitus before 15 years of age

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Summary Islet autoantibodies are early markers for Type I (insulin-dependent) diabetes mellitus. The aim of this study was to establish whether islet autoantibodies were present at birth in children who developed Type I diabetes before 15 years of age. Cord blood sera from 81 children who developed Type I diabetes between 10 months and 14.9 years of age were tested for glutamic acid decarboxylase autoantibodies (GAD65Ab), islet cell antigen 512 autoantibodies (ICA512Ab), insulin autoantibodies (IAA) all by quantitative radioligand binding assays and islet cell autoantibodies (ICA) by indirect immunofluorescence. Cord blood sera from 320 randomly selected matched children were controls. The children who developed Type I diabetes had an increased frequency of cord blood islet autoantibodies compared with control subjects: Glutamic acid decarboxylase autoantibodies were detected in 6 % (5/81) patients and 2 % (5/320) control subjects ($p = 0.03$); islet cell antigen 512 autoantibodies in 5 % (4/73) patients and 1 % (4/288) control subjects ($p = 0.06$); insulin autoantibodies (IAA) in 0 % (0/79) patients and

0.3 % (1/320) control subjects ($p = 0.36$); and islet cell autoantibodies in 10 % (8/81) patients compared with 0.6 % (2/320) control subjects ($p = 0.0001$). Taken together, 17 % (14/81) patients had one or more islet autoantibody compared with 4 % (12/320) control subjects ($p = 0.0001$). Whereas none of the control children had more than one antibody, 4 % (3/81) children who later developed Type I diabetes were double positive ($p = 0.002$). Although glutamic acid decarboxylase autoantibodies' concentrations in cord blood correlated to those in the mothers' blood at the time of delivery, no corresponding correlation was found for the other two types of autoantibodies. The increased frequency of cord blood islet autoantibodies suggests that the Type I diabetes process could already be initiated in utero. [Diabetologia (1999) 42: 181–187]

Keywords Autoimmunity, GAD65 antibodies, ICA-512 antibodies, insulin autoantibodies, islet cell antibodies.

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Abbreviations: GAD65Ab, antibodies to the 65 kDa isoform of glutamate decarboxylase; ICA512Ab, antibodies to the cytoplasmic portion of the ICA512 or IAA-2 protein; IAA, insulin autoantibodies; ICA, islet cell antibodies detected by immunofluorescence on frozen sections; JDF-u, Juvenile Diabetes Foundation units.

Type I (insulin-dependent) diabetes mellitus is a multifactorial disease that develops after exposure to unknown environmental factors in children with specific HLA susceptibility genes. At the time of clinical diagnosis, the majority of the insulin-producing cells in the pancreatic islets have been eradicated in association with autoimmune phenomena including insulinitis [1, 2, 3], lymphocyte proliferation abnormalities [4, 5, 6] and autoantibodies to islet cell antigens [7–9]. Studies in first-degree relatives [7, 8], twins [9], pregnant mothers [10, 11] and school children [12, 13, 14] have found that islet autoantibodies can be present years before clinical diagnosis. Some [15–17], but not

all [9, 18–20] first-degree relatives who are positive for islet autoantibody markers go on to develop diabetes [8, 15]. It is therefore speculated that a primary harmful event leads to beta-cell destruction, subsequent antigen presentation and development of islet autoimmunity long before the appearance of hyperglycaemia. Although the detailed mechanisms of the initiating events leading to autoantibody formation are not understood, current approaches to predict Type I diabetes involve the use of cloned autoantigens in standardised radioligand binding assays to detect autoantibodies of high diagnostic sensitivity and specificity (shown in parenthesis as follows). These autoantigens include glutamic acid decarboxylase (GAD65) (75–85% and 89–99%, respectively) [21–27], ICA512 (IA-2) (48–56% and 95–99%, respectively) [28–30], insulin (28–69% and 99%, respectively) [27, 31–33], as well as ICA (80–90% and 91–97%, respectively) [33–37]. All radioimmunoassays with recombinant autoantigens are predictive of Type I diabetes and it has been suggested that the combination of GAD65Ab, ICA512Ab and IAA can replace the ICA analysis [15, 38].

Recent studies suggest that islet cell autoantibodies can be found at birth in children of mothers who either subsequently develop diabetes [11] or had Type I diabetes during pregnancy [39, 40]. It has, however, not been determined if islet autoantibodies were present at birth in children who later developed Type I diabetes but were born to healthy mothers. We therefore studied cord blood serum samples that had been stored from more than 60,000 newborn babies [11, 41] including 85 (0.14%) children who developed Type I diabetes before they were 15 years of age. Their cord blood serum samples were examined along with the mothers' serum at delivery for GAD65Ab, ICA512Ab, IAA or IA, as well as ICA. The aim of the study was to establish whether islet autoantibodies were present at birth in children who later in life developed Type I diabetes.

Subjects and methods

Patients. The city of Malmö in Sweden has 240,000 inhabitants who are served by Malmö University Hospital, the only hospital in the entire city. All deliveries take place in the Department of Obstetrics of this hospital. In addition, all children with Type I diabetes are treated at the Department of Pediatrics in the same hospital. From 1969 to 1991, umbilical cord blood samples were obtained from the majority of children born at the hospital, along with samples taken from the mothers at the time of delivery [11, 41]. Among children born from November 1969 to December 1991 ($n = 61,146$) and followed until June 1994, there were 106 (0.18%) who developed Type I diabetes before 15 years of age. A total of 85 of these 106 children had cord blood sera saved at the time of birth (1969–1991). Among the 85 children who developed diabetes, 5% (4/85) had a mother and 10% (8/83) a father with Type I diabetes. The information about heredity from the father was

incomplete in two children. Since mothers with Type I diabetes can still be autoantibody positive during pregnancy, the four children who had a mother with Type I diabetes were excluded. All four children had insulin antibodies, two also had GAD65Ab, and one child had ICA512Ab. Three of the four mothers had sera samples saved at delivery, and all three had the same autoantibodies as the children in similar concentrations.

The study comprised 81 children (40 male, 41 female). There was enough cord blood to analyse all four islet autoantibodies (ICA, IAA, GAD65Ab, and ICA512Ab) in sera from 73 children, whereas there was only enough sera for ICA, GAD65Ab and IAA from six patients and only enough for ICA and GAD65Ab from two patients. Sera were also available at delivery from 74 of the 81 mothers. None of the mothers of autoantibody-positive patients have developed Type I diabetes during follow up.

Controls. A total of 320 randomly selected sera (170 male, 150 female) from the bank of cord blood were selected and matched by year of birth to the children who later developed diabetes. There was enough cord blood to analyse all four islet autoantibodies (ICA, IAA, GAD65Ab and ICA512Ab) in sera from 288 children, but only enough sera for ICA, GAD65Ab and IAA analysis in the remaining 32. Information about Type I diabetes among mothers and fathers was not available for the control subjects.

All sera were coded and analysed in the same assay for patients, mothers and control subjects in triplicate. This study was approved by the ethics committee of the Medical Faculty at the University of Lund.

Islet autoantibody assays. We analysed GAD65Ab using a radioligand assay as described previously [23, 26]. The 320 control cord blood sera were used to define the upper limit of normal using the means + 3 SD of the control means. A GAD65Ab index was calculated as described [26]. Our laboratory participated in the international GAD65Ab proficiency test with both sensitivity and specificity of 100%, respectively.

We analysed ICA512Ab in a similar radioligand binding assay as the one described for GAD65Ab [26] using ICA512 cDNA [42] kindly donated by G. Eisenbarth (Denver, Colo., USA) to prepare the ^{35}S -methionine labelled ICA512. The upper limit of normal was defined as for GAD65Ab based on the ICA512Ab index calculated as described [26].

We determined IAA in a competitive binding radioligand assay using monoiodinated insulin as antigen and polyethylene-glycol (PEG) as precipitating agent [32, 31]. Insulin antibodies were considered present if precipitated radioactivity exceeded non-specific binding and was suppressed by the addition of excess unlabelled insulin. Concentrations of IAA were expressed in nU/ml according to an international standard [43]. Values above the means + 3 SD of the control means were considered positive. Our laboratory has participated in the international IAA proficiency programme with both sensitivity and specificity of 100%, respectively.

We analysed ICA with an indirect two-colour immunofluorescence assay [44, 45] and the results were expressed in Juvenile Diabetes Foundation units (JDF-u) by a standard curve based on the international JDF-u reference sera sample [46]. The Malmö laboratory participates in the International Diabetes Workshop proficiency programme, and in the 13th evaluation, our ICA assay showed a sensitivity of 100% and a specificity of 100%.

Statistical Analysis. Comparisons between groups were done with Fishers' exact test (two-tailed), nonparametric statistics

Table 1. Islet autoantibodies in cord blood, sex and the age at diagnosis in children who developed Type I diabetes before 15 years of age

	<i>n</i>	Male/ Female	Age at diagnosis (years)	
			Median	Range
Islet autoantibody				
GAD65Ab	5	3/2	10.6	5.3–14
ICA512Ab	4	1/3	9.4	6.5–11.9
ICA	8	4/4	9.2	3.3–13.1
Antibody negative	67	34/33	8.7	0.9–14.9
All	81	40/41	8.4	0.9–14.9

were done by Mann-Whitney U-test. Correlations were calculated using StatWiew 4.5 software (Abacus Concepts, Berkeley, USA).

Results

The data shows that the age at diagnosis of Type I diabetes ranged from 10 months to 14.9 years (median 8.4 years; Table 1). There was an increased frequency of islet autoantibodies in the cord blood among the 81 children who developed Type I diabetes compared with the 320 control subjects (Tables 1 and 2). There was no significant difference in sex or age at diagnosis of Type I diabetes between patients with islet autoantibodies and those without autoantibodies at birth (Table 1). At least one islet autoantibody was detected in 17% (14/81) of the children who later developed Type I diabetes, compared with 4% (12/320) control subjects ($p = 0.0001$). These data suggest that islet autoantibodies are already common at birth in children who later in life develop Type I diabetes. We next investigated whether any individual type of islet autoantibodies was more prevalent than the others (Table 2). We also analysed the presence of autoantibodies in cord blood compared with the mothers' autoantibodies at the time of delivery (Table 3). For

time clustering, the season of birth and antibody positivity was also analysed (Table 4).

We found GAD65Ab in 6% (5/81) of patients (mean 0.46 GAD65Ab index; range 0.14–1.19) compared with 2% (5/320) among the control subjects ($p = 0.03$) 0.11–0.78 (mean 0.26 GAD65Ab index, range). Sera were available from 4/5 mothers of later diabetic children: two had GAD65Ab at concentrations similar to those of their offspring and a positive correlation was found between the GAD65Ab concentrations in child and mother ($r = 0.825$, $p < 0.0001$). Patients positive for GAD65Ab were more often born in the spring (March-May) than during the rest of the year, compared with the GAD65Ab negative subjects ($p = 0.03$; Table 4).

We detected ICA512Ab in 5% (4/73) of patients (mean 0.03 ICA512Ab index; range 0.02–0.04) and (1%) (4/288) control subjects (mean 0.02 ICA512Ab index; range 0.02–0.04) ($p = 0.06$). Among the ICA512Ab positive children who later developed diabetes, all mothers were ICA512Ab negative, thus no correlation was found between ICA512Ab concentrations in cordblood compared with the mothers' blood ($r = 0.15$, $p = 0.21$).

Insulin autoantibodies (IAA) were not found in any of the children who later developed diabetes compared with IAA in 0.3% (1/320) among the control subjects ($p = 0.36$).

Cord blood ICA were detected in 10% (8/81) of children who later developed diabetes (mean 5.9 JDF-u; range 3–20) compared with 0.6% (2/320) ($p = 0.0001$) in the control group (mean 5.0 JDF-u; range 4–6). None of the seven mothers with available sera had ICA, even though the child was ICA positive. No correlation was found between ICA concentrations in cord blood compared with the mothers' blood ($r = 0.05$, $p = 0.67$). Patients positive for ICA were more often born in the autumn (September–November) than during the rest of the year, compared with ICA negative subjects ($p = 0.04$; Table 4).

Whereas none of the control children had more than one antibody, 4% (3/81) children who later de-

Table 2. Islet autoantibodies in cord blood among children later developing Type I diabetes and in matched control subjects

	GAD65Ab <i>n</i> = 81	ICA512Ab <i>n</i> = 73	IA <i>n</i> = 79	ICA <i>n</i> = 81	Number	
	<i>n</i> = 320	<i>n</i> = 288	<i>n</i> = 320	<i>n</i> = 320	patients	control subjects
Patients						
Control subjects						
	+	–	–	–	3	5
	–	+	–	–	3	4
	–	–	+	–	0	1
	–	–	–	+	4	2
	–	m	–	+	1	0
	+	–	–	+	1	0
	+	m	m	+	1	0
	–	+	–	+	1	0
Total Positive					14 (17%)	12 (4%)
Total Negative	–	– or m	– or m	–	67 (79%)	308 (96%)

m = missing samples

Table 3. Comparison of islet autoantibodies between positive cord blood serum and mothers serum at the time of delivery

Ab (unit)	Total <i>n</i>	Concordant			Discordant		
		<i>n</i>	Ab-levels		<i>n</i>	Ab-levels	
			child	mother		child	mother
GAD65Ab (index)	4	2	0.16–0.53	0.18–0.40	2	0.14–0.21	0.01–0.09
ICA512Ab (index)	4	0	0	0	4	0.02–0.04	0.00–0.01
ICA (JDF)	7	0	0	0	7	3–20	0
Total (samples)	15	2			13		
Total (children)	13	2			11		

Newborns with venous blood missing from mother are excluded

Table 4. Season of birth for children with different islet autoantibodies

Time of birth	Winter (Dec–Feb)	Spring (Mar–May)	Summer (Jun–Aug)	Autumn (Sep–Nov)
all (<i>n</i>)	14	25	19	23
GAD65Ab positive	0	4 ^a	1	0
ICA512Ab positive	0	0	2	2
ICA	1	2	0	5 ^a
Any Ab positive	1	4	3	6

^a denote clustering with $p < 0.05$

veloped Type I diabetes were double positive ($p = 0.002$). Both ICA and GAD65Ab were found in two children who developed Type I diabetes at the ages of 10.5 years and 12.8 years, respectively. One child with a combination of ICA and ICA512Ab was 11.9 years old at diagnosis. Other combinations were not detected. Among control subjects, no child had more than one autoantibody.

The mean age at delivery of the mothers of children with antibodies, who later developed diabetes, was 28.9 years (18–40 years) compared with 27.3 (18–42 years) for the mothers of children without antibodies ($p = \text{NS}$).

Discussion

This investigation was possible because sera had been saved from the majority of children born in the city of Malmö since 1969. Cord blood from 81 children who developed Type I diabetes before 15 years of age were analysed and 17 % (14/81) had at least one islet autoantibody when born. Our study shows that children who develop Type I diabetes have an increased prevalence of cord blood islet autoantibodies compared with control subjects.

It is well known that GAD65Ab, ICA512Ab, IAA or ICA, alone or in combination, predict Type I diabetes in first-degree relatives [8, 37, 47]. The positive predicted value after 3 and 5 years follow-up, respec-

tively, is estimated for GAD65Ab to be 28 % and 52 %, respectively; for ICA512Ab 40 % and 81 %, respectively; for IAA 33 % and 59 %, respectively; and for ICA (> 20 JDF-u) 31 % and 51 %, respectively. Among relatives with two or more of these autoantibodies, the risk of Type I diabetes within 3 years was 39 %, and within 5 years 68 % (if they had ICA > 20 JDF-u), and with all three autoantibodies and ICA > 20 JDF-u, the risk for Type I diabetes within 5 years is estimated to be 100 % [15]. In our study there were 10 % (8/83) fathers and 5 % (4/85) mothers with Type I diabetes. Diabetes in the family was therefore not a prerequisite for cord blood positivity. In future attempts to prevent Type I diabetes in the general population, it will be necessary to determine the positive predictive value following autoantibody screening of newborn babies. Such studies are underway [48].

The cord blood autoantibodies could come from the mother since the fetus has a minor production of IgG and transport of IgG over the placenta barrier is an active process giving the newborn baby an IgG concentration of 150 % of the mother's [49]. Two examples of diseases in the newborn caused by placental transfer of autoantibodies are thrombocytopenia in children born to mothers with autoantibodies against platelets [50] and neonatal thyrotoxicosis [51] caused by transfer of thyrotropin receptor-stimulating autoantibodies. We have shown earlier that mothers who at some time after pregnancy developed Type I diabetes had transplacentally transferred ICA and GAD autoantibodies to their children [11]. This has also been reported for IAA and GAD65Ab [39, 40, 52] and for ICA512Ab [39]. Our study indicates that transplacental passage could differ between islet autoantibodies.

Our study showed IAA was not found in cord blood from any of the later diabetic children included in our series. Four children of mothers with Type I diabetes (excluded from our series because of this), however, had insulin antibodies, two had also GAD65Ab and one ICA512Ab, all at similar concentrations to their mothers in line with the BABY-DIAB study [53].

The correlation found for GAD65Ab between child and mother, indicates that the main reason for

cord blood autoantibodies was transplacental passage. The possibility of fetal-produced autoantibodies is not completely excluded, however. An earlier study implicated fetal-produced antibodies when one of the infants with antibody-positivity at birth continued to be IAA positive at follow-up at 2 years of age [52]. In this study we show that ICA512Ab and ICA only occurred in cord blood and not in the mother. This suggests that these autoantibodies were produced by the fetus. Concordant with this is our observation that the ICA antigen is present in the fetal pancreas which suggests autoimmune reactions against beta cells in utero [54]. It has been reported that ICA512Ab, in contrast to GAD65Ab, are closely associated with Type I diabetes developing before puberty [42, 55, 56]. We speculate therefore that prepubertal diabetes could often be a consequence of intrauterine induced autoimmunity.

The cord-blood autoantibody-positive children in our study did not, however, have an earlier clinical onset of Type I diabetes compared with the antibody-negative children. Intrauterine factors could still have an important role. This is suggested by the findings that congenital rubella is associated with a greatly increased risk of Type I diabetes 10–30 years after the intrauterine infection [57, 58]. Recently, we [59] and others [60] have shown that other viral infections, such as Coxsackie and Echo, during pregnancy increase the risk of Type I diabetes in childhood and adolescence. Possible mechanisms leading to autoantibody formation in congenital infections are viral epitopes sharing determinants with beta cell epitopes resulting in a cross reaction with beta cells [61, 62] or direct damage to the beta cells leading to exposure of normally hidden antigens. The seasonal pattern with higher prevalence than expected of GAD65Ab positivity among children born in the spring and ICA positivity among children born in the autumn could also imply a role for infections.

In conclusion, islet autoantibodies are present at birth more frequently than expected in children who later develop Type I diabetes which suggests that beta cell damage can start in utero. The importance of this is twofold. Firstly, such early beta cell damage underscores the importance of devising safe forms of immunological intervention that can permanently halt damage. Secondly, screening for islet autoantibodies at birth could be a crucial step in identifying those at risk of developing Type I diabetes. Thus, the question is: should early intervention and screening start at birth?

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