Is Presence of Islet Autoantibodies at Birth Associated With Development of Persistent Islet Autoimmunity?

The Diabetes Autoimmunity Study in the Young (DAISY)

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OBJECTIVE — To determine whether the presence of islet autoantibodies in the umbilical cord blood is predictive of subsequent development of islet autoimmunity.

RESEARCH DESIGN AND METHODS — Cord blood sera from 1,118 subjects from the Diabetes Autoimmunity Study in the Young (DAISY) cohort, as well as their venous blood samples taken at follow-up clinic visits, were tested for GAD65 autoantibodies (GAAs), insulin autoantibodies (IAAs), and IA-2 autoantibodies (IA-2As). Venous blood samples taken from mothers of cord blood autoantibody–positive children were analyzed for the same autoantibodies.

RESULTS — At least one of three islet autoantibodies was present in 42 (3.7%) of the cord blood samples tested. The presence of cord blood autoantibodies did not predict the subsequent development of islet autoimmunity (adjusted hazard ratio = 0.73 [0.09, 5.88]). Discordance between cord blood and corresponding maternal autoantibodies was seen in 3 of 36 infants. A strong correlation between levels of autoantibody in cord blood and maternal circulation was found for GAA ($r^2 = 0.93$, $P < 0.001$) and IAA ($r^2 = 0.89$, $P < 0.001$) but not IA-2A ($r^2 = 0.05$, $P = 0.19$). Cord blood autoantibodies in all but one subject disappeared by 9 months of age.

CONCLUSIONS — The presence of cord blood autoantibodies is not predictive of subsequent development of islet autoimmunity. The majority of cord blood autoantibodies appear to result from maternal transmission.

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Type 1 diabetes is usually preceded by the presence of autoantibodies directed toward pancreatic islet cell antigens. The principle autoantibodies found to be associated with type 1 diabetes include GAD65 autoantibodies (GAAs), IA-2 autoantibodies (IA-2As), and insulin autoantibodies (IAAs) (1). These antibodies can be present months to years before the actual clinical diagnosis of diabetes and can serve as predictive markers of type 1 diabetes (2–5). In some children, islet autoantibodies can be detected in the umbilical cord blood. Although maternal transmission of islet autoantibodies to the newborn is not uncommon (6–9), it has been suggested that not all autoantibodies found in cord blood result from transplacental transfer, but may instead result from in utero production of diabetes-associated autoantibodies (7,10,11).

The relationship of cord blood autoantibodies and islet autoimmunity has been explored in previous studies (12–15). However, to assess the predictive value of cord blood autoantibodies in newborns, large prospective studies would be most useful. Since 1993, the Diabetes Autoimmunity Study in the Young (DAISY) has been prospectively investigating the natural history of islet autoimmunity (IA) in infants and children who are at a moderate to high risk of developing type 1 diabetes. Because of the high predictive value of islet autoantibodies, they can be used as a surrogate end point for type 1 diabetes research. This study was able to take advantage of a large bank of stored cord blood sera from DAISY participants, prospectively follow children for development of islet autoantibodies, and apply risk analysis to examine whether the presence of cord blood autoantibodies was predictive of subsequent development of persistent islet autoimmunity.

RESEARCH DESIGN AND METHODS

— Since December 1993, DAISY has prospectively followed children at an increased genetic risk for development of islet autoantibodies and/or type 1 diabetes (16). The Colorado multiple institutional review board approved all protocols. Newborns from Saint Joseph's Hospital in Denver, Colorado, are recruited for entry into the study and are representative of the general population

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Abbreviations: DAISY, Diabetes Autoimmunity Study in the Young; GAA, GAD65 autoantibody; IA, islet autoimmunity; IA-2A, IA-2 autoantibody; IAA, insulin autoantibody.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

of the Denver metropolitan area. Excluded are patients who have difficulty understanding English or whose newborn has a severe congenital malformation or disease. Saint Joseph's Hospital routinely collects cord blood samples during delivery, and ~7 ml of cord blood is obtained for DAISY screening and storage. Informed consent is obtained from parents in the hospital after delivery. A sample of whole blood in EDTA is sent for HLA typing to Roche Molecular Systems in Alameda, California. As of January 2002, >24,000 cord blood samples from children born at Saint Joseph's Hospital have been screened for HLA genotypes. Children are categorized into three HLA risk groups. Children with the high-risk genotype DRB1*04, DQB1*0302/DRB1*0301, and DQB1*0201 (or DR3/4, DQ8) are at ~20 times higher risk for type 1 diabetes compared with the general population (1:16). Among children in the moderate-risk group, those with DRB1*04, DQB1*0302/DRB1*04, DQB1*0302 or DRB1*0301/*0301 or DRB1*04, DQB1*0302/x (where x is neither DRB1*04, DQB1*0302, nor DRB1*0301 nor DR2) are at a sevenfold risk (1:75 in non-Hispanic whites or 1:230 in Hispanics), compared with the general population. Children with all other genotypes were classified at a risk similar to that for the general population (1:300) or lower. All children with the high-risk HLA genotype and a sample of those with the moderate-risk genotype are asked to participate in DAISY.

Newborn and young siblings and offspring of people with type 1 diabetes were also recruited from the Colorado type 1 diabetes registry; from families of diabetic children seen at the Barbara Davis Center for Childhood Diabetes in Denver, Colorado; and through media publicity. These children have also been tested for diabetes-associated HLA genotypes.

The DAISY cohort is prospectively followed from birth. Venous blood samples are obtained for measurement of islet autoantibodies from study participants at clinic visits at the ages of 9, 15, and 24 months and yearly thereafter. Children who test positive for autoantibodies are followed more intensely, at 3–6 month intervals. Children in the DAISY cohort are followed until the clinical diagnosis of type 1 diabetes or until 15 years of age. The median age of follow-up for subjects in this specific analysis is 4.2 years (range 0.6–8.7 years). Additionally, as early as possible during follow-up, at least one venous blood sample is drawn from the parents and siblings of enrolled subjects and tested for autoantibodies.

Subjects for this analysis include children from the DAISY cohort (n = 1,118) who have had at least one complete follow-up visit and who had cord blood sera available for analysis. Additionally, venous blood samples taken from mothers (n = 36) of children with cord blood autoantibodies were analyzed.

The majority of the cohort was non-Hispanic white (n = 763, 68.2%). The remaining children were divided among the following ethnic/racial groups: Spanish/Hispanic (n = 235, 21.0%), biracial (n = 80, 7.2%), African-American (n = 32, 2.9%), Asian (n = 5, 0.4%), and Native American (n = 3, 0.3%). The cohort was 53% (n = 589) male.

Autoantibody assays

Cord blood sera and venous blood sera obtained during follow-up were analyzed for the presence of autoantibodies to GAD (GAA), insulin (IAA), and IA-2 (IA-2A) by radioimmunoassay. All 1,118 cord blood sera samples were sent for testing simultaneously to minimize interassay variability. Additionally, duplicate samples from 6% of subjects were included as blinded quality controls. Concordance between quality control samples was 95%. Maternal samples were sent for testing in triplicate. Sera that tested positive for any of the three autoantibodies were reassayed, and a positive value was confirmed if two of three assays were positive.

All blood sera samples were stored at −20°C before testing and analyzed in the laboratory of Dr. George Eisenbarth at the Barbara Davis Center for Childhood Diabetes. GAA and IA-2A were measured in a combined radioassay using previously described methods. Radioactivity was counted on a TopCount 96-well plate β-counter. The levels of both autoantibodies are expressed as an index, calculated as the sample − negative control)/(positive control − negative control), with the sample and the negative and positive controls measured in counts per minute (cpm) (17). In the 1995 Immunology of Diabetes Society's Combined Autoantibody Workshop, sensitivity for the GAA assay was 82%, specificity was 99%, and the interassay coefficient of variation was 6% (age at diabetes onset <30 years). Sensitivity for the IA-2A assay was 73%, specificity was 100%, and the interassay coefficient of variation was 10% (18). Autoantibodies to insulin were measured by a micro-IAA assay. This assay incorporates competition with unlabeled insulin with precipitation with protein A Sepharose. Sensitivity for the IAA assay was 56%, specificity was 98%, and the interassay coefficient of variation was 10% (18). All samples with GAA, IAA, or IA-2A levels exceeding the 99th percentile and a random 10% of the remaining samples are retested in a blinded manner for quality assurance. The 99th percentile based on testing 198 nondiabetic control subjects aged 0.4–67 years were 0.032 for GAA, 0.01 for IAA, and 0.049 for IA-2A. The single highest value for IA-2A among control subjects, 0.07, was used as the cutoff for positivity for this assay. For GAA and IAA, we used the 99th percentile as the cutoff for positivity.

End point

The end point for this study is the development of current islet autoimmunity (IA). A case of IA is defined as a child who was positive for at least one autoantibody on two or more consecutive visits and who remained positive, or had developed type 1 diabetes, at their most recent visit.

Statistical analysis

Survival analysis was used to determine the risk of developing IA. Hazard ratios (HRs) were estimated using survival analysis with a Weibull distribution (SAS Proc Lifereg). Left, right, and interval censoring was taken into account. All follow-up times began at birth. Variables adjusted for in the analysis included HLA genotype and family history of type 1 diabetes because subjects were selected based on these characteristics. In the present analysis, we grouped low and moderate HLA risk categories together. Differences in proportions were determined by χ^2, and differences in means were determined by a Student's t test. The correlation between antibody levels in cord blood and maternal circulation was calculated using linear regression analysis. Statistical analyses were performed using SAS version 8.

RESULTS

Cord blood autoantibodies

At least 1 of the three islet autoantibodies was present in 42 (3.7%) of the cord

Table 1—*Islet autoantibodies present in cord blood and development of IA during follow-up*

Autoantibody			1st-degree relative without type 1 diabetes		1st-degree relative with type 1 diabetes		Total cohort	
GAA	IAA	IA-2A	*n*	IA	*n*	IA	*n*	IA
−	−	−	987	13*	89	4†	1,076	17
−	−	+	1	0	0	0	1	0
−	+	−	12	0	9	0	20	0
+	−	−	9	1‡	2	0	11	1
−	+	+	0	0	2	0	2	0
+	+	−	0	0	5	0	5	0
+	+	+	0	0	2	0	2	0
Total positive			22	1	20	0	42	1

*Two subjects with type 1 diabetes, 11 subjects with IA; †4 subjects with IA; ‡1 subject with type 1 diabetes.

blood samples tested. Of these positive samples, 30 were positive for IAA, 18 for GAA, and 5 for IA-2A. Only 1 autoantibody was present in 33 sera samples, 2 autoantibodies were present in 7 samples, and all 3 autoantibodies were present in 2 samples. The end point of interest, IA, developed in 18 (1.6%) of the 1,118 children followed. IA developed in a similar proportion of children positive for cord blood autoantibodies (1 of 42 [2.4%]) and in those without detectable autoantibodies (17 of 1,076 [1.6%], $P = 0.6857$). Specifics of islet autoantibody distribution and IA are summarized in Table 1.

The median age at last follow-up was 5.3 years for cord blood autoantibody–positive subjects and 4.2 years for autoantibody-negative subjects ($P = 0.1897$). There was no difference in the distribution of subjects among the different HLA risk categories ($P = 0.4807$). The percentage of positive subjects who had a first-degree relative with type 1 diabetes was significantly greater than the percentage of negative subjects with a first-degree relative with type 1 diabetes (48 vs. 8%, $P < 0.0001$). Of cord blood autoantibody–positive subjects with a first-degree relative with type 1 diabetes, 95% (19/20) were born to mothers with type 1 diabetes. Presumably, many of the autoantibodies detected resulted from maternal transmission.

Corresponding maternal autoantibodies

To determine what proportion of autoantibodies present in cord blood samples are likely to result from maternal transmission, assays of venous blood samples taken from the mothers of positive children were compared with the autoantibodies found in the cord blood. Of the 42 children testing positive for at least one autoantibody, autoantibody information was available for 36 mothers (86%). The median length of time between delivery and maternal blood draw was 1.3 years (range −0.8 to 8.1 years). Blood samples were taken near the end of the first trimester and 1 year after delivery (−0.8 to 1.1 years) in 47% of mothers ($n = 17$), 1.3–3.0 years after delivery in 50% of mothers ($n = 18$), and, in one mother, 8.1 years after delivery.

Individual autoantibody results for each of the 36 subjects with cord blood autoantibodies and corresponding maternal autoantibody statuses are shown in Table 2. Of 16 children with cord blood positive for GAA, 15 of their mothers had GAAs as well. IAA was present in 26 cord blood samples and in 24 of the corresponding maternal samples. Discordance for IAA was seen between one cord blood and maternal sample, and one maternal sample could not be tested for IAA (insufficient volume). A strong linear correlation was detected between the antibody levels in cord blood and in maternal circulation for GAA ($r^2 = 0.933$, $P < 0.001$) (Fig. 1*A*) and IAA ($r^2 = 0.89$, $P < 0.001$) (Fig. 1*B*). Of the four children with cord blood positive for IA-2A, three of their mothers were positive for IA-2A. There was no significant correlation between the level of IA-2A in cord blood and in maternal circulation ($r^2 = 0.05$, $P = 0.19$) (Fig. 1*C*). However, all IA-2A cord blood values were negative except for four low positives, which may account for the apparent lack of a correlation with maternal values. The majority of autoantibodies found in cord blood sera (93% [42 of 45]), appear to result from maternal transmission. Only 3 of 36 children were discordant for autoantibodies with their mothers. None of the children with presumable nonmaternal autoantibodies have developed IA.

Elimination of cord blood autoantibodies

Cord blood autoantibodies disappeared in all but one subject by the age of 9 months. No IAA was detected in samples available at 9 months (0 of 25), 15 months (0 of 21), and 24 months (0 of 22). Similarly, no IA-2A was present in 9-month (0 of 3), 15-month (0 of 4), and 24-month (0 of 4) samples. At 9 months, only 1 of 12 samples remained positive for GAA, but this subject seroconverted to negativity by 15 months. At 9 months, another subject was negative for GAA (value = 0.031) but had developed IAA. By 15 months she seroconverted to positivity for GAA, and she remained positive for GAA and IAA until she developed type 1 diabetes at the age of 1.9 years. All three subjects discordant with maternal autoantibodies had lost their autoantibodies by 9 months of age.

Relationship between cord blood autoantibodies and IA

Both unadjusted and adjusted HRs suggest that the presence of cord blood autoantibodies is not a predictor of IA (unadjusted HR = 1.35 [0.18, 10.16]; adjusted HR = 0.73 [0.09, 5.88]). Adjusting the model for HLA risk and having a first-degree relative with type 1 diabetes did alter the cord blood autoantibody HR, suggesting that there was confounding

Table 2—*Islet autoantibodies in positive cord blood serum and corresponding maternal serum near delivery*

Subject	GAA		IAA		IA-2A		Diabetes during pregnancy
	Cord blood	Maternal	Cord blood	Maternal	Cord blood	Maternal	
00590-0	**0.340**	**0.632**	**0.284**	**0.493**	−0.009	0.052	Type 1
00597-0*	**0.335**	**0.262**	**0.020**	†	**0.377**	−0.055	Type 1
10483-0	**0.073**	**0.116**	−0.009	0.007	0.004	0.021	
11435-0	**0.084**	**0.140**	**0.012**	**0.017**	0.062	**0.105**	Type 1
11499-0	**0.608**	**0.885**	0.004	**0.033**	−0.004	**0.136**	
11621-0	0.024	0.003	**1.994**	**1.656**	**0.071**	**0.177**	Type 1
11865-0	0.017	−0.023	**0.026**	**0.035**	0.007	0.013	
12245-0	−0.004	−0.005	**0.029**	**0.075**	0.029	**0.082**	Type 1
12638-0	−0.001	−0.021	−0.017	0.007	**0.094**	**0.088**	
13313-0	**1.219**	**0.941**	−0.003	0.001	0.021	**0.116**	
13584-0	−0.028	−0.027	**0.016**	**0.030**	−0.007	0.003	
13682-0	**0.797**	**0.955**	**0.026**	**0.061**	0.020	**0.120**	Type 1
13761-0	−0.011	0.008	**0.018**	**0.022**	−0.005	0.017	
14150-0	0.023	0.023	**0.259**	**0.351**	0.023	0.030	Type 1
14961-0*	**0.066**	−0.045	−0.003	−0.004	0.006	0.002	
15179-0	−0.007	−0.003	**1.442**	**1.497**	0.017	**0.135**	Type 1
20035-0	−0.008	−0.037	**0.014**	**0.023**	0.005	0.004	
20907-0	**0.250**	**0.147**	0.005	0.001	−0.013	0.018	
21592-0	−0.002	−0.017	**0.394**	**0.713**	−0.003	0.006	
22013-0	−0.012	−0.032	**0.018**	**0.021**	−0.004	0.009	
22494-0	**1.107**	**1.082**	**0.045**	**0.075**	0.027	**0.183**	Type 1
22536-0	**0.737**	**0.741**	**0.288**	**0.408**	−0.008	**0.089**	Type 1
23099-0	0.015	0.020	**0.075**	**0.252**	0.009	0.019	Type 1
24244-0	**1.133**	**1.125**	0.008	**0.018**	−0.050	**0.145**	
24848-0	−0.020	−0.009	**0.162**	**0.562**	0.002	0.018	Type 1
25205-0	**0.485**	**0.781**	−0.016	0.004	−0.039	**0.110**	
26955-0	**0.955**	**1.206**	0.008	0.001	−0.003	**0.111**	
26995-0	−0.006	−0.040	**0.149**	**0.744**	0.005	0.015	Type 1
30323-0	0.002	0.009	**1.466**	**1.627**	**0.094**	**0.108**	Type 1
30541-0	0.031	0.012	**0.289**	**0.305**	0.019	0.021	Type 1
30816-0*	0.014	−0.017	**0.023**	0.001	0.027	0.004	
30949-0	−0.012	−0.009	**0.162**	**0.562**	0.002	0.018	Type 1
35471-0	−0.001	−0.014	**0.019**	**0.032**	−0.003	0.004	
35659-0	0.002	−0.020	**0.019**	**0.081**	−0.002	0.010	Gestational
36962-0	**0.924**	**0.989**	**0.052**	**0.053**	−0.026	**0.133**	Type 1
50334-0	**0.118**	**0.177**	**−0.012**	**0.017**	−0.001	0.023	

Bold typeface represents positive autoantibody values. Outlined boxes indicate discordant autoantibody values. *Discordance between cord blood autoantibody and maternal autoantibody; †assay result not available (insufficient volume), and subject-maternal pair excluded from IAA analysis for concordance.

between the variables. Both HLA risk (unadjusted HR = 3.76 [1.48, 9.53]; adjusted HR = 4.55 [1.75, 11.85]) and having a first-degree relative with type 1 diabetes (unadjusted HR = 3.19 [1.05, 9.72]; adjusted HR = 4.81 [1.47, 15.70]) predict IA. We also explored the potential relationship between IA and maternal diabetes (gestational, type 1, and type 2 diabetes) and insulin use during pregnancy. However, there were no cases of IA among subjects of affected mothers, so these variables could not be included in the survival analysis models.

CONCLUSIONS — Our results show that the presence of cord blood antibodies does not increase the risk of islet autoimmunity by the age of 5 years in a cohort at increased risk for type 1 diabetes. We observed that having a first-degree relative with type 1 diabetes is associated with both an increased risk of IA and the presence of cord blood antibodies. Our multivariate analyses suggest, however, that the increased risk of IA associated with a family history of type 1 diabetes is independent of the presence of cord blood antibodies in the newborn, suggesting that other genetic and/or environmental factors associated with family history are increasing the risk of IA in these children. The relationship between the presence of cord blood autoantibodies and subsequent development of IA was determined based on the presence of any cord blood autoantibody, regardless of origin. From the examination of maternal venous blood sera taken near the time of delivery of cord blood autoantibody–positive subjects, we can infer that the majority, if not all, of the autoantibodies detected in the cord blood sera of the 42 positive subjects

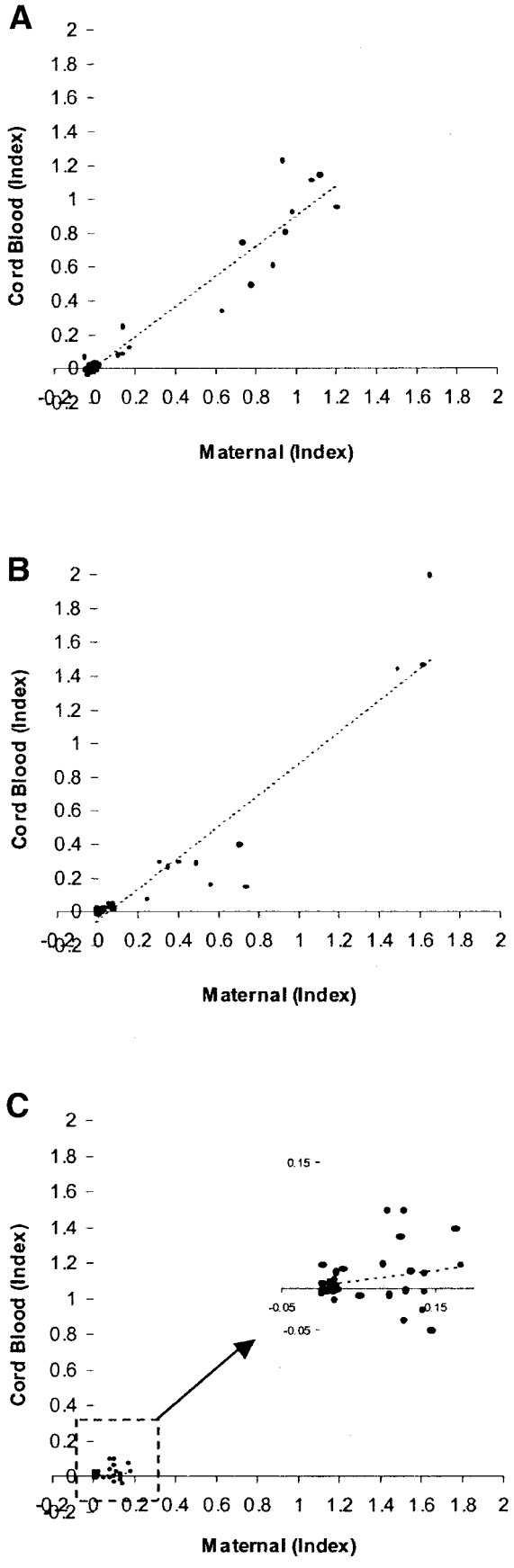

Figure 1—A: *Correlation of GAA in cord blood and maternal circulation* ($r^2 = 0.933$, $P < 0.001$). B: *Correlation of IAA in cord blood and maternal circulation* ($r^2 = 0.89$, $P < 0.001$). C: *Correlation of IA-2A in cord blood and maternal circulation* ($r^2 = 0.05$, $P = 0.19$).

were also from maternal transmission. A small fraction of these autoantibodies may have resulted from in utero production. Because we were interested in investigating the effects of cord blood autoantibodies on the development of IA, regardless of origin, we chose not to distinguish between source when calculating HRs.

Consistent with a transplacental origin of cord blood autoantibodies, we, and others, found that most cord blood autoantibodies will disappear within the first year of life, with the majority of cord blood autoantibodies being eliminated by 9 months of age (6,7,19). Also consistent with maternal transmission of autoantibodies is the strong correlation we found between levels of GAA and IAA in cord blood and maternal circulation. Similar correlations between mother and offspring have been previously reported (6–8,19). Although we did not find a similar correlation for IA-2A, we believe this is attributable to an insufficient number of positive cord blood samples available for comparison.

Lindberg et al. (11) had proposed that cord blood autoantibodies may be of fetal origin and may be indicative of future development of type 1 diabetes. However, our study and other previous studies (12–15) have found that the majority of cord blood antibodies are transplacentally transferred and are not predictive of future development of type 1 diabetes.

It is possible that differences in the length of follow-up may explain the inconsistencies between studies. For example, Lindberg et al. analyzed sera from children who developed diabetes by 15 years of age, whereas previous studies examined subjects that had developed by the age of 2–4 years (13), 5 years (12), and 6 years (15). In our study, the median age of follow-up for children who had been positive for cord blood antibodies was 5.3 years. As DAISY continues to prospectively follow its cohort, it will be informative to see if any additional cord blood autoantibody–positive subjects seroconvert to islet autoimmunity.

Recently, Greeley et al. (20) demonstrated that the absence of maternally transferred autoantibodies in NOD mice protects the NOD progeny from spontaneous progression to diabetes. This suggests that the presence of such autoantibodies plays a critical role in the development of diabetes in the NOD mouse. The equivalent relationship be-

tween islet autoantibodies early in development and the course of diabetogenesis in humans has yet to be determined. We have suggested that diabetes-associated autoantibodies in early development may not have the same pathogenic mechanism in humans as that found in the NOD mouse.

Results of this study suggest that the presence of cord blood autoantibodies do not predict the development of islet autoimmunity by the age of 5 years. Moreover, the majority of cord blood autoantibodies are attributable to transmission of these antibodies from the mother during pregnancy and disappear by the age of 9 months.

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