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Antibodies to islet cell autoantigens, rotaviruses and/or enteroviruses in cord blood and healthy mothers in relation to the 2010–2011 winter viral seasons in Israel: a pilot study

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Abstract

Aims—To determine whether antiviral and/or islet cell antibodies can be detected in healthy pregnant mothers without diabetes and/or their offspring at birth in two winter viral seasons.

Methods—Maternal and cord blood sera from 107 healthy pregnant women were tested for islet cell autoantibodies using radioligand binding assays and for anti-rotavirus and anti-CoxB3 antibody using an enzyme-linked immunosorbent assay.

Results—Glutamic acid decarboxylase (GAD)65 autoantibodies and rotavirus antibodies, present in both maternal and cord blood sera, correlated with an odds ratio of 6.89 (95% CI: 1.01–46.78). For five, 22 and 17 pregnancies, antibodies to GAD65, rotavirus and CoxB3, respectively, were detected in cord blood only and not in the corresponding maternal serum. In 10 pregnancies, rotavirus antibody titres in the cord blood exceeded those in the corresponding maternal serum by 2.5–5-fold. Increased antibody titres after the 20th week of gestation suggested CoxB3 infection in one of the 20 pregnancies and rotavirus in another.

Conclusion—The concurrent presence of GAD65 antibodies in cord blood and their mothers may indicate autoimmune damage to islet cells during gestation, possibly caused by cross-placental transmission of viral infections and/or antiviral antibodies. Cord blood antibody titres that exceed those of the corresponding maternal sample by >2.5-fold, or antibody-positive cord

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Competing interests

None declared.

blood samples with antibody-negative maternal samples, may imply an active *in utero* immune response by the fetus.

Introduction

Type 1 diabetes (T1D) is an autoimmune disease with both genetic and environmental risk factors contributing to its etiology [1]. In several countries epidemiological studies have shown that the season during which children who developed Type 1 diabetes were born differed from that in the general population [2–6]. These findings suggest that the initial trigger for Type 1 diabetes was more likely to occur during autumn and winter, when the incidence of winter viral infections also peak. A virus-infected mother might transmit the virus to the fetus, initiating an autoimmune process against the pancreatic β cells, and/or transmitting anti-virus antibodies to the fetus, thereby providing protection. Rotaviruses and enteroviruses have been implicated in the aetiopathology of Type 1 diabetes, with both viruses showing an islet-cell tropism [7–10].

The aim of the present study was to find out whether antiviral or islet cell autoantibodies can be detected in healthy pregnant mothers without diabetes and/or their offspring at birth during the winter viral season.

Subjects and methods

Subjects

Healthy, pregnant women [$n=107$, mean (range) age 30.7 (19–46) years] from central Israel were enrolled at the time of delivery (January to April 2010, $n=76$, and January to March 2011, $n=43$) to coincide with rotavirus seasons] at the Helen Schneider Women's Medical Center. None had a history of Type 1 diabetes in their families. Matched maternal and cord blood samples were collected for 107 of these women at the time of delivery, with first trimester blood available from 20 of the 107 mothers. The Ethical Review Board of the Belinson Medical Center approved the study and all of the women signed an informed consent form.

Antiviral enzyme-linked immunosorbent assays

Simian rotavirus strain SA11 (ATCC VR-1565; American Type Culture Collection, Manassas, VA, USA) and CoxB3, isolated from a 2009 clinical sample, were used as antigens to measure antiviral immunoglobulin G (IgG) antibody titres using an enzyme-linked immunosorbent assay. The rotavirus antigen was presented after affinity capture using mouse anti-rotavirus IgG (Enco, Cat 0531, Israel) bound to the enzyme-linked immunosorbent assay plates, while CoxB3 was bound directly to the plates. Peroxidase-conjugated goat anti-human IgG (cat 109-035-003; Jackson Immuno Research, West Grove, PA, USA) was used to determine the amount of human IgG bound to the antigens. Supernatant from mock infected MA-104 or human kidney cell cultures, respectively, served as negative controls. The mean OD of negative controls from all assays was determined and clinical samples with OD values two standard deviations above this were considered positive. High, middle and low concentrations of an anti-rotavirus IgG-positive or CoxB3 reference sample were included in all test runs. The relative units (RU) of IgG in all other

clinical samples were determined from the regression curve from the mean ODs of the reference from all test runs for each concentration. Acceptance of analytical data from each run was according to Westgard rules. The inter-test CV for the anti-rotavirus IgG standards was 25.06%, while the intra-test CV for 17 replicates of the same sample was 11%. The inter-test CV for the anti-CoxB3 standards was 14.

Very recent infections were distinguished from infections that occurred in the past by a seroconversion defined by a 4-fold increase in antibody titre between two successive samples, or acquisition of detectable antibody titres in the follow-up sample. (CDC-recommended guidelines for seroconversion).

Radioligand binding assay

Glutamic acid decarboxylase (GAD)65, islet antigen 2 (IA2), and zinc transporter 8 (ZnT8) autoantibodies were determined by radioligand binding assay as previously described [11]. All samples were analysed in triplicate and the mean (range) intra-assay coefficient of variation was 4.5 (0.016–15)%. Our laboratory participated in the Diabetes Autoantibody Standardization Program workshop, where our GAD65 autoantibody assay showed a sensitivity of 86% and specificity of 93%, and the IA2 autoantibody assay showed a sensitivity of 66% and a specificity of 98%. ZnT8 autoantibodies were not included in the workshop.

Thresholds for islet cell antibody positivity

GAD65 antibodies—The positivity of samples was verified in competition assays using recombinant human GAD65 (200ng/ml; Diamyd Medical, Stockholm, Sweden) as previously described [12]. Samples whose binding to radiolabelled GAD65 was reduced by 50% (index 0.5) were considered GAD65 antibody-positive. This threshold is equivalent to 40U/ml.

IA2 autoantibodies—The samples were considered IA2 autoantibody-positive if binding exceeded that of the 98th percentile for healthy controls (30U/ml).

ZnT8 autoantibodies—The threshold was set at 15 RU/ml for autoantibodies to ZnT8R and 26 RU/ml for ZnT8W based on the 98th percentile observed in 50 healthy human control sera.

Odds ratios

Odds ratios with 95% CIs were calculated according to Bland and Altman [13] using an online calculator (<http://www.hutchon.net/ConfidORselect.htm>) prepared by D. J. R. Hutchon (DJRHutchon@hotmail.co.uk).

Results

The prevalence of GAD65, ZnT8 and IA2 autoantibodies in maternal and cord blood sera from the 107 pregnancies at birth is summarized in Table 1. GAD65 antibodies were present in sera from matched cord blood and maternal samples in 5/107 (4.7%) of the pregnancies.

In five pregnancies (4.7%) cord blood samples were positive for GAD65 antibodies, while their respective maternal sera tested antibody-negative. GAD65 antibodies were already elevated in the first trimester for two of the 20 pregnancies, where a sample was collected during the first trimester.

Cord blood antibody titres for rotavirus and CoxB3 ranged between 3.1 and 96.3 RU, respectively, while maternal antibody titres ranged between 3.0 and 49.6 RU. Altogether 37/107 (35.7%) of pregnancies had ≥ 6 RU of rotavirus antibodies in their maternal and/or cord blood samples; 24% (26/107) in cord blood samples alone; 10% (11/107) in both maternal and cord blood samples; and none in the maternal sera alone. Moreover, 10/11 pregnancies with detectable rotavirus IgG in maternal samples, had rotavirus antibody titres in the cord blood, exceeding the maternal antibody titre by 2.5– 5-fold.

Antibody titres to CoxB3 enterovirus ≥ 10 RU were found in 48% (50/105) of pregnancies in maternal and/or cord blood samples, while 33% (36/105) of maternal serum samples and 46% (48/105) of cord blood samples contained antibody titres to CoxB3 enterovirus > 10 RU, and 30% (32/105) of pregnancies were found to have antibodies > 10 RU in both samples. Cord blood antibody titres ranged between 3.0 and 21.7 RU, while maternal antibody titres ranged between 3.4 and 16.4 RU.

For the 20 pregnancies where a first trimester maternal serum sample was available, two showed seroconversion indicative of a very recent infection. The first woman acquired a rotavirus antibody titre of 4.3 RU, while the first trimester sample showed no detectable virus antibody titre. No islet cell autoantibodies were detected in her sera, or in the cord blood, while all three samples had CoxB3 IgG titres ranging between 9.9 to 13.1 RU. The second woman acquired a CoxB3 antibody titre of 9.6 RU, while the first trimester sample showed no detectable virus antibody titre. None of the maternal or cord blood samples from this pregnancy contained detectable antibodies against islet cell antigens or rotavirus.

The ratio of antibody titres in cord blood compared with corresponding maternal blood at delivery is shown in Table 2. When comparing serum samples positive for GAD65 antibodies with sera containing ≥ 6 RU for rotavirus IgG or > 10 RU of CoxB3 IgG, we observed a significant odds ratio of 6.89 (95% CI 1.01–46.78) for pregnancies where both cord blood and maternal serum at birth were positive for GAD65 antibodies and rotavirus IgG.

Discussion

The present study showed that islet cell autoantibodies can be detected at delivery in healthy mothers and/or their offspring. None of the mothers had a family history of diabetes, therefore, the findings should be related to an environmental event occurring during pregnancy, such as a viral infection. Development of islet cell autoimmunity, with the characteristic autoantibodies subsequent to such a viral infection, is likely to occur weeks or months after the virus has cleared the system and virus concentrations drop below the levels of detection by molecular assays. Serological assays for the presence of antibodies to rotavirus and enterovirus are one way to circumvent this difficulty.

We found a significant correlation (odds ratio 6.89; 95% CI 1.01–46.78) between GAD65 antibodies and rotavirus antibodies in pregnancies where both maternal and cord blood samples were positive for GAD65 antibodies and both had ≥ 6 RU of rotavirus IgG. This odds ratio dropped to 4.85, but was no longer significant (95% CI 0.54–51.81) when the threshold for rotavirus IgG titres was set to ≥ 10 RU. Notably, four of the GAD65 antibody-positive cord blood samples with GAD65 antibody-negative mothers had > 10 RU of CoxB3 antibody and the fifth had 9.4 RU.

The finding of GAD65 antibodies in the cord blood of five neonates without the presence of these antibodies in the corresponding mother, and antibody titres to rotavirus and/or enterovirus 2.5–5-fold higher than in the corresponding maternal samples, may be indicative of an independent humoral response [14] to an insult of the fetal pancreas. Similar findings have been reported for GAD65 antibodies [15,16] and independent cellular fetal immune responses to viral infections have been reported [17,18].

A higher frequency of GAD65 antibody-positive cord blood samples compared with those found in a study in Finland [19] and the Diabetes Autoimmunity Study in the Young cohort study in the USA [20] may be attributable to the fact that the samples in the present study were collected specifically from children who were born during peak viral seasons. Another explanation could be related to different thresholds for GAD65 antibody positivity. GAD65 antibody positivity in the present study was confirmed in a specific competition assay [12].

Paired samples with antibodies in both cord blood/maternal samples suggest transplacental transfer of antibodies. Cord blood antibody levels tend to be higher (~60%) than those of the mother at the time of delivery, possibly as a result of active transport of IgG across the placenta [21–23], and/or haemodilution in the mother. We evaluated whether cord blood samples with 2.5–5-fold higher rotavirus and/or GAD65 antibody levels as compared with the respective maternal sample showed corresponding differences in tetanus toxoid antibody levels and total IgG levels in cord blood and mothers (data not shown). Although tetanus toxoid antibody levels in cord blood were on a mean (range) of 1.3 (0.3–4.1)-fold higher than the maternal antibody levels, we found no correlation between the tetanus toxoid antibody ratios and the aforementioned ratios between cord blood and maternal antibodies to rotavirus and GAD65 antibody. Similar results were observed for total IgG. It is therefore unlikely that the observed higher rotavirus and GAD65 antibody levels in cord blood were caused by active transplacental transmission or haemodilution in the mother, but are more likely to have resulted from an independent fetal immune response to viral infection and islet cell autoantigens. The finding of positive GAD65 antibodies in three of the mothers and not in the corresponding cord blood samples is open to speculation.

The present study differs from the TEDDY study [24] in that it investigated preand neonatal conditions, while the TEDDY study investigated post-natal conditions. Another difference is our concept of viral infection as an initiating trigger of the autoimmune process leading to Type 1 diabetes, whereas the TEDDY study investigated whether viral infections immediately preceded the conversion of preclinical to clinical childhood Type 1 diabetes. The observed lack of correlation of enteroviral antibodies and islet cell autoantibodies is

consistent with other studies [25,26]. The significance of the findings of the present pilot study needs to be confirmed.

In conclusion, the present findings may support the hypothesis that maternal rotavirus infections during pregnancy may damage the fetal islet cells and trigger the cascade of events leading to Type 1 diabetes. This may explain the difference in season of birth of children who develop Type 1 diabetes from that of those who do not.

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L.M.S. and C.S.H. contributed equally to this study. Part of this work was in fulfilment of a MS degree at Tel Aviv University by Y.P.

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What's new?

- It has been hypothesized that viral infections initiate islet cell autoimmunity.
- Previous research suggests an association of viral infection *in utero* and islet autoimmunity.
- We found a significant correlation between glutamic acid decarboxylase 65 autoantibodies and anti-rotavirus in healthy mothers at delivery and in cord blood.
- The presence of antibodies in cord blood with antibody-negative mothers suggests an independent fetal immune response.
- Our findings support the hypothesis that viral infections during pregnancy damage fetal islet cells, triggering islet autoimmunity.

Table 1

Maternal and cord blood antibody titres

Antibody-positive * cord blood samples				
	GAD65 (%)	ZnT8R (%)	Rotavirus (%)	CoxB3 (%)
GAD65	10 (9.3)	0/10 (0.0)	3/10 (30)	4/10 (40)
ZnT8R	0/2 (0.0)	2 (1.9)	0/2 (0.0)	0/2 (0.0)
Rotavirus	3/26 (11.5)	0/26 (0.0)	26 (24.3)	14/26 (53.8)
CoxB3	5/48 (10.4)	0/48 (0.0)	15/48(31.3)	48 (44.9)

Antibody-positive * maternal sera at delivery				
	GAD65	ZnT8R	Rotavirus	CoxB3
GAD65	8 (7.5)	0/8 (0.0)	2/8 (25)	4/8 (50)
ZnT8R	0/2 (0.0)	2 (1.9)	0/2 (0.0)	0/2 (0.0)
Rotavirus	2/11 (18.2)	0/11 (0.0)	11 (10.3)	4/11 (36.4)
CoxB3	4/36 (11.1)	0/36 (0.0)	4/36 (11.1)	36 (33.6)

Positive antibody response in maternal sera and cord blood				
	GAD65	ZnT8R	Rotavirus	CoxB3
GAD65	5 (4.7)	0/5 (0.0)	1/5 (20)	1/5 (20)
ZnT8R	0/2 (0.0)	2 (1.9)	0/2 (0.0)	0/2 (0.0)
Rotavirus	2/11 (18.2)	0/11 (0.0)	11 (10.3)	4/11 (37.3)
CoxB3	1/32 (3.1)	0/32 (0.0)	4/32(13.4)	32 (29.9)

GAD, glutamic acid decarboxylase; ZnT8R, xxxx.

* GAD65 index 0.5; ZnT8R 15 relative units; rotavirus 6 relative units; and CoxB3 10 relative units.

Table 2

Ratio of antibodies in cord blood to maternal serum at delivery

Ratio		GAD65	Rotavirus	CoxB3
5		0	1	0
2.5–4.9		0	9	0
0.6–2.4		4	22	32
<0.6		0	0	0
Cord	Maternal			
Positive	NS	5	22	17
NS	Positive	3	0	2
NS	NS	95	56	54
Total number		107	107	105

GAD, glutamic acid decarboxylase; NS, nonsignificant (GAD65) or below detection (rotavirus, CoxB3).