Transmission of Maternal Islet Antibodies and Risk of Autoimmune Diabetes in Offspring of Mothers With Type 1 Diabetes

Kerstin Koczwara, Ezio Bonifacio, and Anette-Gabriele Ziegler

It is suggested that the maternal transmission of islet autoantibodies increases the risk of autoimmune diabetes in mice. The aim of this study was to determine whether fetal exposure to islet autoantibodies modified the risk of type 1 diabetes in humans. Islet autoantibodies were measured at birth in 720 offspring of mothers with type 1 diabetes. Offspring were prospectively followed for the development of multiple islet autoantibodies and diabetes. Offspring who were GAD or IA-2 autoantibody positive at birth (n = 678) had significantly lower risks for developing multiple islet autoantibodies (5-year risk 1.3%) and diabetes (8-year risk 1.1%) than offspring who were islet autoantibody negative at birth (5.3%, P = 0.008; and 3%, P = 0.04, respectively). Risk remained reduced after adjustment for birth weight, gestational age, or maternal diabetes duration (adjusted hazards ratio 0.25, P = 0.007 for multiple islet autoantibodies; 0.25, P = 0.04 for diabetes). Protection in offspring with islet autoantibodies at birth was most striking in offspring without the HLA DRB1*03/DRB1*04-DQB1*0302 genotype. Maternal transmission of antibodies to exogenous insulin did not affect diabetes risk in offspring. These findings suggest that fetal exposure to islet autoantibodies in children born to mothers with type 1 diabetes may be protective against future islet autoimmunity and diabetes. Diabetes 53:1–4, 2004

RESEARCH DESIGN AND METHODS

Offspring of parents with type 1 diabetes (the BABYDIAB study). BABYDIAB prospectively follows offspring of mothers and/or fathers with type 1 diabetes from birth. Cord blood is obtained at birth, and venous blood samples are obtained at age 9 months, and at ages 2, 5, 8, and 11 years (7). Recruitment began in July 1989 and ended in November 2000, and follow-up has continued thereafter. A total of 1,610 children of a parent with type 1 diabetes were recruited at birth and participated in the 9-month follow-up. These included 1,030 children of mothers with type 1 diabetes. Cord blood for determination of all three antibodies (insulin antibody, GADA, and IA-2A) at birth was available in 720 of 1,030 offspring of mothers with type 1 diabetes and in 285 of 580 offspring of fathers with type 1 diabetes and non diabetic mothers. The cord blood volume was insufficient for islet antibody determination in the remaining 290 offspring of mothers with type 1 diabetes and 295 offspring of fathers with type 1 diabetes. After birth, insulin autoantibodies (IAAs), GADAs, and IA-2As were measured in samples from all scheduled follow-up visits. The median follow-up time from birth to last sample was 6.5 years (range 0.75–12.5 years), for a total of 9,773 person-years. HLA DR and DQ genotypes were determined in 602 of the 720 offspring with birth islet autoantibody measurements. Type 1 diabetes diagnosis was defined according to World Health Organization (WHO) criteria. All parents gave written informed consent to participate in BABYDIAB. The study was approved by the ethical committee of Bavaria, Germany (Bayerische Landesarztekammer no. 93537).

Islet antibody measurement. Insulin antibody (IA), GADA, and IA-2A were determined by radiobinding assays as previously described (7,8). The 99th percentile of control children were used to define the upper limits of normal and corresponded to 8.5 local units/ml or 25 WHO units/ml for GADA, 2.5 local units/ml or 4 WHO units/ml for IA-2A, and 1.5 local units/ml for IAA. The assays had sensitivities and specificities of 80 and 94% (GADA), 58 and 100% (IA-2A), and 30 and 98% (IAA), respectively, in the First Diabetes Autoanti-
**TABLE 1**
Cumulative antibody and diabetes risk in offspring of mothers with type 1 diabetes relative to antibody status at birth

<table>
<thead>
<tr>
<th>Antibody status at birth</th>
<th>Multiple islet antibodies (% at 5 years)*</th>
<th>P†</th>
<th>Adjusted HR‡</th>
<th>P§</th>
<th>Diabetes (% at 8 years)*</th>
<th>P†</th>
<th>Adjusted HR‡</th>
<th>P§</th>
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</thead>
<tbody>
<tr>
<td>Insulin antibodies (n [%])</td>
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<tr>
<td>Positive (620 [86])</td>
<td>3.1 ± 0.7</td>
<td>0.7</td>
<td>1.4 (0.3–6.2)</td>
<td>0.63</td>
<td>2.3 ± 0.7</td>
<td>0.15</td>
<td>NA</td>
<td>0.93</td>
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<tr>
<td>Negative (100 [14])</td>
<td>2.1 ± 1.5</td>
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<td>GAD autoantibodies (n [%])</td>
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<tr>
<td>Positive (401 [50])</td>
<td>1.6 ± 0.6</td>
<td>0.1</td>
<td>0.4 (0.1–1.1)</td>
<td>0.08</td>
<td>1.3 ± 0.6</td>
<td>0.21</td>
<td>0.4 (0.1–1.3)</td>
<td>0.11</td>
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<tr>
<td>Negative (319 [44])</td>
<td>4.0 ± 1.2</td>
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<td>IA-2 autoantibodies (n [%])</td>
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<tr>
<td>Positive (267 [37])</td>
<td>1.3 ± 0.7</td>
<td>0.08</td>
<td>0.3 (0.1–1.2)</td>
<td>0.10</td>
<td>1.5 ± 0.9</td>
<td>0.44</td>
<td>0.6 (0.2–2.2)</td>
<td>0.46</td>
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<tr>
<td>Negative (453 [63])</td>
<td>3.5 ± 0.9</td>
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<tr>
<td>GAD or IA-2 autoantibodies (n [%])</td>
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<tr>
<td>Positive (478 [66])</td>
<td>1.3 ± 0.5</td>
<td>0.008</td>
<td>0.25 (0.1–0.7)</td>
<td>0.007</td>
<td>1.1 ± 0.5</td>
<td>0.04</td>
<td>0.25 (0.1–0.8)</td>
<td>0.02</td>
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<tr>
<td>Negative (242 [34])</td>
<td>5.3 ± 1.6</td>
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</table>

Data are means ± SE or HR (95% CI). *Life table risk; †P values calculated using the log-rank test; ‡HR adjusted for maternal diabetes duration, birth weight, and gestational age; §P values calculated for adjusted HR against reference cell; ††unable to calculate HR.

RESULTS

Prevalence of islet antibodies in cord blood of offspring from mothers with type 1 diabetes. At birth, insulin antibodies were detected in 620 of 720 (86%) offspring of type 1 diabetic mothers, GADAs were in 401 of 720 (55.7%) offspring, and IA-2As in 267 of 720 (37%) offspring (Table 1). GADAs or IA-2As were detected at birth in 478 (66%) offspring. Of the 720 offspring, 45 (6.3%) had no islet antibodies in their cord blood, 54 (7.5%) had GADAs (44%) and/or IA-2As (25%) without insulin antibodies, 196 (27.2%) had insulin antibodies only, and 424 (58.9%) had GADAs and/or IA-2As together with insulin antibodies. One of the 285 offspring of fathers with type 1 diabetes had IAA at birth, none had IA-2As, and none had GADAs.

Relationship of birth islet antibody status and subsequent development of diabetes-associated autoimmunity in offspring. Thirty-one offspring of type 1 diabetic mothers developed multiple islet autoantibodies during early childhood, and 16 of these developed diabetes. Autoantibody status at birth significantly affected diabetes risk in offspring of mothers with type 1 diabetes. Offspring who were islet autoantibody positive at birth had a significantly lower risk for the subsequent development of multiple islet autoantibodies (1.3% by age 5 years) and diabetes (1.1% by age 8 years) than offspring who were islet autoantibody negative at birth (5.3%, P = 0.008 for multiple islet autoantibodies; 3.0%, P = 0.04 for diabetes) (Table 1) (Fig. 1). Risks were...
also reduced in the offspring with GADAs or IA-2As at birth after adjustment for maternal diabetes duration, gestational age, and birth weight (adjusted HR for developing multiple islet autoantibodies 0.25, \( P = 0.007 \); adjusted HR for developing diabetes 0.25, \( P = 0.02 \)). The effect of birth autoantibody status on diabetes risk was most striking in children who did not have the high-risk HLA \( DRB1*03/DRB1*04-DQB1*0302 \) genotype (0.5 vs. 3.5% multiple islet autoantibodies by age 5 years, \( P = 0.007 \); 0.3 vs. 2.4% diabetes by age 8 years, \( P = 0.05 \)) and was negligible in HLA \( DRB1*03/DRB1*04-DQB1*0302- \) positive children (17.8 vs. 26.3% multiple islet autoantibodies, \( P = 0.8 \); 18.4 vs. 15.6% diabetes, \( P = 0.7 \) ) (Fig. 2). No significant difference in the risk for developing multiple islet autoantibodies or diabetes was found between offspring with or without cord blood insulin antibodies (Table 1). The risk of developing multiple islet autoantibodies was 4 ± 0.9% (mean ± SE) in the 284 offspring of fathers with type 1 diabetes who were islet autoantibody negative at birth.

**DISCUSSION**

Antibodies bind antigens and can act as cell-surface receptors on B-cells or monocytes and dendritic cells to facilitate or enhance presentation of antigen to T-cells (11–14). As a result, antibodies may play a role in the pathogenesis of autoimmune disease (15). In autoimmune diabetes, however, it has generally been considered that autoantibodies are purely markers of disease because blocking B-cell function in experimental models does not affect the development of disease (16), and removal of immunoglobulin from type 1 diabetic patients has only a minimal effect on disease severity (17,18). While these observations show that autoantibodies alone are insufficient for diabetes development, more recent studies (15,19–21) have demonstrated that B-cell–deficient NOD mice have little insulitis and have a markedly reduced diabetes incidence, indicating that B-cells and perhaps antibodies play a role in the initiation of diabetes. This hypothesis was recently reinforced by the finding (5) that the removal of immunoglobulin during gestation markedly reduced the incidence of diabetes. It remains to be determined whether these observations were due specifically to the presence or absence of autoantibodies to islet antigens in both the NOD mouse and humans.

In humans, the BABYDIAB study provided an opportunity to examine whether fetal and neonatal exposure to autoantibodies and to endogenous autoantigen (GAD or IA-2) or to antibodies against exogenously administered autoantigen (insulin) modified diabetes risk. The BABYDIAB study is the largest study of offspring of parents with type 1 diabetes and includes >1,000 offspring of mothers with type 1 diabetes. We and others have previously shown that islet antibodies in cord blood of offspring from type 1 diabetic mothers correlate highly with the level of antibodies found in maternal blood at delivery, suggesting the transmission of these antibodies through the placenta (1,2) and that these maternally transmitted antibodies usually become undetectable within the first year of life (3,4). Here we determined the prevalence of islet antibodies at birth and followed offspring for the development of persistent diabetes-associated multiple islet autoantibodies and for the development of diabetes. Antibodies to exogenously administered insulin were detected at birth in 86% of offspring of type 1 diabetic mothers, and 66% of offspring had autoantibodies to GAD and/or IA-2 at birth. We found that the presence or absence of insulin antibodies did not affect the risk of developing diabetes or diabetes-associated autoantibodies. Remarkably, however, offspring with autoantibodies at birth had a significantly lower diabetes risk than offspring who were autoantibody negative at birth. The decreased risk did not appear secondary to potential confounders, such as maternal diabetes duration, birth weight, and gestational age.

Assuming that antibodies detected at birth are representative of antibody titers during pregnancy, the findings in the BABYDIAB offspring suggest that fetal exposure to GAD and/or IA-2 autoantibodies may protect against subsequent diabetes. Consistent with this observation is the overall decreased diabetes risk in offspring of type 1 diabetic mothers compared with that of offspring of type 1 diabetic fathers and nondiabetic mothers (22,23) and the previous report (24) of reduced development of islet autoimmunity in offspring of mothers with type 1 diabetes compared with children of fathers with type 1 diabetes. Although many factors are likely to contribute to this difference, it is possible that the fetal autoantibody exposure that occurs in the majority of offspring of type 1 diabetic mothers plays a role in reducing their diabetes risk. Such protection could be due to a more efficient
elimination of autoreactive T-cell clones by antibody-mediated presentation of autoantigen during fetal and neonatal life or to a state of immune ignorance against autoantigen by antibody-mediated blocking or masking the presentation of relevant autoantigen peptides (25). Interestingly, the protection conferred by fetal exposure to autoantibodies was most striking in children who did not have the typical high diabetes risk HLA DRB1*03/DRB1*04-DQB1*0302 genotype, suggesting that the mechanism is unlikely to require HLA restriction of antigen presentation.

Overall, our findings in humans do not support the hypothesis that fetal exposure to islet autoantibodies increases diabetes risk and, if these observations are confirmed, suggest that fetal autoantibody exposure may protect from future endogenous islet autoimmunity and diabetes.

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REFERENCES