Symptoms of Common Maternal Infections in Pregnancy and Risk of Islet Autoimmunity in Early Childhood

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OBJECTIVE — The aim of this study was to test whether symptoms of maternal infections during pregnancy and indicators of postnatal infections predict development of islet autoimmunity in children at genetically increased risk of type 1 diabetes.

RESEARCH DESIGN AND METHODS — A total of 871 children with type 1 diabetes–associated HLA genotypes born in Denver, Colorado, and 391 siblings or offspring of individuals with type 1 diabetes referred from clinics in the Denver metropolitan area were enrolled soon after birth and seen in the clinic at age ≤15 months. Information on indicators of infection was collected by structured interviews soon after birth and at ages 3–15 months. Clinic visits were scheduled at ages 9, 15, and 24 months, and yearly thereafter. The outcome was positivity for one or more islet autoantibodies (to GAD65, insulin, or IA-2/ICA512) at two or more consecutive visits. During a mean follow-up of 4.2 years, 52 children developed islet autoimmunity.

RESULTS — Children whose mother reported at least one symptom of infection during pregnancy (mostly respiratory or gastrointestinal) had a significantly lower risk of islet autoimmunity compared with other children (hazard ratio 0.48; 95% CI 0.27–0.83). After stratification, the association appeared among girls (0.21; 0.09–0.48) but not among boys (1.09; 0.47–2.51) with a P value for interaction of 0.005. Symptoms of neonatal infections, early daycare attendance, exposure to cats or dogs, and household crowding were not related to islet autoimmunity.

CONCLUSIONS — Symptoms of maternal infections in pregnancy predicted a significantly lower risk of islet autoimmunity in young girls, suggesting a protective effect of such infections.

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Type 1 diabetes is among the most common chronic diseases with onset in childhood. The disease results from destruction of the insulin-producing β-cells in the pancreatic islets and is characterized by a preclinical phase of islet autoimmunity (1). The majority of case subjects with type 1 diabetes carry HLA-DR and HLA-DQ susceptibility alleles, but these are not sufficient for the development of the disease. The environmental triggers of autoimmunity and type 1 diabetes are essentially unknown, but evidence is accumulating that putative environmental factors operate early in life. Viral infections are among the prime suspects for environmental triggers of type 1 diabetes (2). Although several different viruses have been linked to type 1 diabetes, most studies have focused on enteroviruses with mixed results (2,3). On the other hand, part of the risk for type 1 diabetes may be due to lack of exposure to protective factors. The so-called “hygiene hypothesis” proposes that the decline in nonspecific infectious and microbial exposure in many populations has caused the concomitant increase in atopic disorders over the past few decades (+), and this hypothesis has recently been extended to autoimmune diseases such as type 1 diabetes (5). Some infections and microbial agents reduce the incidence of autoimmune diabetes in experimental animals (6). A protective effect of childhood infections against type 1 diabetes has also been suggested by a few case-control studies (7–9), but little or no data are available from prospective studies.

The Diabetes Autoimmunity Study in the Young is a prospective study of candidate environmental risk factors for islet autoimmunity and type 1 diabetes in children at genetically increased risk for type 1 diabetes because they have a sibling or parent with type 1 diabetes or carry HLA alleles that confer increased risk of type 1 diabetes (10,11). Autoantibodies to GAD65 (GADA), insulin (IAA), and IA-2 (also called ICA512), often referred to as islet autoantibodies, are markers of islet autoimmunity and are highly predictive of future development of type 1 diabetes (12). The objective of this study was to test whether symptoms of maternal infections during pregnancy, neonatal infections in the child, daycare attendance, exposure to household pets, and household crowding in early childhood can predict development of islet autoimmunity among children at genetically increased risk for type 1 diabetes.

RESEARCH DESIGN AND METHODS — From January 1994 to January 2003, >27,800 cord blood sam-
samples from children born at St. Joseph’s Hospital in Denver have been screened for diabetes-associated HLA genotypes (10). We excluded families in which parents had difficulties understanding English or whose infant had a severe congenital malformation or disease. Eighty-six percent of families approached gave informed consent to genetic screening. Samples of whole blood in EDTA were sent to the HLA typing laboratory at Roche Molecular Systems (Alameda, CA) for PCR-based class II genotyping, as previously described (10). Children were categorized into three groups defined by the odds of developing type 1 diabetes by the age of 20 years. The high-risk group (odds 1:16) was DRB1*04-DQB1*0302/DRB1*0301-DQB1*0201, the moderate risk genotypes (odds 1:75 in non-Hispanic whites and 1:230 in Hispanics) were DRB1*04-DQB1*0302/DRB1*04-DQB1*0302 or DRB1*0301*/0301 or DRB1*04-DQB1*0302/X (in which X does not include DRB1*04, DQB1*0302, DRB1*0301, DQB1*0602, or DR2 [DRB1*15 or 16]). All other genotypes were classified as low risk. For the present analysis, we grouped low- and moderate-risk categories together. Children with at least one sibling or parent with type 1 diabetes were referred from clinics in the Denver metropolitan area and recruited regardless of their HLA genotype. Participants were recruited soon after birth and scheduled for clinic visits at ages 9, 15, and 24 months and annually thereafter. Venous blood was collected at each clinic visit, and children who tested positive for one or more islet autoantibodies were scheduled for more frequent visits. Informed consent was obtained from parents of all children in the study, and the study was approved by the Colorado Multiple Institutional Review Board. Between January 1994 and February 2003, 1,431 children were seen in the clinic at age ≤15 months. Of these, the families of 871 children without and 391 children with a parent or sibling with type 1 diabetes had completed interviews about environmental factors relating to the mother during pregnancy and to the child at ages 3–15 months.

Autoantibody assays and definition of outcome
All measures of autoantibodies in blood were performed in the laboratory of Dr. George Eisenbarth at Barbara Davis Center (Denver, CO). We used radioimmunoassays for insulin, GAD$_{65}$, and IA-2 autoantibodies. Insulin autoantibodies are measured by a micro-IAA assay with sensitivity of 58%, specificity of 99%, and interassay coefficient of variation 11% (13). The combined anti-GAD and –IA-2 radioassay is performed in duplicate on a 96-well filtration plate, and radioactivity is counted on a TopCount 96-well plate β-counter using a modification of a previously reported method. The levels of both antibodies are expressed as an index (sample cpm − negative control cpm)/(positive control cpm − negative control cpm) (14). In the 1995 Immunology of Diabetes Society Workshop, the GADA assay had 82% sensitivity and 99% specificity using sera from new-onset diabetic patients aged <30 years. The interassay coefficient of variation was 6%. The IA-2 assay had 73% sensitivity and 100% specificity, and the interassay coefficient of variation was 10% (15). All samples with IAA, GADA, or IA-2 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 was 0.01 for IAA, 0.032 for GADA, and 0.049 for IA-2. The single highest value for IA-2 among control subjects (0.07) was used as a cutoff for positivity for this assay. For GADA and IAA, we used the 99th percentile as the cutoff for positivity. The definition of islet autoimmunity was positivity for one or more of the three islet autoantibodies at two or more consecutive clinic visits at 1- to 6-month intervals (of the 52 that satisfied this definition, 16 have been negative at one or more subsequent visits and 14 have developed clinical type 1 diabetes up to 28 February 2003). The time of onset of islet autoimmunity was defined as the midpoint between the dates of the two clinic visits when the child was last negative and first positive for at least one autoantibody.

Study variables
Soon after recruitment, the child’s mother completed a mailed questionnaire on relevant factors. Mothers were asked: “When you were pregnant with the study child, did you have any of the following conditions,” with precoded categories: “bad cold or influenza,” “sore throat or tonsillitis,” “sinus infection,” “diabetes/first-degree relatives with type 1 diabetes, for the HLA categories, and for boys and girls by strat-

Statistical analysis
With the cohort defined as above, we calculated based on the logistic model that we could detect a relative risk just under 0.5 with 80% power for exposures with 50–70% prevalence (two-sided tests, 5% significance level). We used Cox regression analysis to estimate the hazard ratio (HR) with 95% CI for the relation between study variables and future development of islet autoimmunity with time from birth to islet autoimmunity or the last clinic visit when the child tested negative as the main time variable (16). We decided a priori to adjust for family history (first-degree relative with type 1 diabetes or not), HLA risk category, and ethnic group. Further adjustment by maternal education, maternal age at delivery, and other variables considered relevant was also done. We investigated whether the HRs were of similar magnitude for children with and without first-degree relatives with type 1 diabetes, for the HLA categories, and for boys and girls by strat-
RESULTS

Maternal infections and islet autoimmunity

<table>
<thead>
<tr>
<th>Maternal infections and islet autoimmunity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Person-years (n)</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>FDR with type 1 diabetes†</td>
</tr>
<tr>
<td>No FDR with type 1 diabetes</td>
</tr>
<tr>
<td>High-risk HLA genotype§</td>
</tr>
<tr>
<td>Non–high-risk HLA genotype</td>
</tr>
<tr>
<td>Hispanic or other ethnic group¶</td>
</tr>
<tr>
<td>Non-Hispanic white</td>
</tr>
<tr>
<td>Males</td>
</tr>
<tr>
<td>Females</td>
</tr>
<tr>
<td>Maternal education &gt;12 years§</td>
</tr>
<tr>
<td>Maternal education ≤12 years</td>
</tr>
<tr>
<td>Breast-fed &lt;3 months</td>
</tr>
<tr>
<td>3–8 months</td>
</tr>
<tr>
<td>≥9 months</td>
</tr>
<tr>
<td>Mean age, first clinic visit (years)</td>
</tr>
<tr>
<td>Mean follow-up (years)#</td>
</tr>
<tr>
<td>Type 1 diabetes**</td>
</tr>
</tbody>
</table>

*Number developing islet autoimmunity during present follow-up. Positive for one or more autoantibodies to GAD, IAA, and IA-2 at two consecutive clinic visits.
†Adjusted for first-degree relative with type 1 diabetes, HLA risk-category, and ethnic group. ‡FDR, first-degree relative (sibling or parent). §The high-risk genotype was DRB1*04-DQB1*0302/DRB1*0301-DQB1*0201. ||Based on self-report. “Hispanic or other” includes 277 Hispanic (77.2%), 42 biracial (11.7%), 30 African American (8.4%), 5 Asian, 1 American Indian, and 2 with missing. ¶Seventy-two children had missing data for maternal education. #Time to last clinic visit where child tested negative for islet autoantibodies or to midpoint between the clinic visits when the child was last negative and first positive. **Developed clinical type 1 diabetes after islet autoimmunity, prior to 28 February 2003.

CONCLUSIONS — Maternal symptoms of common infections during pregnancy predicted a significantly lower risk of developing islet autoimmunity in the young girls in this prospective study.

A role of Th1/Th2 balance has been suggested to explain the lower risk of atopic disorders associated with markers of infections (4,5). Many intracellular infections promote Th1-dominated immune responses (e.g., secretion interferon-γ), which may protect against typically Th2-biased atopic disorders. This concept is probably too simplistic and does not account for associations between parasitic infections, which usually induce predominantly Th2 immune responses, and lower risk of atopic disorders (5). Furthermore, the Th1/Th2 paradigm in its simplest form cannot accommodate a relation between general infectious exposure and lower risk of organ-specific autoimmune diseases, which are thought to be Th1 biased (5,17). Thus, the idea has been proposed that stimulation of the immune system by natural infections, as opposed to vaccinations and a “clean” environment, can prevent development of both atopic and autoimmune diseases (5). Bach (5) has recently summarized potential mechanisms of rele-

ified analyses and testing the respective interactions. Unless reported otherwise, results were similar in these subgroups. The robustness of the analyses against deviations from the proportional hazard assumption and against interval censoring of the outcome was assessed by running the same analyses using logistic regression. All results were essentially the same whether we used Cox or logistic regression. Outcome and exposures were defined before inspecting the relationships between them. A two-sided P value <0.05 or a 95% CI for the HR not overlapping 1.00 was regarded statistically significant.

RESULTS — During the current follow-up, 52 children developed islet autoimmunity, of which 14 subsequently developed clinical type 1 diabetes (Table 1).

Children whose mother reported at least one symptom of infection (mostly respiratory or gastrointestinal) during pregnancy had approximately one-half the risk of islet autoimmunity compared with other children (Table 2). This significant association was essentially unchanged after further adjustment for maternal age at delivery, maternal education, month of birth, or duration of breast-feeding. Maternal respiratory infections and gastrointestinal infections both tended to predict lower risk of islet autoimmunity, although not significantly so when considered separately. Other infections were not related to islet autoimmunity (Table 2). There was no evidence of a dose-response pattern with increasing number of maternal symptoms of infection in pregnancy. However, the association between maternal symptoms of infection and lower risk of islet autoimmunity among children was strong and significant among girls (HR 0.21; 95% CI 0.09–0.48), but not significant among boys (1.09; 0.47–2.51). The test for interaction between maternal infections and sex of the child gave a P value of 0.005 (not shown).

Presence of at least one symptom of neonatal infection (mostly respiratory) and daycare attendance were not related to islet autoimmunity (Table 3). Further analysis of number of other children attending or age at start in daycare did not convey any important additional information. Exposure to cats, dogs, or a combination of the two and household crowding in early life were also not related to islet autoimmunity (Table 3).
Table 2—Symptoms of maternal infections during pregnancy and development of islet autoimmunity among children at genetically increased risk of type 1 diabetes*

<table>
<thead>
<tr>
<th>Reported maternal symptoms during pregnancy</th>
<th>Person-years (n = 5,265)</th>
<th>Cases† (n = 52)</th>
<th>Hazard ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any symptoms§</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n = 818)</td>
<td>3,529</td>
<td>27</td>
<td>0.54 (0.31–0.93)</td>
</tr>
<tr>
<td>No (n = 419)</td>
<td>1,638</td>
<td>24</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>Respiratory infection¶</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n = 675)</td>
<td>2,942</td>
<td>23</td>
<td>0.66 (0.38–1.15)</td>
</tr>
<tr>
<td>No (n = 557)</td>
<td>2,190</td>
<td>27</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>Diarrhea¶</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n = 242)</td>
<td>979</td>
<td>7</td>
<td>0.68 (0.31–1.51)</td>
</tr>
<tr>
<td>No (n = 1,002)</td>
<td>4,213</td>
<td>44</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>Other infection or fever#</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n = 249)</td>
<td>1,078</td>
<td>10</td>
<td>0.97 (0.49–1.94)</td>
</tr>
<tr>
<td>No (n = 994)</td>
<td>4,114</td>
<td>40</td>
<td>1.00 (reference)</td>
</tr>
</tbody>
</table>

*Three hundred ninety-one children with siblings or offspring with type 1 diabetes and 871 children with diabetes-associated HLA genotypes (without first-degree relatives with type 1 diabetes). †Number developing islet autoimmunity during present follow-up. Positive for one or more autoantibodies to GAD, IAA, and IA-2 at two consecutive clinic visits. ‡Adjusted for first-degree relative with type 1 diabetes (yes/no), HLA risk-category, and ethnic group. §Mother respiratory infection, diarrhea, or other symptom of infection or fever during pregnancy with the study child. Twenty-five children had missing data. ¶At least one of the following: “bad cold” (n = 494), “sore throat” (n = 319), “bronchitis” (n = 74), “pneumonia” (n = 8), or “sinusitis” (n = 248). Thirty children had missing data. †Eighteen children had missing data. ‡Kidney or urinary tract infection” (n = 78) or “other infection or fever” (n = 90). Nineteen children had missing data.

vance for autoimmune diseases in this regard, including a role of regulatory T-cells and cytokines (e.g., interleukin 10 and transforming growth factor-β) induced by many types of infections. Infectious agents seem to induce changes in the immune system beyond that of producing antibodies to the specific agent. Another possible mechanism, although not well defined, is “antigenic competition,” whereby immune responses to one antigen are reduced by immune responses to

Table 3—Indicators of early infectious or microbial exposures and development of islet autoimmunity in children at genetically increased risk of type 1 diabetes*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Person-years (n = 5,265)</th>
<th>Cases† (n = 52)</th>
<th>Hazard ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Report of neonatal symptom of infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n = 215)</td>
<td>880</td>
<td>7</td>
<td>0.75 (0.34–1.67)</td>
</tr>
<tr>
<td>No (n = 1,026)</td>
<td>4,298</td>
<td>45</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>Daycare (n = 631)</td>
<td></td>
<td>2,716</td>
<td>25</td>
</tr>
<tr>
<td>No day care (n = 592)</td>
<td>2,495</td>
<td>27</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>No cats or dogs¶ (n = 512)</td>
<td>2,102</td>
<td>18</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>≥1 dog, no cats (n = 376)</td>
<td>1,573</td>
<td>18</td>
<td>1.33 (0.69–2.55)</td>
</tr>
<tr>
<td>≥1 cat, no dogs (n = 173)</td>
<td>720</td>
<td>6</td>
<td>0.98 (0.39–2.46)</td>
</tr>
<tr>
<td>≥1 dog and ≥1 cat (n = 196)</td>
<td>863</td>
<td>10</td>
<td>1.36 (0.63–2.95)</td>
</tr>
<tr>
<td>Likelihood ratio test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Person per room in household#</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.50 (n = 298)</td>
<td>1,412</td>
<td>17</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>0.50–0.69 (n = 539)</td>
<td>2,311</td>
<td>17</td>
<td>0.60 (0.31–1.18)</td>
</tr>
<tr>
<td>≥0.70 (n = 409)</td>
<td>1,505</td>
<td>16</td>
<td>0.85 (0.43–1.68)</td>
</tr>
<tr>
<td>Test for trend</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P = 0.64</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P = 0.77</td>
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<td></td>
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</tbody>
</table>

*Three hundred ninety-one children with siblings or offspring with type 1 diabetes and 871 children with diabetes-associated HLA genotypes (without first-degree relatives with type 1 diabetes). †Number developing islet autoimmunity during present follow-up. Positive for one or more autoantibodies to GAD, IAA, and IA-2 at two consecutive clinic visits. ¶Respiratory problems (n = 126), “cold or runny nose” (n = 70), “meningitis” (n = 3), “pneumonia” (n = 5), “diarrhea” (n = 26), or “other infections or fever” (n = 27). Twenty-one children had missing data. ¶Daycare before age 15 months. Thirty-nine children had missing data. Four children started daycare after developing islet autoimmunity and were censored as nonexposed. ¶Dogs and cats inside the house when child was 0–6 months old. Five children had missing data. Note that categories are mutually exclusive. #Number of people living in household (including index child) divided by number of rooms (counting kitchen but not bathroom) when child was 6 months old. Sixteen children had missing data. Test for trend is Wald test with crowding entered as a continuous variable coded 1, 2, and 3 for the three groups.
other, unrelated antigens. A protective effect of maternal infections in pregnancy rather than of postnatal infections may also point toward a possible role of maternal antibodies (18,19), but our data do not implicate a role of specific infections. Perhaps general infections in the mother during pregnancy also confer protection against related or unrelated diabetogenic postnatal infections in the child. The stronger association between maternal symptoms of infections and lower risk of islet autoimmunity among girls compared with boys detected while checking the data for consistency was not expected, and the explanations for this sex difference are unknown.

Other studies

The few previous studies of general symptoms of maternal infections during pregnancy and risk of type 1 diabetes in children have not found any significant association (20,21), but these case-control studies were prone to recall or selection bias. Specific markers of maternal infections in pregnancy with enterovirus have been associated with increased risk of type 1 diabetes in the children (2), but this could not be confirmed in a recent larger study (22). Infections and respiratory problems in the neonatal period have been associated with increased risk of type 1 diabetes in two case-control studies (21,23). However, some case-control studies have found associations between symptoms of infections in childhood and lower risk of type 1 diabetes (7,9). Indicators of general exposure to microbial and infectious agents, such as daycare attendance and exposure to pets, have been extensively studied in relation to atopic disorders (4) but relatively rarely in relation to type 1 diabetes. A recent meta-analysis concluded that there is some evidence for a lower risk of type 1 diabetes among children who attended daycare centers early in life, although the amount of heterogeneity between studies makes it difficult to draw strong conclusions (24). The present study does not support a role of early daycare attendance in protecting against islet autoimmunity. Children exposed to household pets are likely to be more exposed to a variety of microbial and infectious agents, and such exposure has been associated with lower risk of atopic disorders, although the evidence is not conclusive. The only previous study of exposure to pets and risk of type 1 diabetes did not find any significant association (25), which is consistent with our study of islet autoimmunity.

Strengths and limitations of the present study

Strengths of the current study include that it is prospective with serial follow-up and assessment of exposure in interviews before development of outcome. The fact that symptoms of infections were self-reported and may be difficult to define for mothers is a limitation. However, any possible misclassification is likely to attenuate the association with islet autoimmunity. Many common infections are asymptomatic. It is important to note that our data relates to infectious symptoms and does not answer whether asymptomatic infections are relevant in our context. The infectious symptoms in this study may have been caused by a large number of different pathogens, and it would be impossible to collect blood samples during the symptomatic period for the pregnant women given the design of this study. Future studies may identify and investigate suitable biomarkers of common infectious exposure.

Although we adjusted for a number of factors in the analyses, we cannot rule out the possibility of unmeasured confounding factors. Symptoms of infections in pregnancy may be markers of general hygiene or perhaps of some specific infections. Our results apply to islet autoimmunity and not necessarily to clinical type 1 diabetes, but nearly all children who develop type 1 diabetes first go through the state of islet autoimmunity. Because the cohort consists of children selected to be at genetically increased risk for type 1 diabetes (because they have a sibling or parent with type 1 diabetes or carry HLA alleles conferring increased risk for type 1 diabetes), the results are not necessarily directly applicable to the general population. However, because the majority of children developing type 1 diabetes are at genetically increased risk, and because we did not find evidence for a different association depending on family history or HLA risk category, the present results are likely to be of high relevance at the population level.

In conclusion, symptoms of common maternal infections during pregnancy predicted a significantly lower risk of islet autoimmunity in young girls at genetically increased risk of type 1 diabetes, suggesting a protective effect of such infections.

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References


