No Major Association of Breast-Feeding, Vaccinations, and Childhood Viral Diseases With Early Islet Autoimmunity in the German BABYDIAB Study

MICHAEL HUMMEL, MD
MARTIN FÜCHTENBUSCH, MD
MIKE SCHENKER, MD
ANETTE-G. ZIEGLER, MD

OBJECTIVE — Environmental factors have been suggested to play an important role in the pathogenesis of type 1 diabetes. The aim of this study was to assess the influence of breast-feeding, vaccinations, and childhood viral diseases on the initiation of islet autoimmunity in early childhood.

RESEARCH DESIGN AND METHODS — Data were prospectively collected from questionnaires obtained at birth, at 9 months of age, and at 2 years of age in 823 offspring from parents with type 1 diabetes. By 2 years of age, 31 offspring had islet antibodies, and 10 developed overt diabetes by the time of follow-up.

RESULTS — In offspring from mothers with type 1 diabetes, duration of exclusive and total breast-feeding did not differ between islet antibody–positive and –negative children, regardless of HLA genotype, and breast-feeding of 3 months or longer was not associated with protection from antibody development or diabetes onset. In offspring from diabetic fathers, non–statistically significant reductions in exclusive and total breast-feeding times were observed in the antibody-positive cohort. Neither type nor quantity of vaccinations (including Bacille Calmette-Guérin vaccine; *haemophilus influenzae* vaccine; diphtheria, tetanus, and pertussis vaccine; tick-born encephalitis vaccine; or measles, mumps, and rubella vaccine) were associated with the development of islet antibodies and diabetes. Measles, mumps, and rubella were not reported in children with islet antibodies or diabetes.

CONCLUSIONS — This study showed no evidence that proposed environmental factors affect islet antibody development in the first 2 years of life in offspring from parents with type 1 diabetes.

Diabetes Care 23:969–974, 2000

Environmental factors have been suggested to play an important role in the pathogenesis of type 1 diabetes. Epidemiological studies have reported variations in type 1 diabetes incidence associated with type of early infant diet (1), duration of breast-feeding (1,2), vitamin supplementation (3), vaccinations (4,5), and pre- and postnatal infections (5). However, the epidemiological evidence is inconsistent. Almost all studies accumulate data retrospectively, comparing cases of type 1 diabetes with nonaffected control subjects, and are therefore disadvantaged by recall bias. Moreover, the disease process has a preclinical phase characterized by the presence of autoimmunity to islet antigens, and little is known about the influence of environment on this early induction of islet autoimmunity. BABYDIAB is a prospective study from birth in offspring of parents with type 1 diabetes that evaluates the appearance of islet antibodies in relation to environmental factors and genetic markers (6–9). To determine whether environmental factors may influence the appearance of autoimmunity, we have analyzed in a prospective manner the relationship between breast-feeding duration, vaccinations, and viral infections and the development of islet autoimmunity and progression to clinical disease in BABYDIAB offspring.

RESEARCH DESIGN AND METHODS

Study population

The German BABYDIAB Study is a prospective evaluation of risk and protective factors for the development of type 1 diabetes in offspring of parents with type 1 diabetes. The study is described in detail by Ziegler et al. (6). By design, visits for venous blood sampling were scheduled at birth (sampling from umbilical cord blood) and at 9 months, 2 years, 5 years, and 8 years of age for collection of data on breast-feeding patterns, vaccinations, and viral childhood diseases; for measurements of antibodies to islet cells (ICAs), insulin (IAAs), GAD (GADAs), and IA-2 (IA-2As); and for HLA class II typing. From 1989 until the time of analysis (1999), 1,002 of 1,553 offspring recruited at birth had reached the age of 2 years; 823 (82%) participated in the 2-year follow-up visit and were included into this analysis (568 from a mother, 228 from a father, and 27 from both parents with type 1 diabetes). From these 823 offspring, a 5-year follow-up had been acquired in 155 and a 5- and an 8-year follow-up had been acquired in 46

From the Diabetes Research Institute and the Third Medical Department, Hospital München-Schwabing, Munich, Germany.

Address correspondence and reprint requests to Dr. Anette-G. Ziegler, Institut für Diabetesforschung, Kölner Platz 1, 80804 Munich, Germany. E-mail: anziegler@lrz.uni-muenchen.de.

Received for publication 18 January 2000 and accepted in revised form 20 March 2000.

Abbreviations: BCG, Bacille Calmette-Guérin vaccine; DTP, diphtheria, tetanus, and pertussis vaccine; GADA, GAD antibody; HIB, *haemophilus influenzae* vaccine; IA-2A, antibody against tyrosine phosphatase IA-2; IAA, insulin autoantibody; ICA, islet cell antibody; MMR, measles, mumps, and rubella vaccine; TBE, tick-born encephalitis vaccine.

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

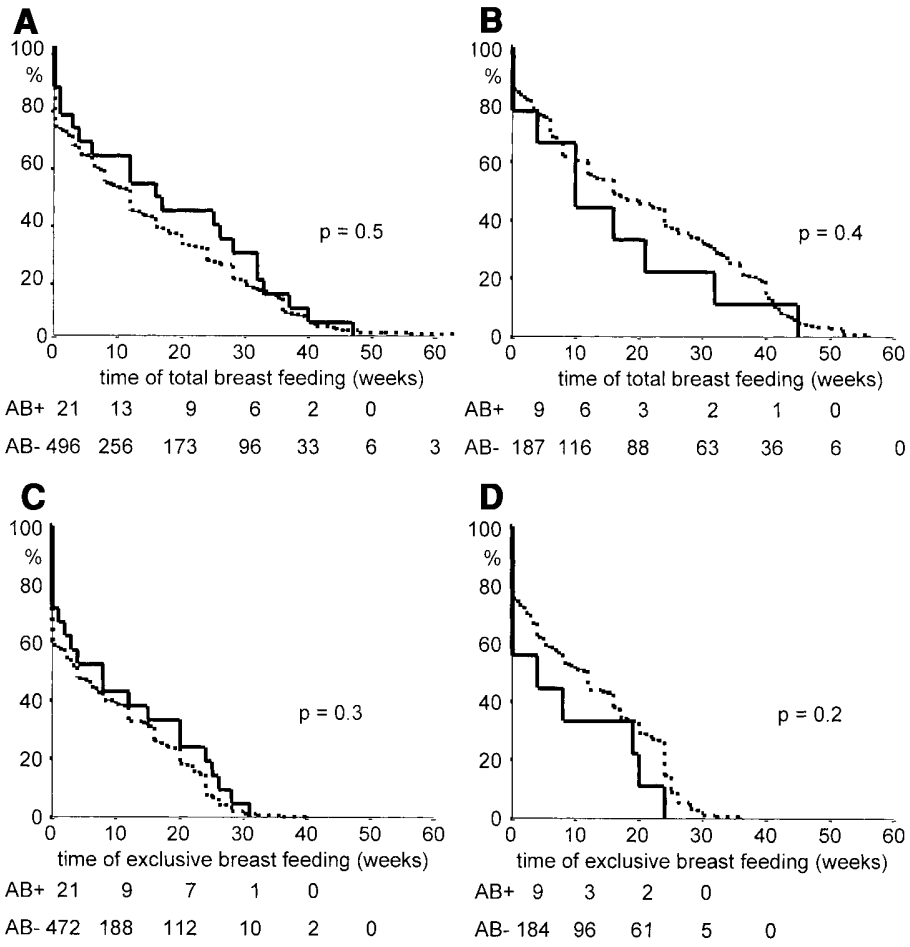

Figure 1—*Life-table analysis of total (*A *and* B*) and exclusive (*C *and* D*) breast-feeding duration in offspring with islet antibodies (solid lines) compared with antibody-negative offspring (broken line) from mothers (*A *and* C*) and fathers (*B *and* D*) with type 1 diabetes. Y-axes represent the proportion of offspring receiving breast milk regardless of other food supplements (*A *and* B*) or exclusively without any other food supplements (*C *and* D*).*

(median follow-up time from birth to last contact or diabetes onset of the total population 3.1 years, range 1.3–9.0). The 179 families (18%) lost to follow-up either had refused participation in the 2-year visit or had moved to an unknown address. A total of 31 (3.8%) offspring developed antibodies to one or more islet autoantigens by the age of 2 years, and 10 (all with islet antibodies) developed type 1 diabetes by the time of follow-up (median onset age 3.3 years, range 1.3–8.5). HLA typing was obtained from 738 of the 823 offspring (8). Of these, 87 offspring had the HLA genotype DRB1*03/04 (DQB1*57non-Asp) or DRB1*04/04 (DQB1*57non-Asp). Of these 87 genetically "high-risk" subjects, 13 (14.9%) developed islet antibodies by the age of 2 years.

Exposure assessment

Data on breast-feeding, vaccinations, and infections were collected by structured questionnaires (available from the authors on request), which were distributed to the families at birth and at 9 months, 2 years, 5 years, and 8 years of age. Questionnaires were completed by the families and returned by mail with vaccination records. Once the questionnaires were returned, families were contacted by phone to ascertain correctness of the completed questionnaires. The following variables were recorded and analyzed:

- The duration of overall (total) breast-feeding (defined as the period when the child received maternal breast milk, regardless of other food supplements) and exclusive breast-feeding (defined as the period when the child received no food other than breast milk)
- Vaccinations against tuberculosis (Bacille Calmette-Guérin [BCG]); *haemophilus influenzae* (HIB); diphtheria, tetanus, and pertussis (DTP); poliomyelitis; tick-born encephalitis (TBE); and measles, mumps, and rubella (MMR). The recommended age for vaccinations were within 6 weeks after birth for BCG; at 3, 4, 5, and 12–15 months for DTP and HIB; at 3, 5, and 12–15 months for polio; at 12 months for TBE; and at 12–18 months and 6 years for MMR
- Data on infection with measles, mumps, or rubella were collected at every follow-up visit

Completed questionnaires were returned from 626 offspring (76.1%). Information concerning total duration of breast-feeding, exclusive time of breast-feeding, vaccinations, and infections was missing from 110, 137, 56, and 68 offspring, respectively.

Antibody assays and HLA determination

IAAs, GADAs, IA-2As, and ICAs were analyzed as previously described (6,7,9). Antibody positivity was defined by the 99th percentile of antibody levels in nondiabetic control children (7,9). HLA-DR and -DQ alleles were determined using polymerase chain reaction–amplified DNA and nonradioactive sequence-specific oligonucleotide probes (10).

Outcome variables

The major outcome variable was the presence (antibody-positive group) or absence (antibody-negative group) of islet antibodies in the 2-year sample. Presence of islet antibody was defined as having at least 1 islet antibody above the 99th percentile of control samples confirmed by repeated measurements (6). Presence of IAA was defined as being positive in the protein A/G-IAA assay (9).

Statistical analysis

The analyses of breast-feeding duration were performed separately for offspring from mothers and fathers with type 1 diabetes. If both parents had diabetes, offspring were included in the mother cohort. The breast-feeding times of islet antibody–positive and –negative offspring were compared with the Mann-Whitney *U* test and Kaplan-Meier life-table analysis. The number of islet antibody–positive and –negative offspring who

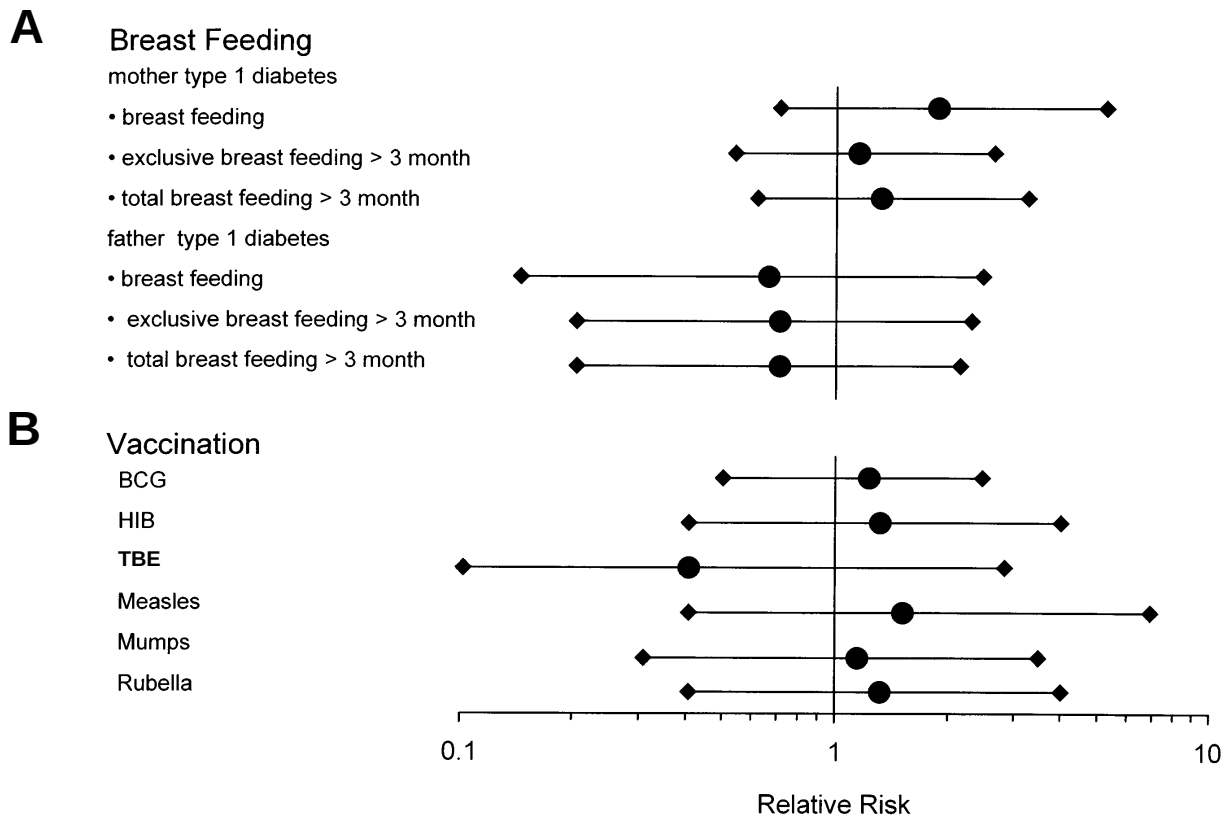

Figure 2—*Risk (odds ratio) for developing islet antibodies with respect to environmental factors. Relative risks (●) are shown with their 95% CI. None of the shown factors had a significant influence on the development of islet antibodies.*

were breast-fed >3 months were compared with Fisher's exact test. The number of islet antibody–positive and –negative offspring receiving specific vaccinations were compared by χ^2 analysis or Fisher's exact test. Offspring who had developed islet antibodies or diabetes before the first immunization with a specific vaccination were excluded from the analysis of that respective vaccine. Odds ratios were calculated as the ratio of the proportion of offspring with islet antibodies in those with breast-feeding times greater and less than 3 months and in those with and without a vaccination event. Statistical methods were performed using version 8.0.1 of the Statistical Package for Social Sciences (SPSS, Chicago).

RESULTS

Breast-feeding

The proportion of offspring who were breast-fed (73%) and the duration of total (median 12 weeks) or exclusive (median 4 weeks) breast-feeding were significantly lower in offspring from mothers with type 1 diabetes compared with offspring from fathers with type 1 diabetes (85% proportion, 16 weeks of total breast-feeding, 11 weeks of exclusive breast-feeding; $P < 0.0001$). In offspring from mothers with diabetes, there were no significant differences in breast-feeding times between islet antibody–positive (median total 16 weeks, exclusive 8 weeks) and islet antibody–negative children (median total 12 weeks, exclusive 4 weeks) by Mann-Whitney *U* comparison or by life-table analysis (Fig. 1*A* and *C*). Of islet antibody–positive offspring, 28.6% compared with 41.5% of islet antibody–negative offspring received supplement feeding from birth (Fig. 1*C*) ($P = 0.33$). Separate analysis in HLA high-risk offspring also showed no differences in breast-feeding patterns between islet antibody–positive and –negative offspring (data not shown). In offspring from fathers with diabetes, a non–statistically significant reduction in exclusive and total breast-feeding times was observed in the islet antibody–positive cohort compared with the islet antibody–negative offspring (median total 10 vs. 16 weeks, $P = 0.41$; median exclusive 4 vs. 12 weeks, $P = 0.31$), and 44% of islet antibody–positive offspring and 25% of islet antibody–negative offspring from fathers with diabetes received supplement feeding from the time of birth (Fig. 1*D*) ($P = 0.36$). Previous studies examined diabetes risk with respect to a breast-feeding time of greater or less than 3 months. The relative risks of developing islet antibodies in the BABYDIAB offspring were 1.25 (95% CI 0.5–3.0) and 1.45 (0.6–3.3) for exclusive and total breast-feeding durations >3 months in offspring from diabetic mothers. In offspring from fathers, the relative risks were 0.65 for both exclusive and total durations (95% CI 0.2–2.5 and 0.2–2.2, respectively) (Fig. 2). All 10 children who developed diabetes were breast-fed, and 5 had a total breast-feeding time >3 months (Table 1).

Vaccinations and viral disease

No significant differences in the prevalence of BCG, DTP, HIB, polio, TBE, or MMR vaccinations were found between islet antibody–positive and –negative offspring. BCG vaccination was performed in 109 (16.6%) of 658 offspring, including 6

Table 1—*Breast-feeding and vaccinations in islet antibody–positive BABYDIAB offspring*

Family ID*	Age at first antibody (years)	IAA	GADA	IA2A	ICA	Age at diabetes onset or last sample (years)	Breast feeding (weeks)		Vaccinations					
							Exclusive	Total	BCG	DTP	HIB	Polio	TBE	MMR
4849-M	0.8	●	●	●	●	1.3 (diabetes)	1	1	●	●●●	●	●	○	○○○
4005-M	0.8	●	●	●	●	3.3 (diabetes)	15	17	●	●●●	●	●	○	†●●●
3941-M	2.0	●	●	●	●	3.8 (diabetes)	2	16	○	●●●	●	●	○	○○○
1872-M	2.0	●	○	○	●	4.9 (diabetes)	12	12	○	●●●	●	●	○	●●●
1032-M	0.8	●	●	●	●	7.1 (diabetes)	0	3	○	●●○	●	●	○	†●○○
1088-M	2.1	●	●	●	●	8.5 (diabetes)	0	1	○	●●○	○	●	○	●●●
2277-F	1.7	●	●	●	●	1.8 (diabetes)	24	32	●	●●●	○	●	○	●●●
4161-F	0.9	●	○	○	●	2.4 (diabetes)	4	4	○	●●●	○	●	○	†●●●
1628-F	2.0	●	●	●	●	3.2 (diabetes)	19	45	○	●●●	○	●	○	●○○
4262-B	0.6	●	●	●	●	2.2 (diabetes)	3	6	○	●●●	●	●	○	†●●●
5006-M	0.8	●	●	○	○	3.3	26	40	○	●●●	●	●	○	○○○
5874-M	2.0	●	○	○	○	2.0	1	8	●	●●●	○	●	○	●●●
1649-M	0.9	●	●	●	●	6.3	24	32	○	●●●	●	●	†●	†●●●
1068-M	0.9	●	●	○	○	8.1	0	0	○	●●●	○	●	○	†●●●
4050-M	0.9	●	●	●	●	3.2	4	4	○	●●●	○	●	○	†●●●
6226-M	1.3	●	●	○	●	2.7	0	32	○	●●●	○	●	○	●●●
4412-M	1.7	●	○	○	○	3.4	8	26	○	●●●	○	●	○	●●●
6354-M	2.1	●	●	●	○	2.1	0	0	○	●●●	○	●	○	●●●
2223-M	2.1	●	●	●	●	5.7	0	0	○	●●○	●	●	○	●●●
4625-M	2.3	●	○	○	○	2.3	47	20	○	●●●	●	●	○	●●●
2905-M	2.4	●	●	●	○	3.6	31	33	○	●●●	○	●	○	○○○
2160-M	2.4	○	●	○	○	2.4	25	37	○	●●●	●	●	○	—
3975-M	2.4	●	●	○	●	2.4	20	25	○	●●●	○	●	○	●●●
3322-M	2.5	●	●	○	●	2.7	—	—	○	●●●	○	●	○	●●●
4000-F	0.8	●	●	○	○	3.8	0	0	●	●●●	●	●	○	†●●○
6637-F	0.9	●	●	○	●	2.3	0	10	○	●●●	○	●	○	○○○
1724-F	1.1	●	●	●	●	5.7	20	21	○	●●●	○	●	○	●●●
5223-F	2.0	●	●	○	○	3.0	0	0	○	●●●	●	●	○	●●●
3929-F	2.1	●	●	●	●	5.3	0	16	○	●●●	●	●	○	●●●
4204-F	2.2	●	●	●	●	4.1	8	10	●	●●●	●	●	○	●●●
4215-B	2.1	●	●	●	○	4.8	28	28	○	●●●	○	●	○	●●●

*B, both parents with diabetes; F, father with diabetes; M, mother with diabetes. ●, Yes; ○, no. †Received vaccination after development of first antibody.

(19.4%) of 31 with islet antibodies. One-third of islet antibody–positive offspring from diabetic fathers and 3 of the 10 offspring who developed diabetes were vaccinated with the BCG vaccine (Table 1). All 3 vaccinated progressors developed diabetes before 4 years of age. Almost all of the offspring received tetanus, diphtheria, and polio vaccines, and the majority also received pertussis vaccines. The frequency of vaccination in offspring with islet antibodies was 100% for tetanus, 100% for diptheria, 100% for polio, and 90.3% for pertussis. In offspring without islet antibodies, the frequency of vaccination was 99.5% for tetanus, 99.6% for diptheria, 98.7% for polio, and 90.7% for pertussis. HIB vaccination was performed in 48% of the offspring with islet antibodies, compared with 42% of the offspring without islet antibodies, and in 6 (60%) offspring who developed diabetes (Table 1). TBE vaccination was infrequent (7.6%), but none of the offspring who developed islet antibodies or diabetes were vaccinated before the appearance of islet autoimmunity (Table 1). MMR vaccination in the second year of life was performed in 81.8 (measles), 81.0 (mumps), and 78.8% (rubella) of all offspring (88, 82, and 82%, respectively, in offspring with islet antibodies). The proportion of offspring who developed islet antibodies after MMR vaccination was similar to antibody prevalence in offspring who were not vaccinated (measles 2.4 vs. 1.4%, mumps 2.3 vs. 2.1%, and rubella 2.3 vs. 1.9%). The effect of vaccinations on antibody development was similar in offspring with high-risk HLA genotypes (data not shown). Overall, antibody-positive and -negative offspring had similar numbers of vaccination events (proportion of completed vaccinations of total possible vaccination events: 68% for islet antibody–positive offspring and 71% for islet antibody–negative offspring). The viral childhood diseases (measles, rubella, and mumps) were reported in only 26 offspring; none of these subjects developed islet antibodies or diabetes.

CONCLUSIONS — Breast-feeding, or lack of it, gives rise to one of the earliest exposures to the environment. Breast-feeding habits were analyzed separately for offspring from mothers and fathers with type 1 diabetes, because babies born to diabetic

mothers were breast-fed less than offspring of diabetic fathers. In offspring from mothers with diabetes, duration of exclusive and total breast-feeding did not differ between islet antibody–positive and –negative children, and breast-feeding of 3 months or longer was not associated with protection from islet antibody or diabetes development. Also, no differences were observed when offspring with HLA high-risk genotypes were analyzed separately. The results of this study, which is the largest study of prospectively followed children of parents with type 1 diabetes, are concordant with a report from the U.S., in which no association between duration of breast-feeding and islet autoimmunity in 18 first-degree relatives who developed islet antibodies in childhood was found (11), and with a study from Australia (12), in which the relationship of breast-feeding and the introduction of cow's milk formula to islet antibody positivity in 322 children of diabetic parents was prospectively determined. Moreover, life-tables between the antibody-positive and -negative cohorts were rather indicative of a longer breast-feeding time in the antibody-positive group. Thus, it is unlikely that a significant major protective effect could be seen with higher numbers. These results indicate that breast-feeding or early introduction of cow's milk are unlikely to be major factors that determine islet autoimmunity in offspring from mothers with type 1 diabetes. Moreover, they suggest that mothers with type 1 diabetes cannot protect their children from autoimmunity by long exposure to breast milk.

In offspring born to a diabetic father and breast-fed from a nondiabetic mother, we observed a slight but not statistically significant reduction in both exclusive and total breast-feeding times in the islet antibody–positive cohort. Therefore, we cannot exclude the possibility that a positive influence of breast-feeding will be revealed when higher numbers of offspring from fathers with type 1 diabetes have been recruited and followed. A tendency toward protection would be consistent with data available from the general population, in which lack of breast-feeding or short duration of breast-feeding is associated with an increased incidence of type 1 diabetes (2). The difference observed between offspring from mothers with type 1 diabetes compared with those from fathers with type 1 diabetes underscores the importance of performing separate analyses of risk associated with breast-feeding patterns.

Vaccinations also represent early exposure to the environment. None of the vaccinations studied (BCG, HIB, DTP, polio, TBE, and MMR) were found to be associated with the development of islet-associated antibodies. In particular, BCG vaccination has been suggested to have a protective effect on the development of type 1 diabetes in humans (13). Our results do not support a protective role of BCG immunization on the development of islet autoimmunity. In contrast, they show that the proportion of offspring who developed autoimmunity and early childhood diabetes after receiving BCG vaccination was relatively high. MMR vaccination has also been suggested to influence diabetes development. Even as a study from Finland observed a halt of the steadily increasing diabetes incidence rate after the introduction of MMR vaccination (4), a report from Germany found that 7 children developed type 1 diabetes shortly after receiving active mumps/measles vaccination (5). In our study, antibodies neither appeared more often after MMR vaccination nor were found more often in vaccinated children compared with offspring that were not MMR vaccinated. Also, no influence by vaccination on the development of islet autoimmunity was found in a cohort of first-degree relatives from the U.S. (14). Overall, the findings in first-degree relatives are consistent with a report from New Zealand that found no associations between year-by-year incidences of childhood diabetes and the introduction, alteration, or abandonment of vaccination programs (15).

In summary, we showed that breast-feeding and vaccination programs had no significant association with the development of islet autoimmunity during early childhood in offspring from parents with type 1 diabetes. Therefore, we suggest that these environmental factors are unlikely to have a major causal influence on initiating islet autoimmunity in such genetically susceptible children.

Acknowledgments — This work was supported by grants from the Bundesministerium für Forschung, Bildung und Technologie (01KD89030 and 1KD9601).

We thank M. Dübell, A. Schimmel, and A. Wagner for data collection and expert technical assistance and all obstetric departments, pediatricians, and family doctors in Germany who participated in the trial for the recruitment and follow-up of families in the BABYDIAB Study.

The following participants contributed data of more than 10 offspring to this analysis: M. Sorger of Medizinische Universitäts-Poliklinik, Bonn; H.U. Janka and C. Kastendiek of Zentralkrankenhaus Bremen-Nord, Bremen; Dr. Kaltofen of Klinikum Chemnitz, Chemnitz; U. Hofmann of Krankenhaus Sachsenhausen, Frankfurt; Dr. Günther of Medizinische Hochschule Hannover, Hannover; H.-J. Gottwald of Städtisches Klinikum St. Georg, Leipzig; M. Schiller, K. Brauch, K. Lohe, W. Rabl, and E. Standl of Städtisches Krankenhaus München-Schwabing and R. Steldinger of Universitäts-Frauenklinik, Munich; and F. Louwen and J. Stüssel of Universitäts-Frauenklinik, Münster, Germany.

This study forms part of the dissertation of M. Schenker of the Ludwig-Maximillians-University of Munich.

References

1. Virtanen SM, Räsänen L, Ylönen K, Aro A, Clayton D, Langholz B, Pitkäniemi J, Savilahti E, Lounamaa R, Tuomilehto J, Åkerblom HK: Early introduction of diary products associated with increased risk of IDDM in Finnish children: the Childhood in Diabetes in Finland Study Group. *Diabetes* 42:1786–1790, 1993
2. Gerstein HC: Cow's milk exposure and type 1 diabetes mellitus: a critical review of the clinical literature. *Diabetes Care* 17:13–19, 1994
3. The EURODIAB Substudy 2 Study Group: Vitamin D supplement in early childhood and risk for type 1 diabetes mellitus. *Diabetologia* 42:51–54, 1999
4. Hyöty H, Hiltunen M, Reunanen A, Leinikki P, Vesikari T, Lounamaa R, Toumilhto J, Åkerblom HK: Decline of mumps antibodies in type 1 (insulin-dependent) diabetic children and a plateau in the rising incidence of type 1 diabetes after introduction of the mumps-measles-rubella vaccine in Finland: the Childhood in Diabetes in Finland Study Group. *Diabetologia* 36:1303–1308, 1993
5. Helmke K, Otten A, Willems WR, Brockhaus R, Mueller-Eckhard G, Stief T, Bertrams J, Wolf H, Federlin K: Islet cell antibodies and the development of diabetes mellitus in relation to mumps infection and mumps vaccination. *Diabetologia* 29:30–33, 1986
6. Ziegler AG, Hummel M, Schenker M, Bonifacio E: Antibody appearance and risk for the development of childhood diabetes in offspring of parents with type 1 diabetes: the German BABYDIAB Study. *Diabetes* 48: 460–468, 1999
7. Roll U, Christie MR, Füchtenbusch M, Payton MA, Hawkes CJ, Ziegler AG: The German multicenter BABYDIAB Study: detection of humoral immune responses to islet antigens in early childhood. *Diabetes* 45:967–973, 1996
8. Schenker M, Hummel M, Ferber K, Walter M, Keller E, Albert E, Janka HU, Kastendiek C, Sorger M, Louwen F, Eisenbarth

GS, Ziegler AG: Early expression and high prevalence of anti-islet antibodies for DR3/4 heterozygous and DR4/4 homozygous offspring of parents with type 1 diabetes: the German BABY-DIAB study. *Diabetologia* 42:671–677, 1999
9. Naserke HE, Bonifacio E, Ziegler AG: Immunoglobulin G insulin antibodies in BABYDIAB offspring appear postnatally: sensitive early detection using a protein A/G-based radiobinding assay. *J Clin Endocrinol Metab* 84:1239–1243, 1999
10. Kimura A, Sasazuki T: 11th International Histocompatibility Workshop reference protocol for the HLA DANN-typing technique. In *HLA*. Vol. 1. Tsuji K, Aizawa A, Sasazuki T, Eds. Oxford, U.K., Oxford University Press, 1992, p. 397–419
11. Norris JM, Beaty B, Klingensmith G, Liping Y, Hoffman M, Chase HP, Erlich HA, Hamman RF, Eisenbarth GS, Rewers M: Lack of association between early exposure to cow's milk protein and β-cell autoimmunity. *JAMA* 276:609–614, 1996
12. Couper JJ, Steele C, Beresford S, Powell T, McCaul K, Pollard A, Gellert S, Tait B, Harrison LC, Colman PG: Lack of association between duration of breast-feeding or introduction of cow's milk and development of islet autoimmunity. *Diabetes* 48: 2145–2149, 1999
13. Shehadeh N, Calcinaro F, Bradley BJ, Bruchlim I, Vardi P, Lafferty KJ: Effect of adjuvant therapy on development of diabetes in mouse and man. *Lancet* 343:706–707, 1994
14. Graves PM, Barriga KJ, Norris JM, Hoffman MR, Yu L, Eisenbarth GS, Rewers M: Lack of association between early childhood immunizations and β-cell autoimmunity. *Diabetes Care* 22:1694–1697, 1999
15. Elliot RB, Bibby NK, McGregor M, Pilcher CC: Childhood vaccines and the aetiology of type 1 diabetes. *Horm Res* 50:117, 1998