Early Infant Feeding and Risk of Developing Type 1 Diabetes–Associated Autoantibodies

Anette-G. Ziegler, MD
Sandra Schmid, PhD
Doris Huber
Michael Hummel, MD
Ezio Bonifacio, PhD

Context Dietary factors modifying type 1 diabetes mellitus (DM) risk have been proposed, but little is known if they trigger the islet autoimmunity that precedes clinical disease.

Objective To determine whether breastfeeding duration, food supplementation, or age at introduction of gluten-containing foods influences the risk of developing islet autoantibodies.

Design and Setting Prospective natural history cohort study conducted from 1989 to 2003 in inpatient/outpatient clinics in Germany.

Participants The BABYDIAB study follows newborn children of parents with type 1 DM. Eligibility requirements were met in 1610 children. Blood samples were obtained at birth, age 9 months, 2, 5, and 8 years. Dropout rate was 14.4% by age 5 years. Breastfeeding data were obtained by prospective questionnaires (91% complete), and food supplementation data were obtained by family interview (72% for food supplementation and 80% for age of gluten introduction).

Main Outcome Measure Development of islet autoantibodies (insulin, glutamic acid decarboxylase, or IA-2 antibodies) in 2 consecutive blood samples.

Results Life-table islet autoantibody frequency was 5.8% (SE, 0.6%) by age 5 years. Reduced total or exclusive breastfeeding duration did not significantly increase the risk of developing islet autoantibodies. Food supplementation with gluten-containing foods before age 3 months, however, was associated with significantly increased islet autoantibody risk (adjusted hazard ratio, 4.0; 95% confidence interval, 1.4-11.5; $P=.01$ vs children who received only breast milk until age 3 months). Four of 17 children who received gluten foods before age 3 months developed islet autoantibodies (life-table 5-year risk, 24%; SE, 10%). All 4 children had the high-risk DRB1*03/04,DQB1*0302 genotype. Early exposure to gluten did not significantly increase the risk of developing celiac disease–associated autoantibodies. Children who first received gluten foods after age 6 months did not have increased risks for islet or celiac disease autoantibodies.

Conclusion Ensuring compliance to infant feeding guidelines is a possible way to reduce the risk of development of type 1 DM autoantibodies.

JAMA. 2003;290:1721-1728 www.jama.com

TYPE 1 DIABETES MELLITUS (DM) is a chronic disease of unknown etiology with a preclinical phase characterized by autoimmunity against pancreatic islet cells.[1,2] A genetic susceptibility is well documented[3] and an environmental influence is assumed.[4] The autoimmunity that precedes type 1 DM can appear in the first years of life, suggesting that environmental agents encountered early in life could be triggers of the disease process.[5] Notably, the early introduction of cow's milk and short duration of breastfeeding have been reported to be associated with increased risk of type 1 DM.[6,7] These findings have created substantial scientific and public interest and have led to several claims that a variety of proteins in cow's milk are immunogenic determinants of type 1 DM.[8-11] However, prospective studies in genetically selected children have not found an association,[12-14] or found only a weak association,[15] between breastfeeding duration or timing of exposure to cow's milk and islet autoimmunity, indicating that an antigenic basis for the association between type 1 DM and cow's milk is unlikely.[16]

The food antigen gluten is an immunological trigger and the driving antigen of the autoimmunity found in celiac disease.[17,18] It is first encountered relatively early in life, most commonly around age 6 months. Changes in the timing of gluten exposure as well as the dose of early exposure have been suggested, but not proven, to modify the risk of celiac disease.[19-22] Gluten also has been suggested to play a role in type 1 DM. The increased association between type 1 DM and celiac disease,[23] the reduced prevalence of type 1 DM autoimmunity after gluten deprivation in patients with celiac disease,[24] and the reduced incidence of autoimmune diabetes in the nonobese diabetic mouse receiving a gluten-free diet[25] are some of the evidence supporting this claim.

The present study investigates whether dietary habits in the first year of life modify risk for the development of type 1 DM-associated islet autoantibodies and celiac disease–associated au-

See also pp 1713 and 1771 and Patient Page.

Author Affiliations: Diabetes Research Institute and Hospital München-Schwabing, Munich, Germany (Drs Ziegler, Schmid, and Hummel, and Ms Huber) and Istituto Scientifico San Raffaele, Milan, Italy (Dr Bonifacio). **Corresponding Author and Reprints:** Anette-G. Ziegler, MD, Diabetes Research Institute, Koelner Platz 1, D-80804 Munich, Germany (e-mail: anziegler@lrz.uni-muenchen.de).

toantibodies in prospectively followed children of parents with type 1 DM. The study specifically asks whether timing of exposures to breast milk, milk formula, solid foods, or gluten-containing foods is associated with an increased prevalence of autoantibodies by age 5 years.

METHODS

Participants

BABYDIAB prospectively follows offspring from birth of mothers and/or fathers with type 1 DM. Venous blood samples and collection of questionnaires data were taken at birth, at age 9 months, and 2, 5, 8, and 11 years.[5] Recruitment into the study began in July 1989 and ended in November 2000. Follow-up (collection of blood samples and questionnaires) continued until July 2003. A total of 1709 offspring were recruited at birth and 1610 participated in the 9-month follow-up thereby meeting eligibility criteria. These offspring included 1010 newborns from mothers with type 1 DM, 572 newborns from fathers with type 1 DM, and 28 newborns from 2 parents with type 1 DM. At the time of analysis (July 2003), the cumulative dropout rate was 7.2% by age 2 years and 14.4% by age 5 years. Islet autoantibodies were measured in blood samples from all scheduled visits and yearly after developing islet autoantibodies. HLA DR and DQ genotypes were determined in 1367 offspring. The median follow-up time was 6.5 years (range, 9 months to 12.5 years) for a total of 9773 subject-years. Antibodies to tissue transglutaminase C (tTGCA) were measured in blood samples from 1161 offspring. Written informed consent was obtained from the parents. The study was approved by the ethical committee of Bavaria, Germany (Bayerische Landesärztekammer No. 95357).

Dietary Questionnaires and Interviews

Families were asked to complete questionnaires at birth, age 9 months, and 2 years with respect to breastfeeding. In the questionnaires, mothers were asked when non–breast milk food supplements were introduced into the child's diet (exclusive breastfeeding duration) and when breastfeeding was discontinued (total breastfeeding duration). Families also were asked to keep food records. Families subsequently were contacted by telephone between September 2001 and December 2001 and asked to supply information from their food records. Parents were asked what food supplements (milk-based and solid foods) were given to their child before age 3 months, and when gluten-containing foods (foods containing wheat, rye, barley, or oat, including breads, biscuits, cakes, porridge, pasta, and flour products) first were given to the child. As a control, parents also were asked to recall the duration of exclusive and total breastfeeding. In all interviews the interviewers were unaware of the responses provided in the prospectively collected questionnaires and of the autoantibody status of the families, whereas parents previously had been informed of the autoantibody status of their child.

Autoantibody Testing

Insulin autoantibodies (IAA), glutamic acid decarboxylase autoantibodies (GADA), and insulinoma antigen-2 (IA-2) were determined by radiobinding assays as described previously.[5,26] The upper limits of normal corresponded to the 99th percentile of control children[5] and corresponded to 25 units/mL for glutamic acid decorboxylase (GAD), antibodies and 4 units/mL for IA-2 antibodies from the World Health Organization, and 1.5 local units/mL for IAA. Using these thresholds for positivity, the assays had sensitivities and specificities of 80% and 94% (GAD antibodies), 58% and 100% (IA-2 antibodies), and 30% and 98% (IAA) in the First Diabetes Autoantibody Standardization Program proficiency evaluation.[27] Measurement of IgA-tTGCA by radiobinding assay and IgA endomysial antibodies by indirect immunofluorescence was performed as described previously.[28] The 99th percentile level in control children without DM and celiac disease was used to define positive tTGCA results. All measurements were performed on coded samples that were operator blinded.

HLA Typing

HLA class II alleles HLA-DRB1, HLA-DQA1, and HLA-DQB1 were determined using polymerase chain reaction–amplified DNA and nonradioactive sequence-specific oligonucleotide probes as described previously.[29]

Statistical Analysis

Time to event methods were used to calculate risks (life-table analysis) and to compare outcome for participants with different covariate categories (Cox proportional hazards model). Positive outcome was defined as 1 or more autoantibodies in at least 2 consecutive blood samples. Status in children with autoantibodies in one sample and no subsequent sample for confirmation was considered a negative result. The age of onset of islet autoantibody positivity was defined as the age at the first positive sample result. Analyses considered censoring in losses to follow-up and in participants with antibody negative status at the follow-up visit age of their last antibody negative sample result. The following dietary variables were analyzed: (1) the duration of breastfeeding (categorized as 0 weeks, 0.1-3 months, 3.1-6 months, >6 months, and unknown); (2) the duration of exclusive breastfeeding (categorized as 0 weeks, 0.1-3 months, 3.1-6 months, >6 months, and unknown); (3) the foods supplemented to the child's diet in the first 3 months of life (categorized as no food supplementation [breast milk only], supplementation with milk-based foods only, supplementation with solid foods that did not contain gluten, supplementation with gluten-containing foods, and unknown); and (4) the child's age at introduction of gluten-containing foods (categorized as <3 months, 3.1-6 months, >6 months, and unknown). Hazards ratios (HRs) were calculated using Cox proportional hazards model. Hazard ratios in Cox proportional hazards model were adjusted

for the potential confounder variables maternal type 1 DM, early gestation age at delivery (<36 weeks, corresponding to the 10th percentile of the study cohort), low birth weight of child (≤2700 g, corresponding to the 10th percentile of the study cohort), and the region of Germany in which the child lived (former East Germany or former West Germany). These data were obtained for all 1610 children from questionnaires at birth and age 9 months. The proportional hazards assumption in the Cox model was tested by examining the log minus log plot of each covariate for parallel curves, and by using a time-dependent Cox regression that included the covariate in question and the interaction between time and that covariate. This interaction was not significant for all covariates indicating that the hazards were proportional. Time to event analyses were performed when the sample size was estimated to have 80% power (2-tailed α of .05) to detect an HR of 1.6 for reduced breastfeeding duration. This estimate was based on a preliminary analysis in 1999 that observed an exposure rate of 50% for breastfeeding less than 3 months and a cumulative 5-year islet autoantibody conversion rate of 6%,[14] and on a previous meta-analysis of type 1 DM risk and breastfeeding duration that reported an HR of 1.6 in children with reduced breastfeeding.[7]

Analysis of autoantibody status and diet also was performed by the 2-tailed Fisher exact test after classification of children into islet autoantibody results of positive or negative by age 2.5 years. The Mann-Whitney *U* test was used to compare breastfeeding duration, gestation age, and birth weight between groups. The introduction of gluten-containing foods was cross analyzed with the duration of exclusive breastfeeding, and the breastfeeding duration reported at interview was compared with those provided prospectively in questionnaires using the Pearson correlation coefficient. All *P* values are 2-tailed (*P*=.05). All statistical analyses were performed using the Statistical Package for Social Science (SPSS Inc, Chicago, Ill).

RESULTS

Questionnaire and Interview Responses

The total number of children enrolled in the study was 1610. For islet autoantibodies, breastfeeding data from prospective questionnaires were obtained for 1460 children (90.7%) and were unknown in 150 children (9.3%). Of these, food supplementation in the first 3 months of life was obtained from telephone interview in 1282 children (80% of enrolled children) and were unknown in 328; all 1282 children who provided food supplementation data also provided prospective breastfeeding data. The age at introducing gluten-containing foods was recorded and retrieved in 1153 of these children (71.6% of enrolled children) and were unknown in 457. For each variable, children with incomplete data did not differ significantly with children with complete data with respect to their year of birth, mean follow-up time, and frequency of islet autoantibodies. As a validation of the food introduction collected by telephone, total and exclusive breastfeeding duration provided in prospective questionnaires were found to be highly concordant with those given at the telephone interview (*r*=0.93 and 0.9, respectively; *P*<.001).

Infant Feeding Habits

Of the 1460 children with known breastfeeding duration, 298 (20.4%) were not breastfed at all, and 433 (29.7%) received non–breast milk food supplements already in the first week of life (TABLE 1). The median duration of total and exclusive breastfeeding were 16 weeks (interquartile range [IQR], 3-32 weeks), and 9 weeks (IQR, 0-22 weeks), respectively. Food supplements other than breast milk were given to 581 (45.3%) of 1282 children in the first 3 months of life. Seventeen (1.3%) of 1282 children received gluten-containing food supplements, another 46 (3.6%) received non–gluten-containing solid food supplements, and 518 (40.4%) received only milk-based food supplements. None of the 17 children who received gluten-containing food supplements and 4 of the 46 children who received non-gluten–containing solid food supplements were still receiving breast milk at the time of introducing these supplements.

The age at which gluten-containing foods were introduced into the diet correlated with the duration of exclusive breastfeeding (*r*=0.45; *P*<.001). Compared with children of mothers without diabetes, children of mothers with type 1 DM were breastfed significantly less (median total breastfeeding duration 12 vs 24 weeks; *P*<.001), had a reduced gestation age (39 vs 40 weeks; *P*<.001), and were heavier at birth (3500 g vs 3390 g; *P*<.001). No significant differences were found for the age of introducing gluten-containing foods with respect to maternal diabetes, gestation age, birth weight, or region of residence.

Islet Autoimmunity

The cumulative risk of developing islet autoantibodies (IAA, GADA, and/or IA-2A) in all 1610 offspring was 5.8% (SE, 0.6%) by 5 years of age. Eighty-five offspring developed islet autoantibodies, and 48 of these were positive for 2 or more islet antibodies. Twenty-two offspring who were autoantibody positive and non who were autoantibody negative developed type 1 DM.

Islet Autoimmunity and Breastfeeding

No significant differences in islet autoantibody risk were observed with respect to the duration of total or exclusive breastfeeding (Table 1). Life-table islet autoantibody risk was lowest in children who were never breastfed (n=298; 5-year risk, 4.2%; SE, 1%) and highest in children exclusively breastfed for longer than 6 months (n=102; 5-year risk, 9%; SE, 3%). The median duration of total breastfeeding was 16 weeks (IQR, 3-32 weeks) in children who were islet autoantibody negative and 17.5 weeks (IQR, 6-33 weeks) in children who were autoantibody positive (*P*=.20), and the median duration of exclusive breastfeeding was 9 weeks (IQR, 0-22 weeks) and 12 weeks (IQR, 1-20 weeks) in children who were autoantibody negative and positive, respectively (*P*=.40).

Islet Autoimmunity and Food Supplements in the First 3 Months

Islet autoantibody risk was significantly increased in children who received gluten-containing foods in their first 3 months of life (Table 1, FIGURE 1). Life-table 5-year autoantibody risk in these 17 children was 24.0% (SE, 10%), compared with 7.0% (SE, 1%) in 701 children who received breast milk only (adjusted HR, 4.0; 95% confidence interval [CI], 1.4-11.5; $P=.01$). This observation was confirmed using an exact analysis (Fisher exact test) of islet autoantibody frequency at age 2.5 years in children who received gluten-containing foods prior to age 3 months (3/17; 17.6%) compared with children who did not receive gluten foods before age 3 months (45/1265; 3.6%; $P=.02$). Four of the 17 children who received gluten prior to age 3 months developed islet autoantibodies. All 4 children had persistent multiple islet autoantibodies (3 had IAA, GADA, and IA-2A and 1 had IAA and GADA); all have remained islet autoantibody positive, and 1 has developed type 1 DM at age 6.9 years; and all have the DR3/DR4-DQ8 genotype. Two children were from mothers with type 1 DM and 2 had fathers with type 1 DM. One of the 46 children who received non–gluten-containing solid foods developed islet autoantibodies. Risk in children who received supplements before age 3 months that were milk-based only was not increased over the risk in children who were exclusively breastfed for at least 3 months (adjusted HR, 0.8; 95% CI, 0.5-1.4).

Islet Autoimmunity and Age at Introduction of Gluten Foods

Islet autoantibody risk was significantly increased in children who received gluten-containing foods prior to age 3 months compared with children receiving gluten between ages 3.1 and 6 months (5-year risk, 24% vs 5.2%; adjusted HR, 5.2; 95% CI, 1.7-15.5; $P=.003$; Table 1 and FIGURE 2). The increased risk was observed for IAA (24% vs 4%; $P=.001$), GADA (24% vs 4%; $P=.001$), and IA-2A (19% vs 3%; $P=.005$). Islet autoantibody risk was not significantly increased in children who first received gluten after age 6 months (5-year risk, 5.9%; adjusted HR, 1.2; 95% CI, 0.7-2.0; $P=.60$), and none of the 25 children who first received gluten-containing foods after age 12 months developed islet autoantibodies.

Islet Autoimmunity and Diet in Children With HLA Genotype

The risk of developing islet autoantibodies is markedly increased in children with the HLA-DRB1*03/04,DQB8 genotype.[29] Of the 1367 children who were HLA typed, 104 had this genotype. Four of these 104 children received gluten-

Table 1. Risk for Islet Autoantibodies in BABYDIAB Cohort Children in Relation to Infant Diet

Feeding Variable	Islet Ab Positive, No.*	5-Year Islet Ab Frequency, % (SE)†	Unadjusted Hazard Ratio (95% CI)‡	Adjusted Hazard Ratio (95% CI)§	*P* Value‖	Overall *P* Value¶
Duration of total breastfeeding, mo						
None (n = 298)	11	4.2 (1)	0.5 (0.2-1.1)	0.6 (0.3-1.2)	.13	
0.1-3 (n = 396)	20	5.0 (1)	0.7 (0.4-1.4)	0.8 (0.4-1.5)	.44	
3.1-6 (n = 274)	19	7.7 (2)	1.0	1.0		.36
>6 (n = 492)	31	7.0 (1)	0.9 (0.5-1.7)	1.0 (0.5-1.7)	.86	
Unknown (n = 150)	4	4.2 (2)	0.4 (0.1-1.3)	0.4 (0.2-1.3)	.14	
Duration of exclusive breastfeeding, mo						
None (n = 433)	19	4.5 (1)	0.7 (0.4-1.2)	0.7 (0.4-1.3)	.35	
0.1-3 (n = 402)	23	6.5 (1)	0.9 (0.5-1.6)	1.0 (0.6-1.7)	.93	
3.1-6 (n = 523)	32	6.6 (1)	1.0	1.0		.55
>6 (n = 102)	7	9.0 (3)	1.2 (0.5-2.7)	1.2 (0.5-2.8)	.60	
Unknown (n = 150)	4	4.2 (2)	0.7 (0.3-1.7)	0.7 (0.3-1.8)	.55	
Food supplementation before age 3 mo						
None (n = 701)	42	7.0 (1)	1.0	1.0		
Milk-based food supplements only (n = 518)	26	4.8 (1)	0.8 (0.5-1.2)	0.8 (0.5-1.4)	.48	
Nongluten solid food supplements (n = 46)	1	2.2 (2)	0.3 (0.04-2.3)	0.3 (0.05-2.5)	.30	.04
Gluten-containing food supplements (n = 17)	4	24.0 (10)	3.5 (1.3-9.8)	4.0 (1.4-11.5)	.01	
Unknown (n = 328)	12	4.4 (1)	0.7 (0.4-1.3)	0.7 (0.4-1.4)	.38	
Age at introduction of gluten foods, mo						
≤3 (n = 17)	4	24.0 (10)	4.8 (1.6-14.2)	5.2 (1.7-15.5)	.003	
3.1-6 (n = 385)	18	5.2 (1)	1.0	1.0		.03
>6 (n = 751)	43	5.9 (1)	1.2 (0.7-2.1)	1.2 (0.7-2.0)	.60	
Unknown (n = 457)	20	5.4 (1)	1.2 (0.6-2.2)	1.1 (0.6-2.2)	.70	

Abbreviations: Ab, antibody; CI, confidence interval.
*Number of islet antibody-positive children in category.
†Life-table 5-year islet autoantibody frequency.
‡Unadjusted hazards ratio from Cox proportional hazards model.
§Hazards ratio obtained after adjusting for the confounder variables maternal type 1 diabetes mellitus, gestation age before 36 weeks, birth weight below 2700 g, and region of residence.
‖For each feeding variable *P* values are calculated for adjusted hazard ratio against reference cell.
¶Overall *P* values for Cox proportional hazard model.

containing food supplements before age 3 months, and all 4 developed islet autoantibodies (adjusted HR, 4.4; 95% CI, 1.2-15.9; $P=.02$ vs children who exclusively received breast milk; TABLE 2). Another 2 children with the DR3/DR4-DQ8 genotype received non-gluten–containing supplements, and 1 of these children developed islet autoantibodies. Islet autoantibody risk was significantly decreased in the 31 children with HLA-DR3/DR4-DQ8 genotype who received only milk-based food supplements (adjusted HR, 0.1; 95% CI, 0.01-0.8; $P=.03$ vs children who exclusively received breast milk).

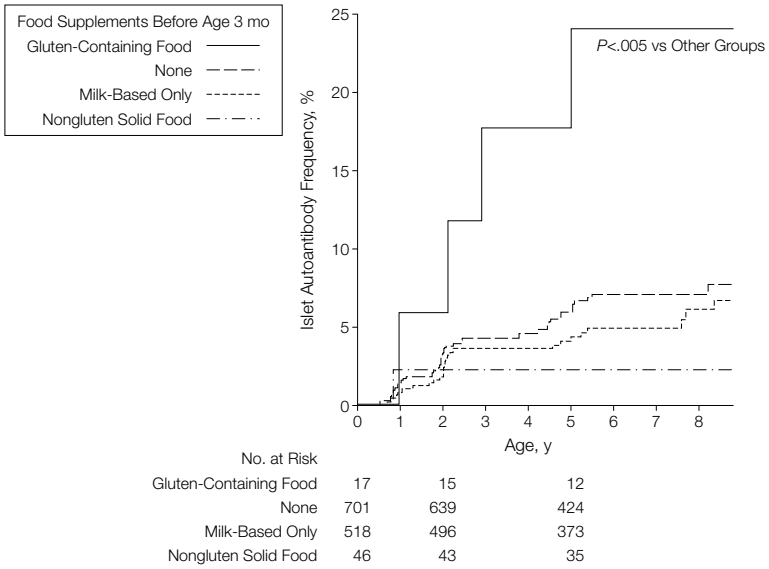
Infant Feeding and Autoantibodies to Tissue tTGCA

Twenty-seven offspring developed IgA-tTGCA, of whom 24 also were IgA-endomysial antibody positive, 21 had at least 1 HLA-DR3 allele, and 2 also had islet autoantibodies. No trend in tTGCA risk was observed for duration of breastfeeding (TABLE 3). Nonsignificant increases in tTGCA risk were found in children who received gluten-containing solid food supplements (adjusted HR, 3.8; 95% CI, 0.5-30.6; $P=.20$) or non–gluten-containing food supplements (adjusted HR, 2.7; 95% CI, 0.6-12.7; $P=.21$) as compared with risk in children who only received breast milk. One child who received gluten-containing foods before age 3 months developed tTGCA. This child also had islet autoantibodies. Two additional children who were introduced to nongluten-containing solid foods before age 3 months developed tTGCA. Neither of these had islet autoantibodies. In total, 7 of 63 children who received gluten- or nongluten-containing solid foods before age 3 months developed islet autoantibodies or tTGCA. Delaying the introduction of gluten-containing foods until after age 6 months did not increase the risk for tTGCA (adjusted HR, 0.7; 95% CI, 0.3-1.8; $P=.46$ vs introduction between ages 3 and 6 months).

COMMENT

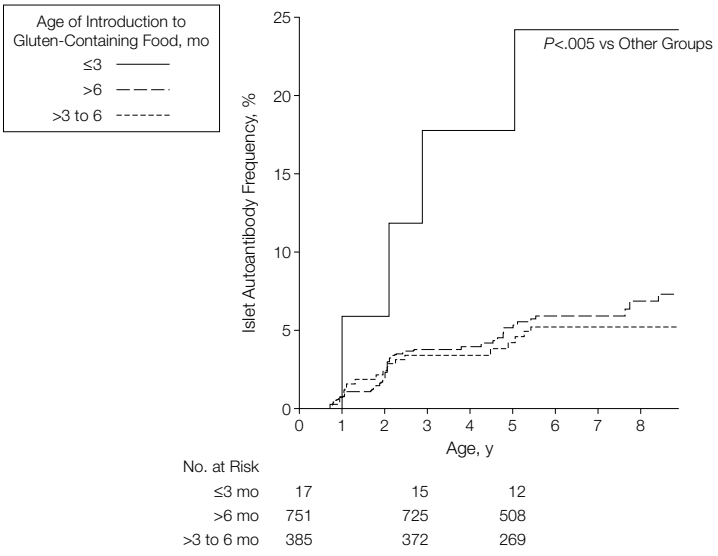
Early introduction of gluten-containing foods was found to be a risk factor for the development of type 1 DM-associated autoantibodies in children of parents with type 1 DM. Exposure to dietary gluten before age 3 months showed a 5-fold higher risk for the development of islet autoantibodies than after age 3 months. All children with the HLA-DR3/DR4-DQ8 genotype who received gluten-containing foods before age 3 months developed multiple

Figure 1. Islet Autoantibody Development in Relation to Early Food Supplementation



Life-table analysis of islet autoantibody frequencies in BABYDIAB offspring of parents with type 1 diabetes mellitus in relation to food supplements received before age 3 months. The *P* value is for the comparison of gluten-containing food vs the other categories.

Figure 2. Islet Autoantibody Development in Relation to Age at Introduction of Gluten-Containing Foods



Life-table analysis of islet autoantibody frequencies in BABYDIAB offspring of parents with type 1 diabetes mellitus in relation to when they first received gluten-containing foods. The *P* value is for the comparison of ≤3 months vs the other categories.

islet autoantibodies. Islet autoantibody risk was not associated with reduced breastfeeding. These findings indicate that early introduction of gluten-containing foods should be avoided in children who are genetically predisposed to type 1 DM.

The accuracy of the findings is dependent on the assumption that the data were not significantly biased by recall error or missing data. Breastfeeding data were prospectively collected from questionnaires. The data on food supplementation and the age of introduction of gluten-containing foods were prospectively recorded, but retrospectively collected by telephone interview. To check the accuracy of the telephone interview data, families again were asked to provide the age at which exclusive and total breastfeeding were discontinued in their child. Telephone retrieved data were highly concordant with those provided prospectively in questionnaires, suggesting that food records were accurate. A limitation of the study was that food supplementation data and the age of in-

Table 2. Risk for Islet Autoantibodies in Children With HLA DR3/DR4-DQ8 Genotype in Relation to Food Supplementation Before Age 3 Months

Food Supplements Before Age 3 mo	Islet Ab Positive, No.*	5-Year Islet Ab Frequency, % (SE)†	Unadjusted Hazard Ratio (95% CI)‡	Adjusted Hazard Ratio (95% CI)§	*P* Value‖	Overall *P* Value¶
None (n = 47)	11	25.6 (7)	1.0	1.0		
Milk-based food supplements only (n = 31)	1	3.5 (3)	0.1 (0.02-1.0)	0.1 (0.01-0.8)	.03	.006
Nongluten solid food supplements (n = 2)	1	50.0 (35)	3.1 (0.4-24.2)	3.0 (0.3-27.2)	.32	
Gluten-containing food supplements (n = 4)	4	100.0 (22)	6.0 (1.9-19.2)	4.4 (1.2-15.9)	.02	
Unknown (n = 20)	3	19.3 (11)	0.7 (0.2-2.4)	0.6 (0.2-2.2)	.44	

Abbreviations: Ab, antibody; CI, confidence interval.
*Number of islet antibody-positive children in category.
†Life-table 5-year islet autoantibody frequency.
‡Unadjusted hazards ratio from Cox proportional hazards model.
§Hazards ratio obtained after adjusting for the confounder variables maternal type 1 diabetes mellitus, gestation age before 36 weeks, birth weight below 2700 g, and region of residence.
‖For each feeding variable *P* values are calculated for adjusted hazard ratio against reference cell.
¶Overall *P* values for Cox proportional hazard model.

Table 3. Risk for Transglutaminase C (tTGCA) Autoantibodies in BABYDIAB Cohort Children in Relation to Infant Diet

Feeding Variable	tTGCA Positive, No.*	5-Year tTGCA Frequency, % (SE)†	Unadjusted Hazard Ratio (95% CI)‡	Adjusted Hazard Ratio (95% CI)§	*P* Value‖	Overall *P* Value¶
Duration of total breastfeeding						
None (n = 236)	5	1.8 (1)	0.9 (0.3-3.0)	0.8 (0.2-2.7)	.67	>.99
0.1-3 mo (n = 298)	8	2.9 (1)	1.2 (0.4-3.7)	1.2 (0.4-3.6)	.77	
3.1-6 mo (n = 202)	6	3.2 (1)	1.0	1.0		
>6 mo (n = 330)	5	1.6 (1)	0.6 (0.2-2.2)	0.6 (0.2-2.0)	.38	
Unknown (n = 95)	3	3.9 (2)	1.4 (0.3-5.9)	1.5 (0.3-6.2)	.60	
Duration of exclusive breastfeeding						
None (n = 346)	8	2.2 (1)	1.0 (0.4-2.8)	1.0 (0.4-2.8)	>.99	.60
0.1-3 mo (n = 291)	7	2.6 (1)	1.1 (0.4-3.1)	1.2 (0.4-3.5)	.68	
3.1-6 mo (n = 369)	8	2.3 (1)	1.0	1.0		
>6 mo (n = 60)	1	2.0 (2)	0.8 (0.1-6.2)	0.9 (0.1-7.2)	.92	
Unknown (n = 95)	3	3.9 (2)	1.5 (0.4-5.3)	1.6 (0.4-6.2)	.49	
Food supplementation before age 3 mo						
None (n = 483)	10	2.2 (1)	1.0	1.0		.50
Milk-based food supplements only (n = 406)	9	2.1 (1)	1.2 (0.4-3.6)	1.0 (0.4-2.5)	.96	
Nongluten solid food supplements (n = 41)	2	5.3 (4)	3.0 (0.4-23.2)	2.7 (0.6-12.7)	.21	
Gluten-containing food supplements (n = 16)	1	6.3 (6)	2.4 (0.5-10.8)	3.8 (0.5-30.6)	.20	
Unknown (n = 215)	5	2.8 (1)	1.0 (0.4-2.5)	1.3 (0.4-4.0)	.62	
Age at introduction of gluten foods						
≤3 mo (n = 16)	1	6.3 (6)	2.3 (0.3-18.2)	2.9 (0.4-24.1)	.32	.55
3.1-6 mo (n = 291)	8	2.9 (1)	1	1.0		
>6 mo (n = 565)	11	2.1 (1)	0.7 (0.3-1.8)	0.7 (0.3-1.8)	.46	
Unknown (n = 289)	7	2.5 (1)	1.0 (0.3-2.6)	1.0 (0.4-2.8)	>.99	

Abbreviation: CI, confidence interval.
*Number of tTGCA positive children in category.
†Life-table 5-year tTGCA frequency.
‡Unadjusted hazard ratio from Cox proportional hazards model.
§Hazard ratio obtained after adjusting for the confounder variables maternal type 1 diabetes mellitus, gestation age before 36 weeks, birth weight below 2700 g, and region of residence.
‖For each feeding variable *P* values are calculated for adjusted hazard ratio against reference cell.
¶Overall *P* values for Cox proportional hazard model.

troducing gluten-containing foods were missing from 20% and 28% of the children, respectively. The study also found a number of potential confounder variables that could affect risks associated with infant feeding. Maternal diabetes affected infant feeding in our cohort and was associated with increased birth weight and reduced gestation age. Adjustment of HRs for these potential confounder variables and for region of residence did not alter the significant relationship between increased islet autoantibody risk and early introduction to dietary gluten. An association with other variables, such as socioeconomic status, cannot be excluded. Finally, 1 of 2 children with DR3/DR4-DQ8 genotype who received non–gluten-containing solid food supplements before age 3 months also developed islet autoantibodies, and we therefore cannot exclude that the association between islet autoantibodies and early introduction to gluten food supplements in children with HLA-DR3/DR4-DQ8 genotype is not due to the early introduction of solid foods per se.

Early introduction of gluten-containing foods was significantly associated with reduced duration of breastfeeding. Despite this, islet autoantibody risk was not increased in children who were breastfed for short duration, and the lowest islet autoantibody frequencies were found in non-breastfed children. These findings were surprising since reduced breastfeeding duration was reported to be associated with increased type 1 DM risk.[6,7] Our data are, however, consistent with those of other prospective studies in children of affected families.[12,13] From these prospective data, it seems unlikely that reduced breastfeeding time is a primary risk factor for islet autoimmunity in children of parents with type 1 DM. Reduced breastfeeding, however, could be a risk factor in combination with, or secondary to, exposure to other factors. Early exposure to cow's milk, for example, has been suggested to increase the risk for type 1 DM. In our study, children who received food supplements in the first 3 months of life that were only milk based did not have an increased islet autoantibody risk compared with children who received only breast milk. Moreover, introduction of milk-based food supplements without other solid food supplements was associated with a significantly reduced islet autoantibody risk in children with the HLA-DR3/DR4-DQ8 genotype. These findings would argue against a role for cow's milk in promoting islet autoimmunity, and even suggest that early exposure may be beneficial in selected individuals. Clearly, the possibility that the association between breastfeeding and type 1 DM incidence observed in some retrospective studies may be secondary to the association with early exposure to dietary foods other than cow's milk should be considered. Differences in breastfeeding habits and breast milk between mothers with and without type 1 DM also could contribute to discrepancies across studies.

The optimal timing of food introduction is debated. It is thought that the timing of first exposure may influence immune tolerance to food antigens, and that there may be an exposure time window that best allows tolerance to be achieved.[30] The Diabetes Autoimmunity Study in the Young also has suggested that islet autoantibody risk is increased in infants who received gluten before age 3 months, but suggests that risk is again increased in children in whom exposure to gluten foods was delayed until after 6 months.[31] Later introduction of gluten did not increase risk of developing islet autoantibodies in our study. For celiac disease, risk was suggested to be increased if gluten exposure is late,[20] although other studies have indicated that increased risk was associated with high dose rather than late exposure.[32] We examined IgA-tTGCA as a marker of celiac disease in our cohort. Risk for tTGCA appeared to be inversely associated with the age of first exposure to gluten-containing foods. The conversion rate to IgA-tTGCA was low, however, and the CIs for tTGCA risk were wide and overlapping. Of interest was the practice of very late (after age 12 months) introduction of gluten adopted by some of the families with prior cases of celiac disease. None of these children developed either type 1 DM or celiac disease associated autoantibodies, suggesting that late introduction of gluten does not increase risk for autoimmunity.

The mechanism by which exposure to gluten or solid food could increase risk of autoimmune DM is unclear. Very early introduction may lead to inflammation in the gut that alters the immune cell repertoire, or lead to changes in islet beta cells that may still be immature in the neonate. Evidence supporting an immune mechanism for the association recently was reported in a gluten-sensitive animal model of autoimmune diabetes.[33] Diabetes development in this model was found to be associated with antibodies against a wheat protein highly homologous to the wheat storage globulin Glb1. Antibodies to a similar protein also were found in children with type 1 DM.

In conclusion, this study finds early introduction of gluten-containing foods to be a risk factor for the development of type 1 DM-associated autoimmunity in children with HLA-DR3/DR4-DQ8 genotype of parents with type 1 DM. Although CIs are large and therefore the magnitude of the contribution to type 1 DM risk cannot be accurately assessed from this study, the data suggest that the prevalence of islet autoimmunity could be reduced if all families complied with infant feeding guidelines and did not introduce gluten-containing and solid foods to infants until after age 3 months. A significant effect on type 1 DM incidence may be expected if the association also is found with type 1 DM risk and if it is found in children of parents without DM.

Author Contributions: *Study concept and design:* Ziegler, Hummel, Bonifacio.
Acquisition of data: Schmid, Huber.
Analysis and interpretation of data: Ziegler, Schmid, Huber, Hummel, Bonifacio.
Drafting of the manuscript: Ziegler, Schmid, Huber, Bonifacio.
Critical revision of the manuscript for important intellectual content: Ziegler, Schmid, Hummel, Bonifacio.
Statistical expertise: Bonifacio.
Obtained funding: Ziegler.
Administrative, technical, or material support: Schmid.
Study supervision: Ziegler.
Funding/Support: This study was supported by grants

from the Juvenile Diabetes Research Foundation (JDRF 1-2000-619), the Stiftung "Das Zuckerkranke Kind," the Bundesministerium für Forschung und Technologie (BMFT 01KD89030), and the Deutsche Diabetesgesellschaft (Dr Buding-Stiftung [grant]).

Acknowledgment: We thank Annette Knopff for expert technical assistance and all pediatricians and family doctors in Germany for participation in the BABYDIAB study.

REFERENCES

1. Eisenbarth GS. Type I diabetes mellitus. a chronic autoimmune disease. *N Engl J Med.* 1986;314:1360-1368.

2. Atkinson MA, Eisenbarth GS. Type 1 diabetes: new perspectives on disease pathogenesis and treatment. *Lancet.* 2001;358:221-229.

3. Davies JL, Kawaguchi Y, Bennett ST, et al. A genome-wide search for human type 1 diabetes susceptibility genes. *Nature.* 1994;371:130-136.

4. Leslie RD, Elliott RB. Early environmental events as a cause of IDDM: evidence and implications. *Diabetes.* 1994;43:843-850.

5. Ziegler AG, Hummel M, Schenker M, Bonifacio E. Autoantibody appearance and risk for the development of childhood diabetes in offspring of parents with type 1 diabetes: the German BABY-DIAB Study. *Diabetes.* 1999;48:460-468.

6. Borch-Johnsen K, Joner G, Mandrup-Poulsen T, et al. Relation between breast-feeding and incidence rates of insulin-dependent diabetes mellitus. *Lancet.* 1984; 2:1083-1086.

7. Gerstein HC. Cow's milk exposure and type I diabetes mellitus. *Diabetes Care.* 1994;17:13-19.

8. Karjalainen J, Martin JM, Knip M, et al. Evidence for a bovine albumin peptide as a candidate trigger of type 1 diabetes. *N Engl J Med.* 1992;327:302-307.

9. Vaarala O, Klemetti P, Savilahti E, Reijonen H, Ilonen J, Akerblom HK. Cellular immune response to cow's milk β-lactoglobulin in patients with newly diagnosed IDDM. *Diabetes.* 1996;45:178-182.

10. Cavallo MG, Fava D, Monetini L, Barone F, Pozzilli P. Cell-mediated immune response to β-casein in recent-onset insulin-dependent diabetes: implications for disease pathogenesis. *Lancet.* 1996;348:926-928.

11. Vaarala O, Knip M, Paronen J, et al. Cow's milk formula feeding induces primary immunization to insulin in infants at genetic risk for type 1 diabetes. *Diabetes.* 1999;48:1389-1394.

12. Norris JM, Beaty B, Klingensmith G, et al. Lack of association between early exposure to cow's milk protein and beta-cell autoimmunity: Diabetes Autoimmunity Study in the Young. *JAMA.* 1996;276:609-614.

13. Couper JJ, Steele C, Beresford S, et al. Lack of association between duration of breast-feeding or introduction of cow's milk and development of islet autoimmunity. *Diabetes.* 1999;48:2145-2149.

14. Hummel M, Füchtenbusch M, Schenker M, Ziegler AG. No major association of breast-feeding, vaccinations, and childhood viral diseases with early islet autoimmunity in the German BABYDIAB Study. *Diabetes Care.* 2000;23:969-974.

15. Kimpimaki T, Erkkola M, Korhonen S, et al. Short-term exclusive breastfeeding predisposes young children with increased genetic risk of type I diabetes to progressive beta-cell autoimmunity. *Diabetologia.* 2001;44:63-69.

16. Atkinson MA, Ellis TM. Infants diets and insulin-dependent diabetes: evaluating the "cows' milk hypothesis" and a role for anti-bovine serum albumin immunity. *J Am Coll Nutr.* 1997;16:334-340.

17. Lundin KE, Scott H, Hansen T, et al. Gliadin-specific, HLA-*DQ(alpha 1*0501,beta 1*0201)* restricted T cells isolated from the small intestinal mucosa of celiac disease patients. *J Exp Med.* 1993;178: 187-196.

18. van de Wal Y, Kooy YM, van Veelen PA, et al. Small intestinal T cells of celiac disease patients recognize a natural pepsin fragment of gliadin. *Proc Natl Acad Sci U S A.* 1998;95:10050-10054.

19. Weile B, Cavell B, Nivenius K, Krasilnikoff PA. Striking differences in the incidence of childhood celiac disease between Denmark and Sweden: a plausible explanation. *J Pediatr Gastroenterol Nutr.* 1995;21:64-68.

20. Ivarsson A, Persson LA, Nystrom L, et al. Epidemic of coeliac disease in Swedish children. *Acta Paediatr.* 2000;89:165-171.

21. Challacombe DN, Mecrow IK, Elliott K, Clarke FJ, Wheeler EE. Changing infant feeding practices and declining incidence of coeliac disease in West Sommerset. *Arch Dis Child.* 1997;77:206-209.

22. Greco L, Mayer M, Grimaldi M, Follo D, De Ritis G, Auricchio S. The effect of early feeding on the onset of symptoms in celiac disease. *J Pediatr Gastroenterol Nutr.* 1985;4:52-55.

23. Cronin CC, Shanahan F. Insulin-dependent diabetes mellitus and coeliac disease. *Lancet.* 1997;349: 1096-1097.

24. Ventura A, Neri E, Ughi C, Leopaldi A, Citta A, Not T. Gluten-dependent diabetes-related and thyroid-related autoantibodies in patients with celiac disease. *J Pediatr.* 2000;137:263-265.

25. Funda DP, Kaas A, Troels B, Tlaskalová-Hogenová H, Buschard K. Gluten-free diet prevents diabetes in NOD mice. *Diabetes Metab Res Rev.* 1999; 15:323-327.

26. Naserke HE, Bonifacio E, Ziegler AG. Immunoglobulin G insulin autoantibodies in BABYDIAB offspring appear postnatally: sensitive early detection using a protein A/G-based radiobinding assay. *J Clin Endocrinol Metab.* 1999;84:1239-1243.

27. Bingley PJ, Bonifacio E, Mueller P. Diabetes antibody standardisation programme: first assay proficiency evaluation. *Diabetes.* 2003;52:1128-1136.

28. Hummel M, Bonifacio E, Stern M, Dittler J, Schimmel A, Ziegler AG. Development of celiac disease associated antibodies in offspring of parents with type 1 diabetes. *Diabetologia*. 2000;43:1005-1011.

29. Schenker M, Hummel M, Ferber K, et al. Early expression and high prevalence of islet autoantibodies for *DR3/4* heterozygous and *DR4/4* homozygous offspring of parents with type 1 diabetes: the German BABYDIAB study. *Diabetologia.* 1999;42:671-677.

30. Strobel S, Mowat AM. Immune responses to dietary antigens: oral tolerance. *Immunol Today.* 1998; 19:173-181.

31. Norris J, Barriga K, Rewers M. Does cereal protein in the infant diet play a role in the etiology of β-cell autoimmunity? the Diabetes Autoimmunity Study in the Young (DAISY). *Diabetes.* 2002;51:A234.

32. Ivarsson A, Persson LA, Nystro L, et al. Epidemic of celiac disease in Swedish children. *Acta Pedriatr*. 2000;89:165-171.

33. MacFarlane AJ, Burghardt KM, Kelly J, Simell T, Simell O, Altosaar I, Scott FW. A type 1 diabetes-related protein from wheat (*Triticum aestivum*): cDNA clone of a wheat storage globulin, Glb1, linked to islet damage. *J Biol Chem.* 2003;278:54-63.