

Original Investigation

Association of Early Exposure of Probiotics and Islet Autoimmunity in the TEDDY Study

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IMPORTANCE Probiotics have been hypothesized to affect immunologic responses to environmental exposures by supporting healthy gut microbiota and could therefore theoretically be used to prevent the development of type 1 diabetes mellitus (T1DM)-associated islet autoimmunity.

OBJECTIVE To examine the association between supplemental probiotic use during the first year of life and islet autoimmunity among children at increased genetic risk of T1DM.

DESIGN, SETTING, AND PARTICIPANTS In this ongoing prospective cohort study that started September 1, 2004, children from 6 clinical centers, 3 in the United States (Colorado, Georgia/Florida, and Washington) and 3 in Europe (Finland, Germany, and Sweden), were followed up for T1DM-related autoantibodies. Blood samples were collected every 3 months between 3 and 48 months of age and every 6 months thereafter to determine persistent islet autoimmunity. Details of infant feeding, including probiotic supplementation and infant formula use, were monitored from birth using questionnaires and diaries. We applied time-to-event analysis to study the association between probiotic use and islet autoimmunity, stratifying by country and adjusting for family history of type 1 diabetes, HLA-DR-DQ genotypes, sex, birth order, mode of delivery, exclusive breastfeeding, birth year, child's antibiotic use, and diarrheal history, as well as maternal age, probiotic use, and smoking. Altogether 8676 infants with an eligible genotype were enrolled in the follow-up study before the age of 4 months. The final sample consisted of 7473 children with the age range of 4 to 10 years (as of October 31, 2014).

EXPOSURES Early intake of probiotics.

MAIN OUTCOMES AND MEASURES Islet autoimmunity revealed by specific islet autoantibodies.

RESULTS Early probiotic supplementation (at the age of 0-27 days) was associated with a decreased risk of islet autoimmunity when compared with probiotic supplementation after 27 days or no probiotic supplementation (hazard ratio [HR], 0.66; 95% CI, 0.46-0.94). The association was accounted for by children with the *DR3/4* genotype (HR, 0.40; 95% CI, 0.21-0.74) and was absent among other genotypes (HR, 0.97; 95% CI, 0.62-1.54).

CONCLUSIONS AND RELEVANCE Early probiotic supplementation may reduce the risk of islet autoimmunity in children at the highest genetic risk of T1DM. The result needs to be confirmed in further studies before any recommendation of probiotics use is made.

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A newborn infant's immune system needs to quickly learn how to tolerate beneficial bacteria and defend against opportunistic pathogens. The intestinal microbiota can influence the balance between proinflammatory and regulatory immune responses.¹ However, there are still unanswered questions as to how the immune system interacts with the microbiota.^{2,3}

A healthy gut microbiota is believed to favorably regulate mucosal barrier function⁴ and reduce intestinal permeability.^{5,6} Abnormalities in gut permeability have been linked to the development of type 1 diabetes mellitus (T1DM).⁷ Healthy gut microbiota may also enhance the overall maturation of the infant immune system^{8,9} and exclude pathogens competitively.¹⁰ Imbalance in gut microbiota and a relative decrease in α -diversity are associated with T1DM according to a recent study.¹¹ A larger proportion of the phylum *Bacteroidetes* has been observed in children with T1DM.¹²⁻¹⁴

Microbial colonization of the infant gut starts in utero,¹⁵ although frequent changes in gut microbiota, mainly in relative abundances of species, have been observed during the first 10 to 12 months of life.¹⁶⁻¹⁹ Early life events, such as mode of delivery, early environment, including hygiene measures, and early feeding, are thought to initially set the trajectory of colonization.^{20,21} Even though α -diversity may be large, strain composition within an individual typically remains constant throughout infancy.¹¹

Probiotics have been defined as live organisms that, when administered in adequate amounts, confer a health benefit on the host.²² Administration of probiotics to healthy infants is considered safe.^{23,24} However, it is still unclear whether probiotics as an early dietary factor could modify the infant gut microbiota trajectory and disease susceptibility.

Studies^{25,26} on manipulation of gut microbiota by probiotics and consequent changes in the risk of developing T1DM-related autoimmunity have mainly used animal models. Probiotics induce favorable immunomodulation, and it has been suggested that probiotic treatment could prevent T1DM. The aim of this study is to examine the association between supplemental probiotic use during the first year of life and islet autoimmunity (IA) among children at increased risk of T1DM.

Methods

The Environmental Determinants of Diabetes in the Young (TEDDY) is a prospective cohort study with the primary goal to identify environmental causes of T1DM. It includes 6 clinical research centers (3 in the United States and 3 in Europe): University of Colorado Health Science Center, Georgia Regents University, Pacific Northwest Diabetes Research Institute, Turku University Hospital, Institute of Diabetes Research, and Lund University. Detailed study design and methods have been previously published.^{27,28} The study was approved by the local institutional review or ethics boards and is monitored by an external advisory board formed by the National Institutes of Health. Written informed consent was obtained for all study participants from a parent or primary caretaker for genetic screening and participation in prospective follow-up.

At a Glance

- Probiotics are live organisms that may confer health benefits on the host.
- The aim of this study was to examine the association between early probiotic exposure and islet autoimmunity among children in The Environmental Determinants of Diabetes in the Young study.
- Early administration of probiotics, during the first 27 days of life, may be associated with reduced risk of islet autoimmunity (hazard ratio [HR], 0.66; 95% CI, 0.45-0.96) among children who were genetically at increased risk for type 1 diabetes mellitus.
- This reduced risk of islet autoimmunity was primarily observed in children with the highest-risk HLA genotype of *DR3/4* (HR, 0.40; 95% CI, 0.21-0.74) but not in children with the other, moderately higher-risk genotypes (HR, 0.97; 95% CI, 0.62-1.54).

Study Population

From September 1, 2004, through February 28, 2010, a total of 424 788 newborns in the university hospitals affiliated with the clinical research centers were screened with parents' consent for T1DM-associated HLA genotypes.²⁹ The screening identified 21 589 eligible infants, of whom 8676 were enrolled in the follow-up study before the age of 4 months. Children who were followed up for less than 12 months ($n = 1032$) or whose islet autoantibody status was indeterminate ($n = 55$) were excluded. Because of HLA ineligibility, 116 additional study participants were removed from the analyses. The final sample for this study consisted of 7473 children with an age range of 4 to 10 years (as of October 31, 2014).

HLA Typing

Infants from the general population, with no first-degree relative (FDR) with T1DM, were eligible for the study if they had any one of the following HLA genotypes: (1) DR4-DQA1*03-DQB1*03:02/DR3-DQA1*05:01-DQB1*02:01, (2) DR4-DQA1*03-DQB1*03:02/DR4-DQA1*03-DQB1*03:02, (3) DR4-DQA1*03-DQB1*03:02/DR8-DQA1*04:01-DQB1*04:02, and (4) DR3-DQA1*05:01-DQB1*02:01/DR3-DQA1*05:01-DQB1*02:01. Acceptable DQB1 alleles in any haplotype listed as DQB1*03:02 also include DQB1*03:04. For the above genotypes, any DR4 of the subtype DRB1*04:03 is ineligible.

Infants who have an FDR with T1DM were eligible for enrollment if they had any of the following HLA genotypes: (1) DR4-DQA1*03-DQB1*03:02/DR3-DQA1*05:01-DQB1*02:01, (2) DR4-DQA1*03-DQB1*03:02/DR4-DQA1*03-DQB1*03:02, (3) DR4-DQA1*03-DQB1*03:02/DR8-DQA1*04:01-DQB1*04:02, (4) DR3-DQA1*05:01-DQB1*02:01/DR3-DQA1*05:01-DQB1*02:01, (5) DR4-DQA1*03-DQB1*03:02/DR4-DQA1*03-DQB1*02, (6) DR4-DQA1*03-DQB1*03:02/DR1-DQA1*01:01-DQB1*05:01, (7) DR4-DQA1*03-DQB1*03:02/DR13-DQA1*01:02-DQB1*06:04, (8) DR4-DQA1*03-DQB1*03:02/DR9-DQA1*03-DQB1*03:03, and (9) DR3-DQA1*05:01-DQB1*02:01/DR9-DQA1*03-DQB1*03:03. Acceptable DQB1 alleles in any haplotype listed as DQB1*03:02 also include DQB1*03:04. All HLA genotypes are referred to in the text by their abbreviated names listing only DR alleles (i.e. DR3/4 for genotype [1] above).

Islet Autoimmunity

The primary outcome of this study was the development of persistent confirmed IA. Blood samples were drawn every 3 months between 3 and 48 months of age and every 6 months thereafter. Persistent IA was defined as confirmed positive antibodies to insulin, glutamic acid decarboxylase, or insulinoma antigen 2, which were analyzed by radiobinding assays,^{30,31} on at least 2 consecutive study visits. All positive islet autoantibodies and 5% of negative islet autoantibodies were confirmed in both central autoantibody laboratories, 1 located in the United States (Barbara Davis Center for Childhood Diabetes at the University of Colorado) and 1 in Europe (University of Bristol). Both laboratories have previously found high sensitivity and specificity³² and concordance. Positive results that were due to maternal IgG transmission when defining the child's IA status led to omission from the IA-positive group. Date of persistent autoimmunity was defined as the draw date of the first of 2 consecutive samples that deemed the child's IA status as persistent that were confirmed positive for a specific autoantibody (or any autoantibody). The mean (SD) age at first IA sampling was 33.4 (23.2) months among the seroconverters ($n = 601$), and the mean (SD) age at the last follow-up for children without IA was 65.6 (28.0) months.

Characteristics and Diet and Health Monitoring of the Study Population

Information about basic demographic characteristics and family history of diabetes was received from the infant screening form. A questionnaire on maternal medications, smoking habits, and probiotic dietary supplement use during pregnancy was mailed to the mothers of enrolled children and completed at 3 to 4 months post partum. After enrollment, parents also received a questionnaire on mode of delivery and child's early diet, including the use of probiotics at 0 to 3 months of age. Parents were advised to consistently maintain a diary after the first clinic visit to collect information on child illnesses and diet. The start age of the probiotic supplement and each type of infant formula were recorded. Information about the mother's educational level and birth order of the child was received from the primary caretaker questionnaire at the 9-month clinical visit. Probiotic exposure was defined as timing of first introduction of probiotics via dietary supplement or infant formula. Clinical center study nurses reviewed the questionnaires and diaries with the parent at clinic visits or over the telephone every 3 months to minimize missing and inaccurate information.

Statistical Analysis

The characteristics of probiotic users for the study children and their mothers were examined one by one using a Cochran-Mantel-Haenszel test and simultaneously using a logistic regression model adjusting for country. The association between the probiotic exposure age and IA was examined among those who were exposed to probiotics during the first year of life. For exploratory analyses, we categorized these individuals according to the probiotic exposure age into 3 equally sized groups (0-27 days, 28-90 days, and 91-365 days) and compared them with the individuals without probiotic exposure during the first year (>365 days) when studying the association with IA. A Cox proportional hazards regression model was applied

to study the association between timing of probiotic exposure and occurrence of IA.

The Cox proportional hazards regression models were simultaneously adjusted for HLA-DR-DQ genotype (DR3/4 vs other), T1DM-related FDR status (yes/no), sex (female vs male), mode of delivery (cesarean delivery vs other), and exclusive breastfeeding duration (≥ 3 vs < 3 months) and stratified for country using the STRATA statement within the model. The models were also adjusted for factors that were associated with probiotic use and could be associated with IA or T1DM: maternal age (≤ 24 , 25-29 [reference], 30-34, and ≥ 35 years), maternal smoking during pregnancy (yes/no), maternal probiotic use during pregnancy (yes/no), birth year, birth order (first born child vs others), diarrheal history, and antibiotic use of the child (yes/no).^{33,34}

Because early IA may have preceded the probiotic exposure, a sensitivity analysis of the association between timing of probiotic exposure and occurrence of IA was conducted by excluding the individuals who developed IA during the first year of life ($n = 106$) to eliminate the effect of the ordering between IA and probiotic exposure. The results from the sensitivity analysis indicated only minor changes in the estimated parameters (hazard ratios [HRs] and P values) and led to the same conclusions as when including all individuals in the model.

All tests for significance were 2-tailed with a significance level of .05. SAS statistical software, version 9.3 (SAS Institute Inc), was used for all statistical analyses.

Results

Probiotic supplementation, from dietary supplements or infant formula, varied by country (Table 1). It was most prevalent in Finland (869 [52.4%]) and Germany (237 [46.8%]) during the first year of life. Most of the Finnish children (827 [95.2%]) had received probiotics from dietary supplements, whereas in Germany, probiotic infant formulas (214 [90.3%]) were the most common sources of probiotics. The median age for first exposure to probiotics was 42 days. Overall, children born at the end of the recruitment period (2009-2010) were 4.9 times more likely ($P < .001$) to be given probiotics than those born in the beginning of the study period (2004-2005) (Table 2).

Participant characteristics that were positively associated with probiotic use during the first year of life were probiotic use during pregnancy ($P < .001$), not smoking during pregnancy ($P = .006$), being first born ($P < .001$), later birth year ($P < .001$), shorter duration of exclusive breastfeeding ($P = .003$), use of antibiotics ($P < .001$), and having diarrhea ($P < .001$) or gastroenteritis ($P < .001$) (Table 2). Only 855 (11.4%) of the children used antibiotics before 3 months of age, whereas 2995 (40.1%) used antibiotics at 3 to 12 months of age. However, probiotic use, from dietary supplements or formula, was strongly associated with antibiotic use ($P < .001$) during the first year of life. Nevertheless, antibiotic use was not associated with IA. By the age of 3 months, 4342 (58.1%) of all TEDDY children had experienced at least one episode of common cold: 1670 (54.8%) in the United States, 757 (45.7%) in Finland, 274 (54.2%) in Germany, and 1641 (72.5%) in Sweden ($P < .001$, χ^2 test). Gastroenteritis was strongly associated with probiotic use ($P < .001$) but not with IA.

Table 1. Distribution of Probiotic Exposure From Dietary Supplements and Infant Formulas During the First Year of Life by Age and Country^a

Variable	Country				
	United States (n = 3046)	Finland (n = 1658)	Germany (n = 506)	Sweden (n = 2263)	All (N = 7473)
Probiotic use					
During first 12 mo	186 (6.1)	869 (52.4)	237 (46.8)	345 (15.2)	1637 (21.9)
During first 3 mo	70 (2.3)	627 (37.8)	123 (24.3)	276 (12.2)	1096 (14.7)
Age at first exposure to probiotics, median (IQR), d	137 (56-244)	28 (14-105)	84 (14-198)	35 (21-70)	42 (14-152)
Source of first probiotic exposure among probiotic users					
Dietary supplements	124 (66.7)	827 (95.2)	11 (4.6)	274 (79.4)	1236 (75.5)
Infant formula	53 (28.5)	25 (2.9)	214 (90.3)	40 (11.6)	332 (20.3)
Both	9 (4.8)	17 (1.9)	12 (5.1)	31 (9.0)	69 (4.2)
Timing of first probiotic exposure for users only, d					
0-27	20 (10.8)	344 (39.5)	74 (31.2)	104 (30.0)	542 (33.1)
28-90	53 (28.5)	283 (32.6)	49 (20.7)	172 (50.0)	557 (34.0)
91-365	113 (60.7)	242 (27.9)	114 (48.1)	69 (20.0)	538 (32.9)

Abbreviation: IQR, interquartile range.

^a Data are presented as number (percentage) unless otherwise indicated.**Table 2. Characteristics of Probiotic Supplement and/or Probiotic Formula Users and Nonusers**

Characteristic	Users (n = 1637)	Nonusers (n = 5836)	P Value ^a	Adjusted OR ^b (95% CI)
Maternal age, y				
≤24	151 (9.2)	746 (12.8)	.003	0.68 (0.53-0.88)
25-29	520 (31.8)	1664 (28.5)		1 [Reference]
30-34	598 (36.5)	2062 (35.3)		1.11 (0.94-1.30)
≥35	368 (22.5)	1364 (23.4)		1.20 (0.99-1.45)
Maternal educational level of high school or more	1407 (87.7)	4534 (79.7)	.001	1.14 (0.93-1.39)
Birth order, first child	799 (50.2)	2442 (43.1)	<.001	1.64 (1.42-1.90)
Antibiotics use during pregnancy	384 (23.7)	1321 (22.9)	.45	1.04 (0.89-1.22)
Probiotics use during pregnancy	116 (7.1)	169 (2.9)	<.001	2.69 (1.95-3.70)
Smoking during pregnancy	180 (11.1)	704 (12.2)	<.001	0.74 (0.60-0.92)
Cesarean delivery	393 (24.0)	1542 (26.4)	.008	1.11 (0.94-1.30)
Birth year				
2004-2005	163 (10.0)	1067 (18.3)	<.001	1 [Reference]
2006	228 (13.9)	1084 (18.6)		1.70 (1.32-2.18)
2007	344 (21.0)	1225 (21.0)		2.79 (2.20-3.52)
2008	379 (23.1)	1149 (19.7)		4.01 (3.17-5.08)
2009-2010	523 (32.0)	1311 (22.4)		4.89 (3.89-6.14)
First-degree relative with T1DM	203 (12.4)	639 (11.0)	.38	0.97 (0.78-1.19)
HLA genotype DR3/4	628 (38.4)	2305 (39.5)	.13	1.08 (0.94-1.23)
Female sex	797 (48.7)	2862 (49.0)	.72	1.04 (0.91-1.19)
Exclusive breastfeeding at least 3 mo	392 (24.0)	1456 (25.0)	<.001	0.79 (0.68-0.92)
Child's antibiotics use during the first 3 mo	910 (55.6)	2435 (41.7)	<.001	1.68 (1.46-1.94)
Diarrhea episode during the first 3 mo	169 (10.3)	494 (8.5)	<.001	1.50 (1.19-1.90)
Common cold during the first 3 mo	910 (55.6)	3432 (58.8)	.04	1.14 (0.99-1.31)
Gastroenteritis (infectious or noninfectious) during the first 12 mo	582 (35.6)	1723 (29.5)	<.001	1.38 (1.20-1.59)

Abbreviations: OR, odds ratio; T1DM, type 1 diabetes mellitus.

^a P value from the Cochran-Mantel-Haenszel test for the association between probiotics and respondent characteristics; analyses adjusted for country.^b Adjusted OR (95% CI) from multiple logistic regression; analyses adjusted for country.

Kaplan-Meier curves of developing IA suggested that the earliest exposure of probiotics had the lowest risk of IA whereas the exposure of probiotics during 91 to 365 days had the highest risk of IA. However, the associations were not statistically significant ($P = .08$, log-rank test) (Figure). The estimated HRs and P values from the adjusted Cox proportional hazards regression models are listed in Table 3. Early exposure to pro-

biotics during the first 27 days of life ($n = 540$) revealed decreased risk of IA (HR, 0.66; 95% CI, 0.45-0.96) in the TEDDY children when adjusting for FDR status, HLA-DR-DQ genotype (DR3/4 vs other), sex, birth order, mode of delivery, maternal age, maternal probiotic use, smoking during pregnancy, exclusive breastfeeding duration, birth year, child antibiotic use, and diarrhea. Results of the exploratory analy-

Figure. Islet Autoimmunity Risk by First Probiotic Exposure Age of the Child

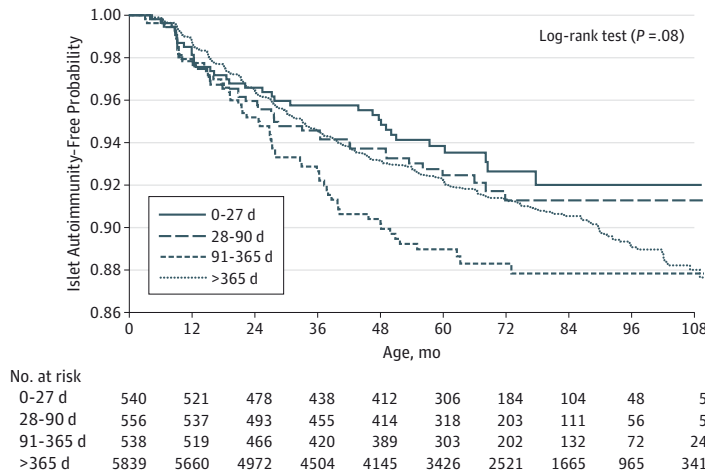


Table 3. First Probiotic Exposure of the Child via Infant Formula and/or Dietary Supplement During the First Year of Life and Risk of IA

Variable	No. (%) of Infants		HR (95% CI) ^a
	Developed IA (n = 601)	Did Not Develop IA (n = 6872)	
Country			
United States	201 (33.4)	2845 (41.4)	... ^b
Finland	151 (25.1)	1507 (21.9)	...
Germany	46 (7.7)	460 (6.7)	...
Sweden	203 (33.8)	2060 (30.0)	...
Timing of first probiotic exposure, d			
0-27	34 (5.7)	506 (7.4)	0.66 (0.45-0.96)
28-90	41 (6.8)	515 (7.5)	0.85(0.61-1.19)
91-365	57 (9.5)	481 (7.0)	1.16 (0.86-1.57)
After 1 year or no exposure	469 (78.0)	5370 (78.1)	1 [Reference]
FDR with T1DM	126 (21.0)	716 (10.4)	2.30 (1.87-2.84)
High-risk HLA-DR- DR3/4	304 (50.6)	2629 (38.3)	1.76 (1.50-2.07)
Female sex	262 (43.6)	3397 (49.4)	0.79 (0.67-0.94)

Abbreviations: FDR, first-degree relative; HR, hazard ratio; IA, islet autoimmunity; T1DM, type 1 diabetes mellitus.

^a The HRs were adjusted for FDR status, HLA-DR genotype, sex, and the following nonsignificant covariates: birth order, mode of delivery, exclusive breastfeeding duration, birth year, child antibiotic use, diarrhea, maternal age, maternal probiotic use, and maternal smoking during pregnancy and stratified for country.

^b Ellipses indicate data not applicable.

sis suggested that very early exposure to probiotics may be important in relation to IA (Table 3). Therefore, we decided to focus on the early exposure (at 0-27 days) and the risk of IA in our further analyses. Early exposure of probiotics was associated with decreased risk of IA (HR, 0.66; 95% CI, 0.46-0.94) when compared with exposure after 27 days or no exposure and adjusted for FDR status ($P < .001$), HLA-DR-DQ genotype ($P < .001$), sex ($P = .006$), birth order ($P = .15$), mode of delivery ($P = .46$), maternal age ($P = .98$), maternal probiotic use ($P = .82$), smoking during pregnancy ($P = .15$), exclusive breastfeeding duration ($P = .42$), birth year ($P = .70$), child antibiotic use ($P = .75$), and diarrhea ($P = .61$).

Our analyses also revealed an interaction ($P = .02$) between early probiotic exposure (at 0-27 days) and HLA genotype in relation to IA. Separate analyses by HLA-DR-DQ genotype revealed a strong inverse association between early probiotic exposure and IA among those with an HLA genotype of DR3/4 (HR, 0.40; 95% CI, 0.21-0.74) but not among other genotypes (HR, 0.97; 95% CI, 0.62-1.54).

We did not find a statistically significant interaction between early probiotic exposure (0-27 days) and country ($P = .34$). The country-specific HRs were not heterogeneous (United States: HR, 0.98; 95% CI, 0.14-7.02; Finland: HR, 0.60; 95% CI, 0.38-0.97; Germany: HR, 0.98; 95% CI, 0.38-2.50; Sweden: HR, 0.73; 95% CI, 0.32-1.67), reflecting a possible protective association between early probiotic exposure and IA.

Discussion

In this multinational cohort study of children at increased genetic risk of T1DM, we observed a reduction in the risk of IA in the children who had received probiotics via dietary supplements and/or via fortified infant formula before or at the age of 27 days compared with those who had first received probiotics after 27 days or not at all. Early probiotic exposure was associated with 60% decrease in the risk of IA among children with the DR3/4 genotype but not among other genotypes.

The strengths of the study included a large international sample with consistent recording of the information on child diet, including probiotics, and health conditions covering the whole first year of life. A limitation of the study was that the species and amounts of microbes from probiotics were not studied. Most of the supplements used by TEDDY children contained mixtures of various *Lactobacillus* and *Bifidobacterium* species along with other commonly used probiotics. Therefore, the effect of specific species could not be evaluated as has been done in controlled clinical trials.³⁵ In addition, the stability of probiotic bacteria is dependent on many factors (eg, storage conditions).^{36,37} Therefore, it would not have been possible to compare doses of probiotics.

Probiotic use varied between TEDDY countries. Giving probiotic supplements to neonates is a fairly new trend, particularly in the United States.³⁸ Even today probiotics are not recommended as a routine supplementation in the United States even though adverse events associated with probiotic use are extremely rare.^{23,39,40} Smaller population-based interventions on probiotic use and health outcomes among infants in Finland⁴⁰ have received considerable attention by the media, which may have boosted the consumption of probiotics in that country.

Antibiotic medication use was common at the ages of 3 to 12 months, and probiotic use was often associated with administration of antibiotics. There is also evidence that antibiotic use may increase the risk of T1DM.³⁴ This could contribute to the fact that we did not find a protective association between later introduction of probiotics and the risk of IA. We also have to consider that the probiotics may be able to modify gut microbiota only in early life because after introduction of solid foods, diet may have an overly dominant effect on gut microbiota composition, making probiotic supplementation less successful.⁴¹ Young infants are particularly susceptible to infectious diseases.⁴² Lönnrot et al⁴³ also noticed that respiratory infections during early life in TEDDY were often accompanied by gastrointestinal symptoms, such as diarrhea, which we found to be strongly associated with probiotic use. The very early weeks of life may open a window for probiotics to modify gut microbiota favorably, thus helping the immune system of an infant's gut to gain the maturity needed for correctly processing new environmental exposures, such as pathogens.

Approximately 70% of all gastroenteritis cases are caused by a virus,⁴⁴ making an antibiotic treatment ineffective. Probiotics are often prescribed instead to shorten diarrheal episodes.^{44,45} Probiotic use was strongly associated with gastroenteritis in TEDDY. Shortened duration of gastroenteritis by probiotic treatment may protect infant gut from extended inflammation and adverse immunity-suppressing

consequences.⁴⁵ This may partly explain why we did not find an association between gastroenteritis and IA even though such an association has been suggested earlier.^{46,47}

Both T1DM and T1DM-related autoimmunity have multifactorial origins.^{33,48,49} Contributing factors and their interplay with probiotics could also vary among countries. The recent observation of a plateau in the incidence of T1DM in Finland⁵⁰ suggests changes in environmental exposures, for example, in serum 25-hydroxyvitamin D concentrations.⁵¹ The increasing trend of probiotic use^{40,52} also preceded the plateauing rates of T1DM. Respiratory tract infections at an early age have been found to be associated with IA.⁵³ Early use of probiotics and relatively lower rates of the common cold before 3 months of age in Finland also warrant further study. However, the changes in the exposures and their putative connection to the incidence of IA and T1DM in Finland still lack conclusive evidence.

Finding the larger protective association between early probiotic exposure and IA among children with the DR3/4 genotype, when compared with other genotypes, suggests a gene-environment interaction. The genotype may influence the interaction of the host immune system with the bacteria present in the probiotic supplement. An earlier study⁵⁴ has also suggested that the HLA genotype may modify the association between the timing of dietary exposure and IA.

This is the first time, to our knowledge, that the association between probiotic use and T1DM-related IA has been studied in a longitudinal, observational setting among genetically high-risk children. Of importance, a protective association between early probiotic use and T1DM-related IA has been observed. Previous studies^{11,12,14} have provided evidence that imbalance in gut microbiota may be connected with autoimmune disorders, such as T1DM, and that changes in the microbiota precede the pathogenic condition. However, studies reporting a successful manipulation of gut microbiota by probiotics in humans are scarce. In any case, influencing the gut microbiota with ingested probiotics would be expected to be more effective very early in life, as we observed.

Conclusions

Early exposure to supplemental probiotics may decrease the risk of IA among children at elevated risk of T1DM. However, a randomized clinical trial should confirm the association, and mechanistic analyses are needed to identify potential environmental factors (eg, infections that could mediate the association). These results have to be confirmed before making recommendations on the use of probiotic supplementation.

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