Prospects for Primary Prevention of Type 1 Diabetes by Restoring a Disappearing Microbe

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Running Title: Prevention of Type 1 Diabetes

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Abstract

Prevention of childhood-onset type 1 diabetes has become more urgent with its marked increased incidence in recent decades in the modern world. Temporally associated with the rising incidence of type 1 diabetes, as well as other autoimmune and allergic diseases in childhood in modern times, is the disappearance of *Bifidobacterium* and specifically *Bifidobacterium longum* subsp. *infantis* (*B. infantis*) predominance in the intestinal microbiota of breastfed, vaginally delivered infants. *B. infantis* efficiently metabolizes human milk oligosaccharides without cross-feeding free sugar monomers to other commensals or pathogens and thereby dominates the intestinal microbiota of breastfed infants. Increased levels of short chain fatty acids, which

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stimulate both immunoregulation and healthy intestinal and pancreatic β-cell function, are generated by *B. infantis*. Based on recent observations of the intestinal microbiota in early life in young children who develop type 1 diabetes and demonstration of the robust preventive effects of short chain fatty acids in animal models of autoimmune diabetes, we hypothesize that restoring a *B. infantis* dominant microbiota early in infancy will prevent islet autoimmunity and childhood-onset type 1 diabetes.

Key Words: type 1 diabetes, prevention, probiotics, microbiome, short chain fatty acids

Introduction

An increasing contribution of the environment to the etiology of childhood-onset type 1 diabetes is evidenced in recent decades by a 2-4% increase in annual incidence, disease presentation at an earlier age in some European countries (1), and increased prevalence in lower HLA-risk genotypes (2). The significant incidence, daily burden, morbidity, mortality, and economic costs of type 1 diabetes have catalyzed widespread efforts for its prevention. Primary prevention of islet autoimmunity associated with childhood-onset type 1 diabetes requires the development of a highly safe intervention since neonates and/or infants will be the target population because the peak age incidence of islet autoantibody seroconversion is approximately 9-24 months (3; 4). Even with targeting high risk genotype infants for primary prevention, the majority will never develop clinical disease in their lifetime. Furthermore, an intervention for primary prevention that is safe and cost-effective, could be used for public health-based universal infant administration, an approach that would eliminate the need to screen for genetic risk and provides more widespread prevention than targeting a genetic at-risk childhood population. Clinical trials for primary prevention of childhood-onset type 1 diabetes have not however shown efficacy to date (5).

Development of Healthy Immune Regulation and Trained Innate Immunity

In addition to the rising incidence of childhood-onset type 1 diabetes, there has been an increased incidence and prevalence of several other childhood diseases in the developed and rapidly developing world, including allergic disease as well as celiac disease (6). A common feature of these diseases is defective immune regulation with recognition of either self-antigens or hyper-responsiveness to environmental triggers, suggesting that immunoregulation or immune

training may not be as complete and robust as in prior decades. Immunoregulation develops early in life through a symbiotic relationship between the host and the microbes residing at mucosal surfaces, especially in the intestine (7). The intestinal microbiota evolves over the first two to three years of life before it starts to resemble the adult microbiota (8). During this period of development, the microbiota of the infant and young child changes markedly with weaning from breast milk to cow's milk formula, which generates the prominent major shift, introduction of complementary food, infections, or exposure to antibiotics (8).

The basis of immune education or training from the microbiota early in life has not been completely elucidated, but a homeostatic relationship and dialogue between the intestinal microbiota and the immune system, which coevolved, establishes the immunological tone of tissues both in the local environment and systemically (9). When these interactions are disrupted, disease may ensue. Signaling from the microbiota through bacterial structural components and metabolites shapes a regulatory landscape and suppresses immune responses to commensals and dietary food antigens (10). This regulation of cellular components of innate immunity is mediated in part by epigenetic effects on their chromatin landscape (11), which results in innate immune memory or enhancement of innate immune function and altered immunological tone or set-points, representing trained innate immunity (12).

In the mouse, and likely in humans, there are critical periods of time or developmental windows for induction and establishment of healthy microbiota-induced immunoregulation, which if missed, increases the likelihood of development of pathogenic immune responses and susceptibility to disease (13). Furthermore, the temporal pattern of microbiota development and

the role and level of colonization of keystone, pioneer microbes in establishing and maintaining the microbial ecology early in life have been shown to dictate health and susceptibility to disease (13; 14).

Disappearing Microbes Are No Longer Colonizing the Intestinal Microbiota in Early Life

A difference in the microbiota of breastfed and formula fed infants was recognized almost 100 years ago with the breastfed microbiota colonized with higher levels of *Bifidobacterium.* The so-called "bifido factor" in breast milk that promotes colonization with *Bifidobacterium* is now recognized as human milk oligosaccharides (HMOs), which are comprised of more than 150 complex, branching sugar molecules. The HMOs represent ~ 15% of the energy content of breast milk, are not metabolized by the infant, and serve as a prebiotic primarily for *Bifidobacterium*, which actively metabolize HMOs to short-chain fatty acids (SCFA) (15).

In recent decades, several environmental changes have contributed to both altered infant microbiota and health. Delivery by Cesarean section, which increases the risk of type 1 diabetes by approximately 20% (16), has become more common worldwide, and bestows an intestinal microbiome associated with decreased colonization with *Bifidobacterium* even in breastfed infants (17) and with overall effects on the intestinal microbiota lasting throughout infancy (8). Antibiotic use in the intrapartum and perinatal period and during infancy and early childhood has increased in recent decades, alters the infant microbiota with significant reduction in colonization with *Bifidobacterium*, and predisposes children born by Cesarean section to type 1 diabetes (18).

The microbiota of vaginally-delivered, breastfed neonates and infants has changed in recent decades. Historically, *Bifidobacterium* dominated the intestinal microbiota of vaginally-delivered, breastfed infants, but this predominance of *Bifidobacterium* is not as frequent today (19-22). In a recent study in the United States, approximately 30% of infants at two months of age who were exclusively breastfed had no detectable *Bifidobacterium* and only 30% had *Bifidobacterium* representing greater than 50% of the total bacterial community, with sampling ten times in the first two months of life and analysis by 16S rRNA sequencing and quantitative PCR (22). A decrease in infant colonization with Bifidobacterium has also been observed in Finland in comparison to HLA-matched infants in Karelia, Russia in the DIABIMMUNE study (23), as detailed below.

Among all bacterial species, *Bifidobacterium longum* subsp. *infantis* (*B. infantis*) has a unique ability of efficiently metabolizing HMO molecules to simple sugars that are then converted to SCFA because it contains a unique gene cluster encoding genes for binding, transporting and hydrolyzing HMOs (24). As a consequence of this unique ability to scavenge and metabolize HMOs, *B. infantis* has historically predominated in the intestinal microbiota of breastfed infants. Remarkably, the near disappearance of *B. infantis* in the infant microbiota in the developed world overshadows the decrease in colonization with *Bifidobacterium* noted above (20; 25). Approximately 70% of breastfed infants from Ghana (n=32) were found to be colonized with *B. infantis* compared to none of 40 breastfed infants from New Zealand and the United Kingdom, as analyzed by PCR (26). In breastfed, vaginally delivered infants in New Zealand, Australia and Southeast Asia, 15% or less of infants were colonized with *B. infantis* at 8 weeks of age as detected by quantitative PCR (20; 27). Detectable intestinal colonization with *B. infantis* in

breastfed vaginally-delivered infants with multiple sampling (up to 10 times) and using state-of-the-art methods, as described above, is infrequent (10% or less of infants) in the United States in recent times (22; 28). The rarity (<5% prevalence) of *B. infantis* colonization of Japanese women late in pregnancy (n= 100) of whom 100% were colonized with Bifidobacterium contrasts markedly with the frequency of detection of other species of Bifidobacterium (29). Concomitant with disappearance of intestinal colonization with *Bifidobacterium* and *B. infantis* in infancy in the developed world, is an increase in infant fecal pH by as much as 1.5 pH units in breastfed infants over the last century (30), which likely reflects the decreased production of SCFA and lactate arising from the reduction in *Bifidobacterium* colonization and absence of *B. infantis,* which was historically their major source in stools of breastfed infants (20-22; 24; 30).

The low frequency of *B. infantis* colonization in breastfed vaginally-delivered infants in recent decades reflects a transgenerational effect on low levels of maternal colonization with *B. infantis* likely arising from exposure to antibiotics, Cesarean section, changes in the food chain, or exclusive formula feeding in infancy. This loss of infant intestinal colonization with *B. infantis* best fits with the so-called "disappearing microbe hypothesis" (31), which is a variant of the hygiene hypothesis (6) and applies to several different bacteria, including *Helicobacter pylori*, and may account for defective immunoregulation and transgenerational effects that are contributing to the modern day proliferation of autoimmune disease, allergic disease, and obesity in the developed world. Beneficial effects of a high abundance of *Bifidobacterium longum*, (possibly *B. longum* subsp. *infantis)* in the intestinal microbiota have been demonstrated for several immune-mediated phenomena and diseases. Increased *B. longum* abundance in the early microbiota at 3-6 months of age has been observed to promote highly activated Treg cells in the

maturation of the Treg cell population, and the relative abundance of *B. longum* at 3 months of age appears to be inversely associated with subsequent atopic sensitization, number of allergen-specific IgEs, and development of allergies. (Ruohtula T et al. submitted). Treatment for 12 weeks with a combination of *B. longum, B. lactis* and *Lactobacillus casei* reduced the severity of atopic dermatitis and the use of topical steroids (32). In addition *B. longum* has been reported to restore mucus growth and protect against diet-induced, microbiota-mediated colonic mucus deterioration (33).

Alteration of the Infant Intestinal Microbiota Precedes Development of Childhood Onset Type 1 Diabetes

Alteration of the infant bacterial microbiome has been associated with the development of childhood-onset type 1 diabetes, with some differences among these relatively small studies but a relatively consistent finding of a decrease in overall bacterial diversity and stability, decreased *Bifidobacterium* species, increased *Bacteroides* species, and reduced abundance of butyrate and overall SCFA-producing bacteria and of bacterial genes encoding fermentation pathways and short-chain fatty acid biosynthesis (34; 35).

The DIABIMMUNE study compared the microbiome of infants in Karelia, Russia, who sustain a high rate of infectious disease and low rate of type 1 diabetes and other autoimmune and allergic disease, to the microbiome of infants in Finland and Estonia, who have lower rates of infectious disease and higher rates of autoimmune and allergic diseases and higher exposure to antibiotics in the first three years of life (36). The microbiota of Finnish children had decreased levels of colonization with *Bifidobacterium* and failed to establish stable, single-strain *Bifidobacterium*

communities compared to Russian children (23). The relative abundance of *Bifidobacterium longum*, representing predominantly the subspecies *B. infantis*, in the infant intestinal microbiota during the breastfeeding period showed a strong inverse correlation with the incidence of type 1 diabetes in the three countries (r = -0.98) (Figure 1). In the Russian children, HMO utilization genes were conferred primarily by *Bifidobacterium*, which contrasted with Finnish children who had HMO utilization predominated by *Bacteroides*, which also was more abundant throughout the first three years of life. A lack of breastfeeding did not account for the low levels of *Bifidobacterium* in the microbiota of the Finnish children. The Russian children were predominantly exposed to *E. coli* LPS, while the Finnish children were predominantly exposed to *Bacteroides* LPS. The two forms of LPS have a different structure, and Bacteroides LPS was less immunostimulatory than *E. coli* LPS and blunted the immunostimulatory properties of *E. coli* LPS. In an animal model of type 1 diabetes, *E. coli* LPS but not *Bacteroides* LPS was protective. The DIABIMMUNE results suggest that low colonization with *Bifidobacterium* is allowing *Bacteroides* to create a foothold in the infant intestine and decreasing healthy microbial immunostimulation and immunoregulation for prevention of autoimmune and allergic disease.

There are multiple mechanisms how an altered microbiota with decreased *Bifidobacterium* and reduced SCFA could contribute to susceptibility to childhood-onset type 1 diabetes (Figure 2). First, as discussed above, an altered microbiota (i.e. dysbiosis) could compromise immunoregulatory "tone" and immune education, and thus, decrease the threshold for developing autoimmunity after exposure to environmental triggers in infants with genetic risk. An altered microbiota will be more susceptible to perturbations from diet or infections. The intestinal microbiota affects the pathogenicity of viruses, including enteroviruses (37), which

have been implicated in the etiology of human type 1 diabetes, and confers colonization resistance to bacterial pathogens. Additionally, an altered intestinal microbiota may lead to decreased intestinal mucosa barrier function arising from altered or decreased direct signaling of metabolites, such as SCFA (38), and such a leaky gut has been implicated in early stages of human type 1 diabetes (39). A leaky gut could contribute to disease susceptibility by facilitating translocation of microbiota or microbial products across the intestinal barrier to activate innate and adaptive immunity. This pathway has been demonstrated to play a role in the streptozotocin mouse model of diabetes, where intestinal dysbiosis leads to translocation of microbes or their products into the pancreatic draining lymph nodes that activate the intracellular pattern-recognition receptor nucleotide-binding oligomerization domain containing 2 (NOD2) in myeloid cells to contribute to onset of diabetes (40). The absence of high levels of SCFA with absence of *Bifidobacterium* or *B. infantis* may have effects beyond intestinal barrier function. SCFA activate release of cathelicidin-related antimicrobial peptides from β-cells that act in a paracrine manner on β-cells to modulate β-cell inflammatory responses and apoptosis and convert an inflammatory into a regulatory milieu in the pancreas in autoimmune diabetes (41).

In fact, increasing the levels of intestinal acetate and butyrate with acetylated and butyrylated starch diets robustly prevents autoimmune diabetes in NOD mice by inducing immunoregulation of islet antigen-specific cytotoxic T cells and generating regulatory T cells (42). Of interest, a retrospective analysis of the uncontrolled administration of probiotics containing primarily *Lactobacillus* and or *Bifidobacterium* in The Environmental Determinants of Diabetes in the Young (TEDDY) study that was initiated within in the first 27 days of life was associated with a decreased frequency of islet autoantibody seroconversion specifically in children with the HLA

DR3/DR4 genotype, who have a higher risk of type 1 diabetes, with initiation at a later time period proving ineffective (43).

Restoring a Disappearing Microbe to Prevent Type 1 Diabetes

The dysbiosis and decreased colonization of *Bifidobacterium* associated with childhood-onset type 1, the historic alteration of colonization of the intestinal microbiota of breastfed infants associated with increased prevalence of childhood onset type 1 diabetes, and the beneficial effects of high levels of SCFA, lead us to hypothesize that establishing high-level, durable infant intestinal colonization with a high SCFA-producing bacteria, such as *Bifidobacterium,* may prevent childhood-onset type 1 diabetes by generating a stable microbiota that will be resistant to perturbations, induce healthy immunoregulation and immune training, maintain intestinal barrier integrity, and promote survival of pancreatic β-cells (41; 42; 44).

Although many bacterial species can generate SCFAs, *B. infantis* is ideal for this purpose because of its natural historical predominance, current disappearance in breastfed, vaginally-delivered infants in developed countries, and its unique ability to import and metabolize breastmilk HMOs without cross-feeding other commensals or pathogens and to generate high levels of SCFA. Other *Bifidobacterium* do not have the same characteristics, and thus, do not closely mimic the "natural" microbiome of the past (24). Some Bacteroidaceae can metabolize HMOs, but they metabolize HMOs in a manner different from *B. infantis* in that they use extracellular glycosyl hydrolases that metabolize complex glycans outside the bacterial cell and thus can cross-feed other bacteria, which is not ideal for a probiotic, use different HMOs, and fail to generate SCFAs and consume HMOs to the same degree as a *B. infantis*-dominant infant

intestinal gut microbiota (22; 45-47). Also Bacteroidaceae may not prove ideal for immune education, as suggested by the DIABIMMUNE study (23). *B. infantis,* in fact, has been demonstrated to enhance intestinal integrity, increase IL-10 and Tregs, suppress IL-17 production, induce oral immune tolerance in mouse models, and modulate other pathways of immunoregulation (22; 26; 48).

Furthermore, in contrast to most bacteria that have been used as probiotics, *B. infantis* has been shown to stably colonize the neonatal intestine of breastfed infants. In a clinical trial in breastfed infants, daily administration of *B. infantis* (Strain EVC001) to neonates from day 7-28 of life safely, quickly, and robustly (100%) induced predominant colonization (>80% of the bacterial microbiota) that persisted in the absence of further administration of the probiotic as long as breastfeeding continued, which reflects the prebiotic properties of breastmilk HMOs for *B. infantis* (22; 49). Colonization was equally robust in both vaginally-delivered and Cesarean-delivered breastfed infants. A decrease in stool pH, increase in stool acetate and lactate, 10-fold higher consumption of HMOs, and increased microbiome stability was associated with *B. infantis* colonization (22).

The duration of high level colonization with *B. infantis* that must be maintained to confer effective immunoregulation to prevent type 1 diabetes is presently unknown. Based on the maturation of immune responses in infants to infections and vaccines (50), however, three to six months of colonization will likely prove sufficient for induction of effective immunoregulation. Durable high level colonization with *B infantis* will require the presence of HMOs in a quantity found in breast milk or a prebiotic resembling such at similar concentrations. Despite

recommendations that infants be exclusively breastfed for the first six months of life, only approximately 50% of infants in the United States are receiving breast milk at six months of age (CDC Breastfeeding Report Card 2016). A prebiotic that substitutes for HMO (51) that is administered either in cow's milk formula or as a supplement would need to be administered at concentrations high enough to maintain robust *B. infantis* colonization with early weaning or to initiate abundant colonization in exclusively formula fed neonates and infants.

Proving efficacy of prevention of childhood-onset type 1 diabetes with a *B. infantis* probiotic will require conducting human intervention trials. For practical purposes, the initial prevention trial should target HLA genotype high-risk breastfed neonates identified preferably at birth (3), with administration of the probiotic beginning in the first two weeks of life. The infants would need to be breastfed for three to six months to maintain high level *B. infantis* colonization during that period. The development of Stage 1 type 1 diabetes, defined as the presence of multiple islet autoantibodies and associated with high risk of progression to symptomatic type 1 diabetes (52), could be used as the trial primary endpoint, with a secondary endpoint of prevention of symptomatic type 1 diabetes.

Conclusion

Safe and cost-effective approaches for the prevention of childhood-onset type 1 diabetes that could ultimately be administered to all infants in a public health manner are required. The keystone commensal microbe *B. infantis* has disappeared as the predominant microbe in the infant microbiota in the developed world (20-22; 30), and we hypothesize that restoring *B. infantis* colonization early in infancy will prevent childhood-onset type 1 diabetes through effects

on immune training, prevention of dysbiosis and altered intestinal permeability, and enhanced β-cell function and survival. In contrast to most probiotics, *B. infantis* stably and safely colonizes the intestine at high abundance in breastfed infants delivered either vaginally or by Caesarean section (22; 49). A *B. infantis* probiotic primary prevention clinical trial can be conducted in a relatively short time frame in HLA genotype high-risk breastfed infants with a primary endpoint of islet autoantibody seroconversion (Stage 1 type 1 diabetes) with follow-up for long-term prevention of onset of clinical disease. Demonstration of proof-of-concept in a high-risk cohort would catalyze a public health-based clinical trial in a general childhood population, which could also inform prevention of other childhood diseases (e.g. allergic disease, celiac disease, childhood obesity) in addition to type 1 diabetes.

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References

1. Harjutsalo V, Sjoberg L, Tuomilehto J: Time trends in the incidence of type 1 diabetes in Finnish children: a cohort study. Lancet (London, England) 2008;371:1777-1782
2. Steck AK, Armstrong TK, Babu SR, Eisenbarth GS: Stepwise or linear decrease in penetrance of type 1 diabetes with lower-risk HLA genotypes over the past 40 years. Diabetes 2011;60:1045-1049
3. Krischer JP, Lynch KF, Schatz DA, Ilonen J, Lernmark A, Hagopian WA, Rewers MJ, She JX, Simell OG, Toppari J, Ziegler AG, Akolkar B, Bonifacio E: The 6 year incidence of diabetes-associated autoantibodies in genetically at-risk children: the TEDDY study. Diabetologia 2015;58:980-987
4. Ilonen J, Hammais A, Laine AP, Lempainen J, Vaarala O, Veijola R, Simell O, Knip M: Patterns of beta-cell autoantibody appearance and genetic associations during the first years of life. Diabetes 2013;62:3636-3640
5. Jacobsen L, Schatz D: Current and future efforts toward the prevention of type 1 diabetes. Pediatric diabetes 2016;17 Suppl 22:78-86
6. Bach JF: The hygiene hypothesis in autoimmunity: the role of pathogens and commensals. Nature reviews Immunology 2018;18:105-120
7. Hooper LV, Littman DR, Macpherson AJ: Interactions between the microbiota and the immune system. Science (New York, NY) 2012;336:1268-1273
8. Backhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, Li Y, Xia Y, Xie H, Zhong H, Khan MT, Zhang J, Li J, Xiao L, Al-Aama J, Zhang D, Lee YS, Kotowska D, Colding C, Tremaroli V, Yin Y, Bergman S, Xu X, Madsen L, Kristiansen K, Dahlgren J, Wang J: Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life. Cell host & microbe 2015;17:690-703
9. Belkaid Y, Harrison OJ: Homeostatic Immunity and the Microbiota. Immunity 2017;46:562-576
10. Tanoue T, Atarashi K, Honda K: Development and maintenance of intestinal regulatory T cells. Nature reviews Immunology 2016;16:295-309
11. Shih HY, Sciume G, Mikami Y, Guo L, Sun HW, Brooks SR, Urban JF, Jr., Davis FP, Kanno Y, O'Shea JJ: Developmental Acquisition of Regulomes Underlies Innate Lymphoid Cell Functionality. Cell 2016;165:1120-1133
12. Netea MG, Joosten LA, Latz E, Mills KH, Natoli G, Stunnenberg HG, O'Neill LA, Xavier RJ: Trained immunity: A program of innate immune memory in health and disease. Science (New York, NY) 2016;352:aaf1098
13. Gensollen T, Iyer SS, Kasper DL, Blumberg RS: How colonization by microbiota in early life shapes the immune system. Science (New York, NY) 2016;352:539-544
14. Torow N, Hornef MW: The Neonatal Window of Opportunity: Setting the Stage for Life-Long Host-Microbial Interaction and Immune Homeostasis. Journal of immunology (Baltimore, Md : 1950) 2017;198:557-563
15. Bode L: Human milk oligosaccharides: every baby needs a sugar mama. Glycobiology 2012;22:1147-1162
16. Cardwell CR, Stene LC, Joner G, Cinek O, Svensson J, Goldacre MJ, Parslow RC, Pozzilli P, Brigis G, Stoyanov D, Urbonaite B, Sipetic S, Schober E, Ionescu-Tirgoviste C, Devoti G, de Beaufort CE, Buschard K, Patterson CC: Caesarean section is associated with an increased risk

of childhood-onset type 1 diabetes mellitus: a meta-analysis of observational studies. Diabetologia 2008;51:726-735
17. Penders J, Thijs C, Vink C, Stelma FF, Snijders B, Kummeling I, van den Brandt PA, Stobberingh EE: Factors influencing the composition of the intestinal microbiota in early infancy. Pediatrics 2006;118:511-521
18. Clausen TD, Bergholt T, Bouaziz O, Arpi M, Eriksson F, Rasmussen S, Keiding N, Lokkegaard EC: Broad-Spectrum Antibiotic Treatment and Subsequent Childhood Type 1 Diabetes: A Nationwide Danish Cohort Study. PloS one 2016;11:e0161654
19. Turroni F, Milani C, Duranti S, Ferrario C, Lugli GA, Mancabelli L, van Sinderen D, Ventura M: Bifidobacteria and the infant gut: an example of co-evolution and natural selection. Cellular and molecular life sciences : CMLS 2018;75:103-118
20. Tannock GW, Lee PS, Wong KH, Lawley B: Why Don't All Infants Have Bifidobacteria in Their Stool? Frontiers in microbiology 2016;7:834
21. Lewis ZT, Mills DA: Differential Establishment of Bifidobacteria in the Breastfed Infant Gut. Nestle Nutrition Institute workshop series 2017;88:149-159
22. Frese SA, Hutton AA, Contreras LN, Shaw CA, Palumbo MC, Casaburi G, Xu G, Davis JCC, Lebrilla CB, Henrick BM, Freeman SL, Barile D, German JB, Mills DA, Smilowitz JT, Underwood MA: Persistence of Supplemented Bifidobacterium longum subsp. infantis EVC001 in Breastfed Infants. mSphere 2017;2
23. Vatanen T, Kostic AD, d'Hennezel E, Siljander H, Franzosa EA, Yassour M, Kolde R, Vlamakis H, Arthur TD, Hamalainen AM, Peet A, Tillmann V, Uibo R, Mokurov S, Dorshakova N, Ilonen J, Virtanen SM, Szabo SJ, Porter JA, Lahdesmaki H, Huttenhower C, Gevers D, Cullen TW, Knip M, Xavier RJ: Variation in Microbiome LPS Immunogenicity Contributes to Autoimmunity in Humans. Cell 2016;165:842-853
24. Underwood MA, German JB, Lebrilla CB, Mills DA: Bifidobacterium longum subspecies infantis: champion colonizer of the infant gut. Pediatric research 2015;77:229-235
25. Tannock GW, Lawley B, Munro K, Gowri Pathmanathan S, Zhou SJ, Makrides M, Gibson RA, Sullivan T, Prosser CG, Lowry D, Hodgkinson AJ: Comparison of the compositions of the stool microbiotas of infants fed goat milk formula, cow milk-based formula, or breast milk. Applied and environmental microbiology 2013;79:3040-3048
26. Young SL, Simon MA, Baird MA, Tannock GW, Bibiloni R, Spencely K, Lane JM, Fitzharris P, Crane J, Town I, Addo-Yobo E, Murray CS, Woodcock A: Bifidobacterial species differentially affect expression of cell surface markers and cytokines of dendritic cells harvested from cord blood. Clinical and diagnostic laboratory immunology 2004;11:686-690
27. Lawley B, Munro K, Hughes A, Hodgkinson AJ, Prosser CG, Lowry D, Zhou SJ, Makrides M, Gibson RA, Lay C, Chew C, Lee PS, Wong KH, Tannock GW: Differentiation of Bifidobacterium longum subspecies longum and infantis by quantitative PCR using functional gene targets. PeerJ 2017;5:e3375
28. Lewis ZT, Totten SM, Smilowitz JT, Popovic M, Parker E, Lemay DG, Van Tassell ML, Miller MJ, Jin YS, German JB, Lebrilla CB, Mills DA: Maternal fucosyltransferase 2 status affects the gut bifidobacterial communities of breastfed infants. Microbiome 2015;3:13
29. Mikami K, Takahashi H, Kimura M, Isozaki M, Izuchi K, Shibata R, Sudo N, Matsumoto H, Koga Y: Influence of maternal bifidobacteria on the establishment of bifidobacteria colonizing the gut in infants. Pediatric research 2009;65:669-674

30. Henrick BM, Hutton AA, Palumbo MC, Casaburi G, Mitchell RD, Underwood MA, Smilowitz JT, Frese SA: Elevated Fecal pH Indicates a Profound Change in the Breastfed Infant Gut Microbiome Due to Reduction of Bifidobacterium over the Past Century. mSphere 2018;3
31. Blaser MJ: The theory of disappearing microbiota and the epidemics of chronic diseases. Nature reviews Immunology 2017;17:461-463
32. Navarro-Lopez V, Ramirez-Bosca A, Ramon-Vidal D, Ruzafa-Costas B, Genoves-Martinez S, Chenoll-Cuadros E, Carrion-Gutierrez M, Horga de la Parte J, Prieto-Merino D, Codoner-Cortes FM: Effect of Oral Administration of a Mixture of Probiotic Strains on SCORAD Index and Use of Topical Steroids in Young Patients With Moderate Atopic Dermatitis: A Randomized Clinical Trial. JAMA dermatology 2018;154:37-43
33. Schroeder BO, Birchenough GMH, Stahlman M, Arike L, Johansson MEV, Hansson GC, Backhed F: Bifidobacteria or Fiber Protects against Diet-Induced Microbiota-Mediated Colonic Mucus Deterioration. Cell host & microbe 2018;23:27-40.e27
34. Knip M, Siljander H: The role of the intestinal microbiota in type 1 diabetes mellitus. Nature reviews Endocrinology 2016;12:154-167
35. Knip M, Honkanen J: Modulation of Type 1 Diabetes Risk by the Intestinal Microbiome. Current diabetes reports 2017;17:105
36. Kallionpaa H, Laajala E, Oling V, Harkonen T, Tillmann V, Dorshakova NV, Ilonen J, Lahdesmaki H, Knip M, Lahesmaa R: Standard of hygiene and immune adaptation in newborn infants. Clinical immunology (Orlando, Fla) 2014;155:136-147
37. Kuss SK, Best GT, Etheredge CA, Pruijssers AJ, Frierson JM, Hooper LV, Dermody TS, Pfeiffer JK: Intestinal microbiota promote enteric virus replication and systemic pathogenesis. Science (New York, NY) 2011;334:249-252
38. Koh A, De Vadder F, Kovatcheva-Datchary P, Backhed F: From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. Cell 2016;165:1332-1345
39. Li X, Atkinson MA: The role for gut permeability in the pathogenesis of type 1 diabetes--a solid or leaky concept? Pediatric diabetes 2015;16:485-492
40. Costa FR, Francozo MC, de Oliveira GG, Ignacio A, Castoldi A, Zamboni DS, Ramos SG, Camara NO, de Zoete MR, Palm NW, Flavell RA, Silva JS, Carlos D: Gut microbiota translocation to the pancreatic lymph nodes triggers NOD2 activation and contributes to T1D onset. The Journal of experimental medicine 2016;213:1223-1239
41. Sun J, Furio L, Mecheri R, van der Does AM, Lundeberg E, Saveanu L, Chen Y, van Endert P, Agerberth B, Diana J: Pancreatic beta-Cells Limit Autoimmune Diabetes via an Immunoregulatory Antimicrobial Peptide Expressed under the Influence of the Gut Microbiota. Immunity 2015;43:304-317
42. Marino E, Richards JL, McLeod KH, Stanley D, Yap YA, Knight J, McKenzie C, Kranich J, Oliveira AC, Rossello FJ, Krishnamurthy B, Nefzger CM, Macia L, Thorburn A, Baxter AG, Morahan G, Wong LH, Polo JM, Moore RJ, Lockett TJ, Clarke JM, Topping DL, Harrison LC, Mackay CR: Gut microbial metabolites limit the frequency of autoimmune T cells and protect against type 1 diabetes. Nature immunology 2017;18:552-562
43. Uusitalo U, Liu X, Yang J, Aronsson CA, Hummel S, Butterworth M, Lernmark A, Rewers M, Hagopian W, She JX, Simell O, Toppari J, Ziegler AG, Akolkar B, Krischer J, Norris JM, Virtanen SM: Association of Early Exposure of Probiotics and Islet Autoimmunity in the TEDDY Study. JAMA pediatrics 2016;170:20-28
44. Schroeder BO, Backhed F: Signals from the gut microbiota to distant organs in physiology and disease. Nature medicine 2016;22:1079-1089

45. Marcobal A, Barboza M, Sonnenburg ED, Pudlo N, Martens EC, Desai P, Lebrilla CB, Weimer BC, Mills DA, German JB, Sonnenburg JL: Bacteroides in the infant gut consume milk oligosaccharides via mucus-utilization pathways. Cell host & microbe 2011;10:507-514
46. Yu ZT, Chen C, Newburg DS: Utilization of major fucosylated and sialylated human milk oligosaccharides by isolated human gut microbes. Glycobiology 2013;23:1281-1292
47. Marcobal A, Sonnenburg JL: Human milk oligosaccharide consumption by intestinal microbiota. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 2012;18 Suppl 4:12-15
48. Konieczna P, Akdis CA, Quigley EM, Shanahan F, O'Mahony L: Portrait of an immunoregulatory Bifidobacterium. Gut microbes 2012;3:261-266
49. Smilowitz JT, Moya J, Breck MA, Cook C, Fineberg A, Angkustsiri K, Underwood MA: Safety and tolerability of Bifidobacterium longum subspecies infantis EVC001 supplementation in healthy term breastfed infants: a phase I clinical trial. BMC pediatrics 2017;17:133
50. Kollmann TR, Kampmann B, Mazmanian SK, Marchant A, Levy O: Protecting the Newborn and Young Infant from Infectious Diseases: Lessons from Immune Ontogeny. Immunity 2017;46:350-363
51. Bode L, Contractor N, Barile D, Pohl N, Prudden AR, Boons GJ, Jin YS, Jennewein S: Overcoming the limited availability of human milk oligosaccharides: challenges and opportunities for research and application. Nutrition reviews 2016;74:635-644
52. Insel RA, Dunne JL, Atkinson MA, Chiang JL, Dabelea D, Gottlieb PA, Greenbaum CJ, Herold KC, Krischer JP, Lernmark A, Ratner RE, Rewers MJ, Schatz DA, Skyler JS, Sosenko JM, Ziegler AG: Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. Diabetes care 2015;38:1964-1974
53. Kondrashova A, Reunanen A, Romanov A, Karvonen A, Viskari H, Vesikari T, Ilonen J, Knip M, Hyoty H: A six-fold gradient in the incidence of type 1 diabetes at the eastern border of Finland. Annals of medicine 2005;37:67-72
54. Teeaar T, Liivak N, Heilman K, Kool P, Sor R, Paal M, Einberg U, Tillmann V: Increasing incidence of childhood-onset type 1 diabetes mellitus among Estonian children in 1999-2006. Time trend analysis 1983-2006. Pediatric diabetes 2010;11:107-110

Legends for the Figures:

Figure 1. Relative abundance of *Bifidobacterium longum (B.longum)* species in the intestinal microbiota during the breastfeeding period in Finnish, Estonian and Russian Karelian infants (blue bars, based on the DIABIMMUNE study) (23) and the annual incidence of type 1 diabetes among children under the age of 15 years in the three countries (orange bars). The relative abundances of *B. longum* are significantly different between the countries ($P < 2 \times 10^{-5}$, repeated measures ANOVA). There is an inverse correlation between the rate of type 1 diabetes and the relative abundance of *B. longum* ($r = -0.98$). The incidence rate of type 1 diabetes is for the period of 1990-1999 in Finland and Russian Karelia (53), and for the period 1999-2006 in Estonia (54).

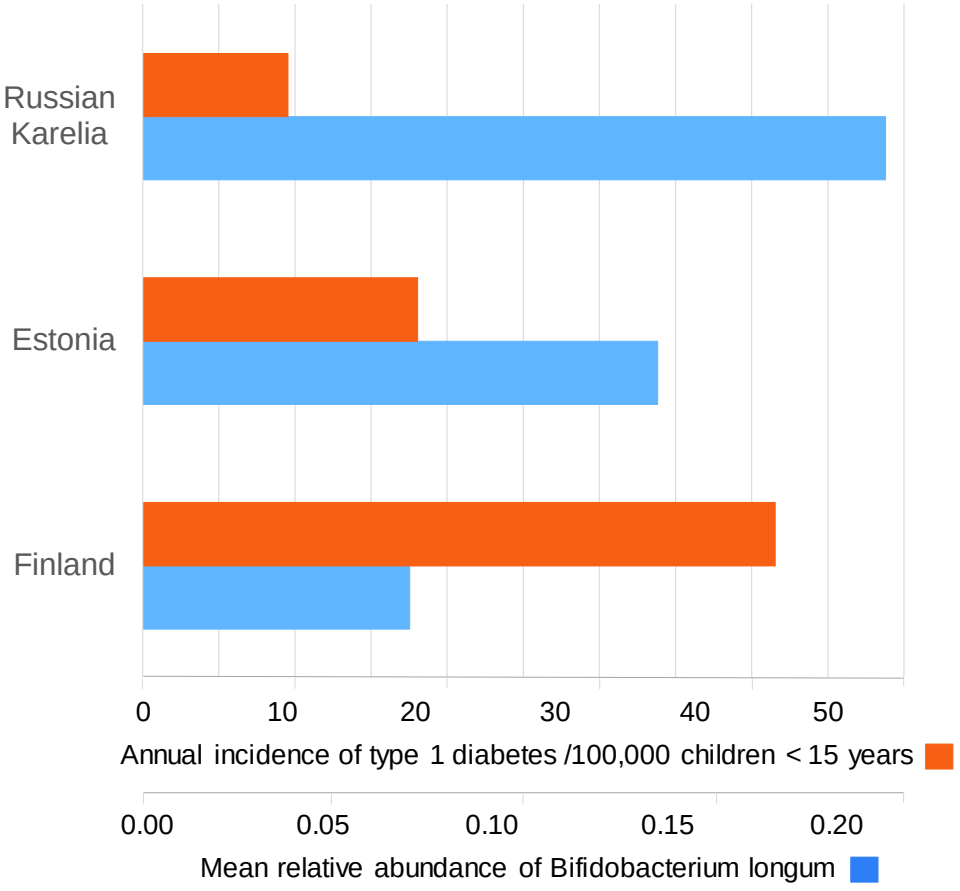

Figure 2. Expected impact of the combination of supplementation with *Bifidobacterium longum* subsp. *infantis* (*B. infantis*) and human milk oligosaccharides (HMO) from breast milk. The supplementation results in a stable intestinal microbiota with abundance of *B. infantis* that will prevent colonization with bacterial pathogens by creating colonization resistance. The combination also leads to high production of short-chain fatty acids, which support preservation of the intestinal barrier and active immunological education in the gastrointestinal tract as well as promoting survival of the β-cells in the pancreatic islets.