Probiotics for the Prevention of Beta Cell Autoimmunity in Children at Genetic Risk of Type 1 Diabetes—the PRODIA Study

MARTIN LJUNGBERG,[a] RIITA KORPELA,[b] JORMA ILONEN,[c] JOHNNY LUDVIGSSON,[a] AND OUTI VAARALA[a,d]

[a]*Division of Pediatrics and Diabetes Research Centre, Department of Molecular and Clinical Medicine, Linköping University, S-58 185 Linköping, Sweden*

[b]*Valio Ltd, 00037 Helsinki, Finland*

[c]*Department of Clinical Microbiology, University of Kuopio, Kuopio, Finland and Immunogenetics Laboratory, University of Turku, 20520 Turku, Finland*

[d]*Department of Viral Diseases and Immunology, National Public Health Institute, 00037 Helsinki, Finland*

ABSTRACT: The final aim of the PRODIA study is to determine whether the use of probiotics during the first 6 months of life decreases the appearance of type 1 diabetes mellitus (T1DM)-associated autoantibodies in children with genetic risk for T1DM. A pilot study including 200 subjects was planned to show whether the use of probiotics during the first 6 months of life is safe and feasible. The prevalence of autoantibodies among the study subjects at 6, 12, and 24 months of age was at levels close to the expected and the clinical follow-up did not either indicate problems in the feasibility of the study.

KEYWORDS: type 1 diabetes; probiotics; autoantibodies

INTRODUCTION

Type 1 diabetes mellitus (T1DM) is considered to be an autoimmune disease leading to destruction of the insulin-producing beta cells in pancreas. Interaction between genetic factors and environmental factors are believed to trigger the autoimmune response finally causing T1DM.[1] About 90% of the children

Address for correspondence: Martin Ljungberg, Centre of Clinical Experimental Research, Division of Pediatrics and Diabetes Research Centre, Department of Molecular and Clinical Medicine, Faculty of Health Sciences, Linköping University, S-581 85 Linköping, Sweden. Voice: +46-13-229538; fax: +46-13-127465.
e-mail: martin.ljungberg@imk.liu.se

Ann. N.Y. Acad. Sci. 1079: 360–364 (2006).
doi: 10.1196/annals.1375.055

developing T1DM carry high-risk genes (human leukocyte antigen [HLA] DQB1*0302 and/or HLA DQB1*0201), but less than 10% of the children with these risk genes actually develop T1DM.[2] A number of environmental factors have been suggested to be associated with T1DM. Early exposure to cow milk proteins and wheat gluten in the infant diet, as well as enteral virus infections, together with aberrant development and maturity of the gut immune system, are candidate risk factors.[3] Enterovirus and rotavirus infections have been associated with the appearance of beta cell autoantibodies.[4,5] In animal models the incidence of T1DM has been shown to be highest in a low microbial load environment.[6] Microbiotic colonization of the newborn infant's gut ecosystem by specific bacterial species may be important in the initial regulation of the developing immune system.[7] Development of T1DM has been associated with intestinal immune activation and enhanced immunity to food antigens.[8,9] Probiotics, defined as nonpathogenic cultures of living bacteria with a health-promoting effect,[10] have *in vitro* showed to activate monocytes, macrophages, and dendritic cells and thus influence the immune system.[11–13] Probiotics also have been shown to support the maturity of the gut immune system and could therefore support oral tolerance and protection against enteral virus infections, that is, risk factors of T1DM.[14] Beta cell autoantibodies occurring years before clinical onset of T1DM are predictive for the disease in individuals with genetic risk. The three major islet autoantibodies are those against glutamic acid decarboylase (GADA), tyrosine phosphatase (IA-2A), and insulin (IAA). Children developing T1DM are mostly positive for at least two of these markers.[15] The PRODIA study started in February 2003 at the Faculty of Health Sciences at Linköping University as a pilot study primarily to test feasibility and safety of the protocol. The final aim of a main study will be to determine whether the use of probiotics during the first 6 months of life decreases the appearance of beta cell autoantibodies in children with increased genetic risk for T1DM. Affected factors involved could be the reduced occurrence of enteral virus infections, enhanced maturation of the gut immune system, reduced immunization to dietary insulin, or induced immune regulation.

SUBJECTS AND METHODS

The PRODIA study was approved by the Research Ethics Committee at the Faculty of Health Sciences, Linköping University. PRODIA is a double-blind randomized placebo controlled study. Between February 2003 and June 2005 all parents to newborn infants at Linköping University Hospital were informed about the PRODIA pilot study. After informed consent by the parents, 1200 children were screened for HLA genotypes associated with risk for T1DM. HLA risk genotypes were defined as the presence of HLA-(DR3)-DQA1*05-DQB1*02 and/or (DR4)-DQB1*0302 haplotypes without protective haplotypes DQB1*06301 and DQB1*0301. When combined with HLA-(DR3)-DQA1* 05-DQB1*02 also DQB1*0603 and DQA1*0201-

DQB1*02 were considered protective. All children with risk genotype were randomized to receive either probiotics or placebo during the first 6 months of life. The probiotics, distributed once a day by parents at home in soluble capsules, consist of a cocktail of Lactobacillus rhamnosus GG (5×10^9 cfu), Lactobacillus rhamnosus LC705 (5×10^9 cfu), Bifidobacterium breve Bbi99 (2×10^8 cfu), and Propionibacterium freudenreichii ssp. Shermani JS (2×10^9 cfu). Blood samples are taken at 6, 12, and 24 months of age. Fecal samples are collected at home at 3 months intervals and the introduction of new foods is recorded. The diet of the children is not manipulated. We analyze the occurrence of beta cell autoantibodies (GAD, IA-2, and IAA) and measure monocyte and T cell-derived cytokines and chemokines in plasma and supernatants of *in vitro*-stimulated cells. We also study the expression of intracellular signal proteins (T bet, STAT-4, STAT-6, GATA-3) in peripheral blood mononuclear cells (PBMC). Expression of monocyte activation markers is analyzed in fresh whole blood and in whole blood stimulated with lipopolysaccharide (LPS) and lipoteichoic acid (LTA). The phytohemagglutinin (PHA) and IAA-induced T cell responses are studied. Microbiological analyses of feces samples are done to control incidence of enteral virus infections and compliance. Enterovirus infections are followed also by isolation of enterovirus RNA in blood samples and by serological tests. Venous blood samples were collected at 6, 12, and 24 months and measurements of beta cell autoantibodies GAD, IA-2, and IAA were done as previously described.[16] The cutoff for positivity was determined as 99th percentile level of autoantibodies in healthy 5-year-old children.

RESULTS

About 60% of the parents asked to participate gave their informed consent. As expected we found 264 children with risk genes among the tested infants. The dropout rate has been steady between 15% and 25%. TABLE 1 shows the detection of autoantibodies in various samples. One sample was detected positive for IAA at 6 months of age. No sample was detected positive at 12 months of age. However, at 24 months of age one sample was detected positive for GADA and another one for IA-2A. No sample was detected positive for more than one autoantibody.

TABLE 1. Positivity to autoantibodies in plasma samples at 6, 12, and 24 months of age

	GADA	IA-2A	IAA	Pos>1 a.a
Age	pos/tot	pos/tot	pos/tot	pos/tot
6 months	0/170	0/170	1/168	0/168
12 months	0/151	0/151	0/146	0/146
24 months	1/61	1/61	0/61	0/61

pos = positive; tot = total; a.a. = autoantibody

DISCUSSION

The PRODIA protocol seems to be feasible, although quite many parents do not want to participate and the dropout rate is quite high. We expected to find roughly a 2% prevalence of at least one of the measured autoantibodies at 24 months of age in the study group. In the Finnish Diabetes Prediction and Prevention (DIPP) study follow-up cohort a frequency of 2.9% of children were found persistently positive for IAA, 1.7% for GADA, and 1.2% for IA-2A at the same age.[17] The DIPP study was similarly based on genetic screening of general population, but the lower risk group of children selected based on the presence of (DR3)-DQA1*05-DQB1*02 haplotype was not included. This group with approximately half of the risk ratio to that of moderate risk group with (DR4)-DQB1*0302[18] covers around a third of PRODIA group, which is taken into account by lower expected autoantibody positivity. The detected number of autoantibodies, 1/168 for IAA at 6 months of age, 1/61 for GADA, and 1/61 for IA-2A at 24 months of age, is close to the expected and there is no evidence that the intervention would increase the appearance of beta cell autoimmunity in the children who participate in the PRODIA study. We conclude that the PRODIA study protocol seems to be safe and that the study protocol is feasible for the families. The mechanistic studies of the effect of probiotics on the development of the immune system and occurrence of enterovirus infections are ongoing.

ACKNOWLEDGMENTS

We are grateful to all the participating children and parents in the PRODIA study. Many thanks to the PRODIA team in Linköping: coordinator Anneli KR Johnsson, nurses Eva Isacsson and Ann-Marie Sandström, and laboratory technicians Gosia Smolinska and Ingela Johansson. Appreciation goes to Rosaura Casas PhD and Anna Lindström MSc in the PRODIA study group. This study was generously supported by Swedish Child Diabetes Foundation (Barndiabetesfonden), Swedish Research Council (K2002-72X-11242-08A and K2003-72X-14690-01A), Swedish Diabetes Association, and the Foundation for Strategic Research. Support from Valio Ltd. is very much appreciated.

REFERENCES

1. KIM, M.S. & C. POLYCHRONAKOS. 2005. Immunogenetics of type 1 diabetes. Horm. Res. **64:** 180–188.
2. BERZINA, L., A. SCTAUVERE-BRAMEUS, J. LUDVIGSSON & C.B. SANJEEVI. 2002. Newborn screening for high-risk human leukocyte antigen markers associated with insulin dependent diabetes mellitus: the ABIS study. Ann. N. Y. Acad. Sci. **958:** 312–316.

3. AKERBLOM, H.K., O. VAARALA, H. HYOTY, *et al*. 2002. Environmental factors in the etiology of type 1 diabetes. Am. J. Med. Genet. **115:** 18–29.
4. LONNROT, M., K. KORPELA, M. KNIP, *et al*. 2000. Enterovirus infection as a risk factor for beta-cell autoimmunity in a prospectively observed birth cohort: the Finnish Diabetes Prediction and Prevention Study. Diabetes. **49:** 1314–1318.
5. HONEYMAN, M.C., B.S. COULSON, N.L. STONE, *et al*. 2000. Association between rotavirus infection and pancreatic islet autoimmunity in children at risk of developing type 1 diabetes. Diabetes **49:** 1319–1324.
6. DELOVITCH, T. & B. SINGH. 1997. The nonobese diabetic mouse as a model of autoimmune diabetes: immune dysregulation gets the NOD. Immunity **7:** 727–738.
7. SALVINI, F., L. GRANIERI, L. GEMMELLARO & M. GIOVANNINI. 2004. Probiotics, prebiotics and child health: where are we going? J. Int. Med. Res. **32:** 97–108.
8. WESTERHOLM-ORMIO, M., O. VAARALA, P. PIHKALA, *et al*. 2003. Immunologic activity in the small intestinal mucosa of pediatric patients with type 1 diabetes. Diabetes **52:** 2287–2295.
9. VAARALA, O. 2002. The gut immune system and type 1 diabetes. Ann. N. Y. Acad. Sci. **958:** 39–46.
10. LIN, D. 2003. Probiotics as functional foods. Nutr. Clin. Pract. **18:** 497–506.
11. VECKMAN, V., M. MIETTINEN, S. MATIKAINEN, *et al*. 2003. Lactobacilli and streptococci induce inflammatory chemokine production in human macrophages that stimulates Th1 cell chemotaxis. J. Leukoc. Biol. **74:** 395–402.
12. POCHARD, P., P. GOSSET, C. GRANGETTE, *et al.*2002. Lactic acid bacteria inhibit TH2 cytokine production by mononuclear cells from allergic patients. J. Allergy Clin. Immunol. **110:** 617–623.
13. CHRISTENSEN, H.R., H. FROKIAER & J.J. PESTKA. 2002. Lactobacilli differentially modulate expression of cytokines and maturation surface markers in murine dendritic cells. J. Immunol. **168:** 171–178.
14. VAARALA, O. 2003. Immunological effects of probiotics with special reference to lactobacilli. Clin. Exp. Allergy **33:** 1634–1640.
15. KULMALA, P., K. SAVOLA, J.S. PETERSEN, *et al.*1998. Prediction of insulin-dependent diabetes mellitus in siblings of children with diabetes. A population-based study. The Childhood Diabetes in Finland Study Group. J. Clin. Invest. **101:** 327–336.
16. HOLMBERG, H., O. VAARALA, V. SADAUSKAITE-KUEHNE, *et al.* 2006. Higher prevalence of autoantibodies to insulin and GAD65 in Swedish compared to Lithuanian children with type 1 diabetes. Diabetes Res. Clin. Pract. **72:** 308–314.
17. KIMPIMAKI, T., P. KULMALA, K. SAVOLA, *et al*. 2002. Natural history of beta-cell autoimmunity in young children with increased genetic susceptibility to type 1 diabetes recruited from the general population. J. Clin. Endocrinol Metab. **87:** 4572–4579.
18. NEJENTSEV, S., M. SJOROOS, T. SOUKKA, *et al*. 1999. Population-based genetic screening for the estimation of type 1 diabetes mellitus risk in Finland: selective genotyping of markers in the HLA-DQB1, HLA-DQA1 and HLA-DRB1 loci. Diabet. Med. **16:** 985–992.