

Title: Impact of probiotic use on immune cell function in children.

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Protocol Version Date: Version 5/1/2017

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Funding Source: Department of Pediatrics
DuPont™ Danisco® will be providing probiotic

Project Summary

Probiotics are microorganisms that are believed to provide health benefits when consumed. The term probiotic is currently used to name ingested microorganisms associated with beneficial effects to humans and animals. Probiotics are popularized in the lay literature for many different clinical problems. They have been studied in infants and children as a preventive or treatment for a variety of infections. Studies on the medical benefits of probiotics have yet to reveal a cause-effect relationship, and their medical effectiveness has yet to be conclusively proven for most of the studies conducted thus far. The putative benefit of probiotics in the prevention of infection relates to potential benefits to the innate and adaptive immune systems of infants.

The goals of this investigation are to study immune system cell function and microbiome in children who are taking probiotics.

To accomplish this goal, we propose a pilot study for which we will obtain blood and nasopharyngeal and stool samples prior to and post probiotic use in children greater than 12 months-36 months over a 27-38 day period.

Background and Significance

Acute upper respiratory viral infections (URIs) are a common, important and expensive clinical problem. The acute URI is the most frequent infection experienced by children and adults. URI is frequently complicated by bacterial infections such as acute otitis media, acute sinusitis or pneumonia especially in children. Furthermore, the practitioner, uncertain about the role of bacteria in URI, often feels compelled to prescribe antibiotics. The use of antibiotics for both imagined and actual complications of URI contributes greatly to local and global problems of antibiotic resistance. In addition, viral URI may be the most important trigger for acute episodes of wheezing and exacerbations of reactive airways disease and asthma.¹ Asthma, with its tremendous attendant morbidity and mortality, in both children and adults, continues to increase in frequency. The population of the US experiences approximately 500 million URIs per year with an economic impact of \$40 billion annually including a loss of an estimated 22 million school days and 20 million work days. Despite such a high prevalence, serious health consequences and huge economic burden there are very few prevention or treatment options for URI. Accordingly, there has been much interest in the use of probiotics to prevent URI and/or to shorten the duration of symptoms across all age groups.

There is evidence that probiotics prevent and ameliorate URI: Various probiotics have been studied in the prevention of URI in children. Several studies have shown a decrease in the number of URIs²⁻⁸ and a reduction in duration and severity of symptoms^{2,3,9-16} A recent large meta-analysis showed significantly fewer days of illness per person, shorter illness episodes by almost a day and fewer numbers of days absent from school/work or day care.¹⁷ Furthermore, a methodologically robust study by Leyer et al, evaluated the combination product *Lactobacillus acidophilus* NCFM and *Bifidobacteria animalis* Bi-07 in children 3 to 6 years of age. They reported a 34% risk reduction of fever, duration of coughing and rhinorrhea as well as reduced antibiotic use and days absent from group child care and work (for parents).¹¹ These results strongly influenced our selection of probiotic.

The mechanisms by which probiotics reduce the frequency and severity of URI are not well understood. There is evidence that probiotics enhance immune function in children and that this enhancement may account for the decrease in frequency and severity of viral URIs observed in children receiving probiotics (*L. acidophilus* NCFM and *B. lactis* Bi-07) in comparison to those receiving placebo.¹⁸⁻²⁴ To date, the underlying immune mechanism(s) that account for this protective health benefit has not been clearly delineated. However, the most likely mechanism is via their impact on various immune cell numbers and function.²⁴ Probiotics are known to regulate the balance between pro and anti-inflammatory cytokines and the activation of Treg cells and the resultant skewing of Th1, Th2 and Th17 cell activities. For example, Ghadimi et al demonstrated in an in vitro assay (using peripheral blood mononuclear cells (PBMCs)) of allergic and non-allergic patients that incubation of PBMCs with various lactic acid producing bacteria decreased the presence of Th2 cytokines (IL-4 and IL5) and enhanced the production of IFN- γ (known for its antiviral effect).²⁵ In a series of clinical studies performed by the same group, healthy adults were randomized to receive probiotic or placebo for several months.^{19,23} Episodes of respiratory infection were shortened significantly in

those who received probiotic and significantly higher levels of CD8+ T cells, CD4+ T cells, and monocytes were demonstrated in the probiotic-treated group. Furthermore, there have been numerous studies in the murine model showing that probiotics are effective in preventing influenza in those infant rodents fed a variety of lactic acid producing bacteria.²⁶⁻²⁸

The immune assays used in this study are similar to those currently being used in a large NIH-funded birth cohort (PI: Gern, Co-I: Seroogy) aimed at determining the impact of environmental exposures on immune maturation and protection from severe viral respiratory illnesses (Wisconsin Infant Study Cohort; WISC). Interim analysis from the WISC project (first 38 study subjects) demonstrates qualitative differences in the anti-viral responses of plasmacytoid dendritic cells (pDC)s in the cord blood of infants born into farming

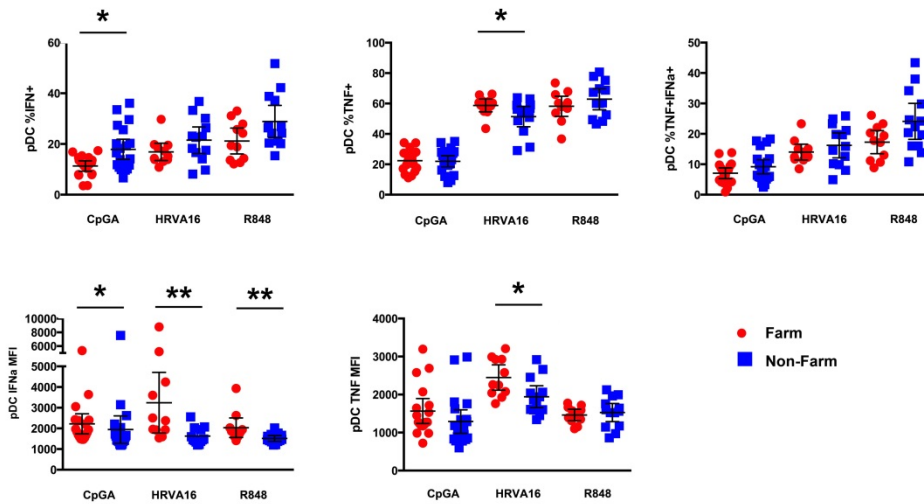
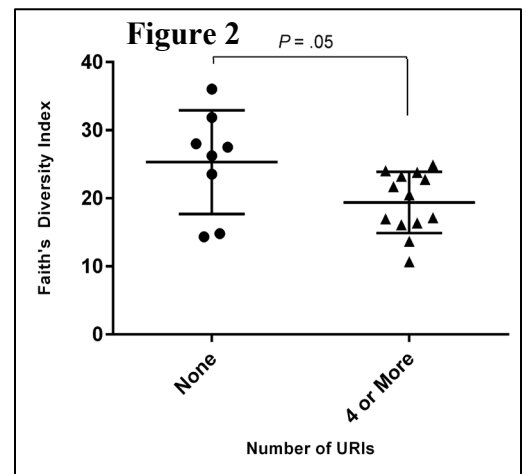


Figure 1: Toll-like receptor (TLR) responses to varied viral-associated TLRs and HRVA16 from WISC Study subjects. TLR9 (CpGA);TLR7/8 (R848). Mean plotted with 95%CI. CpGA Farm n=17, Non-Farm n=21; HRVA16 and R848 Farm n=11, Non-Farm n=13. * p ≤ 0.05; ** p ≤ 0.01. Non-parametric Mann-Whitney test. Top row is percent of pDC expressing cytokine. Second row is mean fluorescent intensity (MFI) of pDCs.

environments (manuscript in preparation) vs those born into non-farming environments (figure 1).

We are currently in the 4th year of a large NIH funded observational study (Wald –PI, DeMuri Co-I) to determine the viral and bacterial antecedents of acute bacterial sinusitis in children 4-7 years of age after onset of an URI. We have shown that there are significant differences in the composition of the nasopharyngeal (NP) microbiome in children with frequent URI vs those with no URIs. Figure 2 shows the differences in Faith’s Diversity Index, an indicator of a robust microbiome, in children with no URIs during the 1 year study period vs those with frequent URI. These data support the hypothesis that changes in the NP microbiome, specifically the loss of key immunomodulatory species that influence host responses to pathogens, are associated with the frequency of URI.



Our preliminary data indicate that changes in the microbial environment affect the frequency of URI and immunological function. This is conceptualized in figure 3. We hypothesize that supplementation with the combination probiotic will have similar effects on URI frequency and immunological function due to changes in the microbial environment. In the proposed pilot study we will test the hypothesis that probiotic supplementation will lead to an increase in the number and enhanced antiviral function of both pDCs and Treg cells.

This pilot study will provide essential preliminary data to guide the selection of immune markers to be measured in a larger prospective controlled trial of children receiving probiotic compared to those receiving placebo. Immune function markers that show a significant change after probiotic supplementation will be chosen in the controlled study as primary outcomes. For instance, if expression of TNF-alpha is higher in post-probiotic samples, this will be used as the major outcome in the larger trial.

Specific Aims/Study Objectives

The objective of this pilot study is to delineate the alterations in immune function in children who receive a combination probiotic (*L. acidophilus* NCFM and *B. lactis* Bi-07) supplement daily. Children ages >12-36 months will receive probiotic orally for 30 days. The qualitative and quantitative response of innate immune cells stimulated with various toll like receptor (TLR) agonists and human rhinovirus and quantification of T regulatory cells (Treg) will be determined in study subjects at initiation and conclusion of the study (baseline and at 30 days) using an optimized multi-parameter flow cytometry assay. **We hypothesize that probiotic supplementation will lead to an increase in the number and enhanced antiviral function of both plasmacytoid dendritic cells (pDC) and Treg cells.** We also hypothesize that probiotic supplementation will lead to changes in the nasopharyngeal microbiome.

Specific Aim 1: To determine the impact of probiotic supplementation on immune cell function through the assessment of quantitative responses of whole blood immune cells stimulated with various toll like receptor agonists .and

Specific Aim 2: To demonstrate the impact of probiotic supplementation on the nasopharyngeal (NP) and fecal microbiome.

The study duration is 1 year.

Research Design and Methods

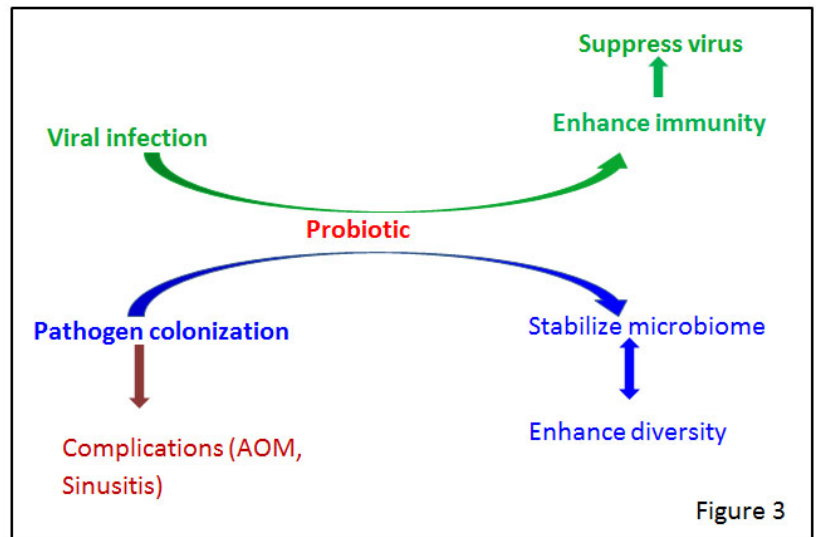
Eligibility and enrollment:

Inclusion Criteria

- Healthy children greater than 12 months-36 months of age
- English speaking parent
- Child has not received a live vaccine such as MMR or Varicella in the past 2 weeks

Exclusion Criteria

- Asthma/allergic rhinitis
- Premature birth (\leq 36 weeks gestation)
- Known to be lactose intolerant
- Immunodeficiency or any underlying problem requiring the use of steroids or other immunosuppressive agents
- Use of nonsteroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen in the last 2 weeks
- Currently taking probiotics, have received probiotics in the previous two weeks or are unwilling to refrain from the use of non-study probiotics during the next 30 days



- Any antibiotic treatment in the last 2 weeks
- Conditions which might interfere with dispersion of the probiotic after oral administration such as short gut or anomalies of the digestive tract
- Concurrently participating in another clinical study, in which the child has been or will be exposed to an investigational or a non-investigational product (pharmaceutical product or device).

Up to 40 patients total may be recruited to have a total of 30 analyzable patients. This includes a buffer of up to 10 patients to replace any that do not complete the study. Children will be enrolled from two large pediatric practices. Informed consent will be obtained from a parent or legal guardian.

This age group was chosen because of the high incidence of URI. Children of this age bear the highest burden of URI and its complications.

Health Link IT will be asked to run a bi-monthly list of potential subjects who are greater than 12 months-36 months of age and have a clinic appointment for a well-child clinic visit at 20 S Park Pediatrics or West Towne Pediatrics. A letter of introduction will be signed by the primary pediatrician and mailed to each family. The research nurse will put a reminder note on the door after the child is roomed at the clinic to remind the pediatrician that this is a potential subject for the study. The reminder note will have inclusion/exclusion criteria and a brief synopsis of study participation.

During the visit, the clinic staff or pediatrician will ask the family if they are interested in learning more about the study. If yes, the study nurse will be summoned at the end of the clinic visit to discuss the study with the family.

All children will receive a daily dose of probiotic (10^9 cfu of *L.acidophilus* NCFM and *B. lactis*, Bi-07), a commercially available probiotic. The product is stable at room temperature for 6 months. *L. acidophilus* NCFM and *B. lactis*-07 are classified by the FDA as a Generally Recognized as Safe (GRAS) food ingredient. This particular combination probiotic was chosen because it has shown efficacy in published clinical trials for URI in children and adults. Parents will give probiotic in 1 ounce of milk or water once a day for 30 days in an open label fashion.

A baseline blood sample (5-10 ml) will be obtained for immunologic studies (see below). If the child is having standard of care lab work done, the research staff will accompany the family to the lab with the research kit. Parents will be texted every week to determine if any illnesses have occurred in the interval and if the child has received any antimicrobials. In addition, a daily diary will be completed recording the presence of respiratory or gastrointestinal symptoms or signs and the administration of the investigational product. This will suffice to record illness events or any adverse events experienced by the participants.

Repeat blood testing for immunologic studies will be performed after the child has taken probiotic for at least 30 days. Compliance will be assessed by the study nurse at the final visit by counting the remaining sachets and reviewing the daily diary.

Note that each child will serve as his/her own control. The baseline sample will serve as the control sample. We have not included a placebo-control group at this time due to funding limitations in this exploratory pilot study. If our pilot data suggests a significant effect of probiotic, this study will be expanded to include a control group. We are aware that there may be changes in immune function in the 4 weeks between samples, however, this is the most efficient use of limited resources for this pilot investigation.

For the microbiome analysis NP swabs and stool samples will be obtained at study entry and at 30 days.

a) Visit 1. The table above shows procedures according to visit. At enrollment, a blood sample and nasopharyngeal swab and stool sample will be obtained. The following data will be collected: age, previous illnesses, immunization status, number of siblings (and their health status), attendance at daycare (defined as 3 or more children for at least 10 hours of contact time/week), parental age and health status, address and size of dwelling, pets in the home and dietary intake. Diaries will be distributed to parents to track administration of the probiotic. Probiotics will be given to the family with instructions on daily administration. A container and “toilet hat” (for toilet trained children) will be provided to parents to collect stool samples. The family will either return the stool sample to clinic or it will be picked up by study staff. If the parent does not collect the sample or the sample is not usable study staff will encourage the parent to re-collect. If this is not possible the patient will not be used in final analysis of stool microbiome.

c) Visit 2. We will review the medical history for any changes that may have occurred from the last visit. We will obtain a blood sample, a nasopharyngeal swab and stool sample. We will collect the used probiotic box. We will review the diary card and inquire about any symptoms the child may be experiencing.

d) Weekly Text Message

Parents will be texted every week to determine if any illnesses have occurred in the interval and if the child has received any antimicrobials. The text will contain a link to UW’s REDCap so that they can complete a short survey. Detailed instructions will be provided to the parent by study staff. In addition, a daily diary will be completed recording the presence of respiratory or gastrointestinal symptoms or signs or any other adverse events and the administration of the investigational product. Study nurses will review each weekly report on REDCap and will report adverse events to the investigators. These will be documented in REDCap and followed-up on by the study team. This will suffice to record illness events or any adverse events experienced by the participants.

Probiotics

L. acidophilus NFCM and *B. lactis-07* are classified by the US FDA as a Generally Recognized as Safe (GRAS) food ingredient. Furthermore, *B. lactis* and *L. acidophilus* have been present in human food for decades and are listed in the Inventory of Microorganisms with Documented History of Use in Human Food²⁹. European Food Safety Authority (EFSA) has also added these species to the Qualified Presumption of Safety list³⁰. No harmful or toxigenic activities are associated with *B. lactis* or *L. acidophilus*. Furthermore, Bi-07 and NFCM can be genetically identified on species and strain level^{31,32}.

An overview of the safety of probiotics was reported in 2011.³³ Although 622 studies were included in the review, only nonspecific safety statements were made in 235 studies; 387 reported the presence or absence of specific adverse events. The authors concluded that the available evidence in randomized controlled trials does not indicate an increased risk. The two probiotics to be used in this study (*L. acidophilus* NFCM and *B. lactis – 07*) have been used singly or in combination, in several published studies encompassing 399 adults and 222 children^{11,34,35} without any evidence of an increase in adverse events. In a trial by West and al, an exceptionally thorough safety and tolerability analysis was conducted for NFCM&Bi-07.³⁴ No participants selected for the safety analysis reported any clinically significant adverse events.

Some subjects taking probiotics may experience mild gastrointestinal symptoms like bloating that have disappeared once the use of the probiotic has been stopped.

There has been extensive experience with probiotics in this age group and even in younger children with an excellent safety record.^{2-15,36-42} In fact, probiotics are routinely added to commercial infant formulas (Gerber Good Start Protect Plus, Nutramagen with Enflora and others) and are marketed toward children of all ages.

Some subjects taking probiotics may experience mild gastrointestinal symptoms such as bloating that have disappeared once the use of the probiotic has been stopped. Overall adverse events are mild or non-existent. A recent evaluation of the safety of a probiotic in 6 month old infants found no adverse events when compared with placebo.⁴⁰

The study product is 1 daily sachet containing 5×10^9 *L. acidophilus* and 5×10^9 *B. lactis* provided as a white crystalline powder.

The study product will be supplied by DuPont™ Danisco® in individual sachets containing 5×10^9 *L. acidophilus* and 5×10^9 *B. lactis*. The contents of one sachet will be added to 1 ounce of milk or water each morning. The product will be packaged and labelled by Danisco and shipped to the research pharmacy of the University of Wisconsin where it will be stored in the refrigerator. A 36 supply will be given to each subject.

Subjects will ingest one sachet daily in 1 ounce of milk or water.

There is no randomization. All patients receive the study product. The research pharmacy and study coordinators will be responsible for reconciling the product.

Laboratory Testing

Blood samples: All subjects will undergo blood draws at Visits 1 and 2. The pediatric research staff will obtain the blood either at a clinic visit or a home visit. The tubes will be transported by the research staff to the UW Research Lab located in WIMR (Dr. Seroogy's Lab). Total blood volume will be between 5-10 mL following the maximum allowable amount in the AFCH guideline. The initial processing of blood samples will be done in Dr. Seroogy's lab. Cytokine analysis will be measured fee-for-service by RBM Myriad (Austin, TX).

Nasopharyngeal swabs: All subjects will have a nasopharyngeal swab obtained at Visits 1 and 2. The swabs will be placed into RNA Later medium and frozen within 7 days of collection in the pediatric -80C freezer located in H4/4. Nasopharyngeal swab samples for assessment of the nasopharyngeal microbiome will be collected at enrollment and at the follow-up visit. (Copan Diagnostics, Inc. Murrieta California). The soft strands of nylon on the swab result in tremendously improved patient comfort and efficiency in specimen collection. The velvet brush-like texture collects liquid by capillarity and rapidly and efficiently dislodges cells, providing an optimal approach to sampling for this study. The swab is inserted into the nostril along the floor of the nasal cavity until the posterior wall is reached. The swab is held in place for 5 seconds while rotating gently. Stool sample collection kits will be given to parents at the start of the study. Stools will be collected at study entry and after 30 days. Stool samples will be frozen at -80C until analysis.

Stool and NP samples will be batch shipped on dry ice to Dr. Lynch's laboratory at University of California-San Francisco for analysis. Dr. Lynch's lab will be performing a service of doing 16s sequencing for microbiome analysis. NP and stool samples will not be banked.

Data and Safety Monitoring Plan

Dr. Wald and Dr. DeMuri are board certified infectious disease pediatricians. Dr. Seroogy is board certified in pediatric allergy and immunology. All are experienced researchers. All will be available to the study staff and to families to address any concerns or unanticipated problems.

In this pilot study adverse events will be monitored by the study investigators and not an independent data and safety monitoring board. Based on published data, there are very minimal if any adverse events expected in children receiving probiotics.⁴⁰ In addition the number of subjects is small and will be monitored closely by the study team.

Parents will be texted every week to determine if any illnesses have occurred in the interval and if the child has received any antimicrobials. The text will contain a link to UW's REDCap so that they can complete a short survey. Detailed instructions will be provided to the parent by study staff. In addition, a daily diary will be completed recording the presence of respiratory or gastrointestinal symptoms or signs or any other adverse events and the administration of the investigational product. Study nurses will review each weekly entry to REDCap and will report adverse events to the investigators. These will be documented in REDCap and followed-up by the study team. This will suffice to record illness events or any adverse events experienced by the participants.

Any unexpected or unanticipated event will be reported to the IRB and DuPont per policy.

Specific Aim 1: To determine the impact of probiotic supplementation on immune cell function

Quantitative response of innate immune cells stimulated with a toll like receptor (TLR) agonist and other stimulants will be determined in study subjects at initiation and conclusion of the study (baseline–before probiotic and 30 days after the initiation of probiotic supplementation). Thus each subject will serve as their own control.

Method:

Immunoprofiling of antigen stimulated blood. The TruCulture system (Myriad RBM) will be used to assess immune function pre and post probiotic supplementation.⁴³ This is a syringe-based device designed for point of care use. The tubes contain 1 ml of cell culture medium which will include resiquimod R848 and possibly other stimulants. R848 is a synthetic agonist of TLR7 and TLR8. In addition a control (null) tube will contain only cell culture medium. One ml of whole blood will be mixed with medium in each tube and incubated at 37 C in a dry heat-block for 24-48 hours. After incubation, cells are separated by a valve separator component. The supernatant is stored at -20 C until analysis. The cellular component will be banked for future studies. Cytokine levels on supernatants will be sent to and measured by a commercial laboratory (RBM Myriad-Austin, TX). Measured cytokines will include GCSF, IL-2, IL-3, IL-5, IL-6, IL-7, IL-8, IL-10, MIP-1 alpha and beta, MCP-1, TNF-alpha, TNF-beta, IFN-alpha, IP-10 and others. Dr. Seroogy's lab will perform initial processing of blood specimens. Cytokine determination will be performed fee-for-service by RBM Myriad (Austin, TX).

Data analysis, interpretation, and expected outcome: The primary endpoint for this aim is determination of quantitative responses of whole blood leukocytes to varied agonists. We expect that probiotic exposure will be associated with enhanced production of IL-10, TNF α and IFN α . The changes found will be attributed to the probiotic intervention since precision testing using this assay by others has demonstrated good sample replication⁴³.

Specific Aim 2: To demonstrate the impact of probiotic supplementation on the NP and fecal microbiome.

The impact of probiotic consumption on the NP and fecal microbiota will be determined by assessing study subjects at initiation and conclusion of the study. We hypothesize that the administration of probiotic will be associated with an increase in the richness and diversity of the microbiota after 1 month of probiotic supplementation as measured by Shannon-Weiner and unweighted UniFrac 1 indexes.

Methods: NP and stool samples will be collected immediately and stored at -80 °C until analysis. Total bacterial DNA will be extracted from stool and NP samples as described.^{44,45} The 16S V1-V2 region will be amplified using primers containing sample-specific barcodes and sequenced. This analysis will be performed by the Lynch laboratory at UCSF.

Data analysis, interpretation, and expected outcome: Data analysis will be performed in QIIME with Operational Taxonomic Units (OTUs) chosen based upon 97% sequence similarity. Statistical differences between treatment arms and between samples collected pre- and post-administration of probiotic will be examined using the Monte-Carlo test in R with the Benjamini-Hochberg procedure applied to control the false discovery rate.⁴⁶ We hypothesize that the administration of probiotic will be associated with a significant increase in the richness and diversity of microbiota as measured by Shannon-Weiner and unweighted UniFrac 1 indexes.

Statistical Considerations (Aim 1 and 2): Endpoints: The primary outcome will include innate immune cell function parameters, adaptive and NK cell function parameters, and Treg cell number/function parameters.

Sample Size and Power Calculation: A sample size of 30 subjects is proposed for this pilot study. In our previous study involving intracellular cytokine and surface staining of PBMCs samples, the observed effect sizes for evaluating differences in the primary monocytes and mDCs parameters between various subgroups ranged from 0.52 to 1.68. The proposed sample size of 30 subjects is adequate for detecting anticipated moderate (0.5-0.8) to large (>0.8) effect sizes for the changes from the baseline to the one month follow-up assessment in the primary innate immune cell function parameters with sufficient power. The following table shows that attainable power levels for detecting moderate effect sizes (ES) ranging from 0.5 to 0.8 using a two-sided paired t-test with a false-discovery rate (FDR) of 0.05, assuming a sample size of 30 subjects with an attrition rate of 10% and a panel of 30 target parameters where the true mean differences (D) exceed the effect size in K=10 to 20 parameters.

Table: *Attainable power levels for detecting moderate to large effect sizes with proposed sample size of 30 subjects*

	Effect size for change in outcome parameters from baseline to the 3-month follow-up assessment											
	0.5			0.6			0.7			0.8		
K (#D>ES)	10	15	20	10	15	20	10	15	20	10	15	20
Power (%)	55	73	89	78	89	96	91	96	99	99	99	99

Hence, under various scenarios, a moderate effect size for the changes in the target parameters will be detected with 55-99% power while a large effect size will be detected >99% power. In summary, the proposed sample size of 30 subjects is sufficient for detecting differences in the primary target parameters which will provide preliminary data for conducting a larger validation study. It is unknown whether this effect size has clinical relevance at this time in terms of prevention of infection. We hope to test this in a larger controlled trial.

Statistical Analysis Plan: All outcome parameters will be summarized using standard descriptive statistics in terms of means, standard deviations, medians and ranges. Absolute and percentage changes from the baseline to the month 3 assessment will be calculated and evaluated using a paired t-test or nonparametric Wilcoxon signed rank test. The Benjamini-Hochberg method will be utilized to control the false discovery rate. Subgroup analyses will be conducted by compliance rates (at least 50%, 75% or 90% compliant). Pearson or nonparametric Spearman's rank correlation analyses will be conducted to evaluate associations of changes between outcome parameters. These analyses will be considered exploratory.

Data and Record Keeping

The master subject key will be maintained by the research staff and will be stored in an excel document on the Department of Pediatrics password-secure server. The code will be a unique number. The coded data will be maintained by the research staff and will be stored in a locked drawer in the research staff's locked office.

The excel file containing the data collection variables will be stored on the Department of Pediatrics password-

secured server. Only research staff will have access to this file. The file will contain the unique subject code but will not have any identifiers such as name, date of birth, or MRN#.

Specimens sent to UCSF will include a de-identified specimen number. Specimens sent back from UCSF will include microbiome profiles linked to the same de-identified specimen number. Specimens sent to RBM Myriad will include a de-identified specimen number. Specimens sent back from RBM Myriad will include cytokine profiles linked to the same de-identified specimen number.

An excel document containing name and MRN# will be maintained in a separate folder in the Department of Pediatrics password-secured server. This document will be used for the purpose of tracking when a potential patient is approached for the study.

The consent documents, questionnaires, and the subject screening eligibility documents will be stored in a locked drawer in the research staff's locked office. The questionnaires will be coded with the subject ID and will not have any identifiers.

The samples will be labeled with a de-identified specimen number. A log of the specimens obtained will be maintained in an excel document on the Department of Pediatrics password-secured server.

Banking Specimens for Future Use

The blood samples that are banked will be used for potential future immune system research. No genetic testing will be done.

The samples will be stored in Dr. Seroogy's lab located in WIMR and will be labeled with the deidentified sample number. Only her lab staff will have access to the samples. The master code will be maintained by the research staff stored electronically on the Dept of Pediatric's password protected server (separately from the data).

The data and samples will remain in a coded fashion as described above for up to 5 years. Data and samples may be withdrawn within the 5 year window by contacting the PI. After the 5 years, the data and samples will be anonymized and the master code will be destroyed/deleted.

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