A SCIENTIFIC TOOL SUPPORTED BY DANONE

Issue N°6
Oct 2011

PROBIOTICS WATCH

Contents

This probiotics watch is designed as a time-saving tool for scientists and clinicians interested in probiotic research. In an interactive format, the quarterly report provides timely, quasi-exhaustive lists of the scientific publications of the previous three months. It sorts them by topic and highlights some of the most relevant results. Readers can also check out upcoming scientific events and regular bibliometric analyses.

Objectivity is a strong commitment, that's why the articles are selected by an editorial committee, composed of renowned scientists in the field. Editorial committee members also comment on what they believe are the quarter's most relevant publications.
Last but not least each issue features an editorial by a probiotic expert, which offers special insight into this fascinating field of science.

EDITORIAL

PROBIOTICS AND IRRITABLE BOWEL SYNDROME: FROM EVIDENCE TO GUIDELINES

by **Professor Heiner Krammer**, *University Medical Center, Mannheim, Germany*

"Probiotics have been used on an empiric basis within the management of irritable bowel syndrome (IBS) for many years..."

> Read more

NOTEWORTHY PUBLICATIONS

- Evidence for molecular mechanisms involved in bifidobacteria adaptation to infant gut environment
- A *Bifidobacterium* locus shown to be an essential host-colonization factor
- Immunomodulation of human dendritic cells by a lactobacilli is dose-dependent
- A probiotic against urogenital infections in women
- Enteral supplementation of probiotics prevents enterocolitis in preterm infants: a result from a meta-analysis
- Lactobacilli intake during pregnancy prevents atopic eczema in children: a result from a meta-analysis
- LTA-deficient lactobacilli regulates colonic inflammation in mice
- Variations in the composition of infant gut microbiota in relation to age and to sensitization state
- Temporal and dynamic analysis of intestinal flora in healthy adults

Method

> **Monitoring period**: 01/03/2011 to 15/08/2011 > **Database**: Medline > **Result**: 609 publications

> **Keywords**: probiotic / lactic acid bacteria / streptococcus thermophilus / lactobacilli / fermented milk / bifidobacteria

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PROBIOTICS AND IRRITABLE BOWEL SYNDROME: FROM EVIDENCE TO GUIDELINES

by **Professor Heiner Krammer**
University Medical Center, Mannheim, Germany

Probiotics have been used on an empiric basis within the management of irritable bowel syndrome (IBS) for many years. As our understanding of the gastrointestinal microbiota has increased in recent years, scientists and clinicians have focused research endeavours on IBS pathogenesis and on the effects of different probiotic strains on the cardinal symptoms of this clinical disorder. Indeed there is now sufficient evidence supporting the beneficial effects of some probiotics, that a number of clinical practice guidelines see fit to include probiotics within management paradigms. Most recently, the 2011 German consensus guidelines on IBS recommend that selected probiotics can be used in the treatment of IBS, with the strain being selected according to the symptoms, and in the UK, where probiotics have been supported within National Institute for Health and Clinical Excellence (NICE) guidelines since 2008, the most recent, 2010 Map of Medicine treatment pathway, endorsed by the Royal College of Physicians, identified that specific strains such as *Bifidobacterium lactis* DN-173 010, *Bifidobacterium infantis* 35624 and the probiotic cocktail VSL#3 have shown clinical trial evidence for efficacy in controlling symptoms such as bloating distention and flatulence in IBS patients.[1-3]

As a high proportion of the abstracts selected for inclusion within this issue of Probiotics Watch highlight, there continues to be a great research focus on the role for probiotics in IBS, building our evidence-base and giving new insights into the science behind the disease. Scientists exploring the possible mechanisms by which probiotics may exert their effects on IBS symptomatology have developed a number of animal models of IBS in which to study the effects of different probiotic organisms. Johnson et al, for example[4], describe a model designed to mimic the inflammation and visceral hypersensitivity that characterizes IBS and contributes to symptoms of bloating and distension, which they have used to evaluate the effects of orally administered *Bifidobacterium infantis* 35624. Their study found that this probiotic strain significantly reduced and normalized hypersensitive responses to colonic distention in rats with laboratory-induced-colitis.

In order to understand how probiotic organisms effect changes in IBS pathophysiology, we need to know more about how gut microbiota differ between otherwise healthy subjects and patients with IBS, and how these differences impact on symptoms. In an elegant study involving deep molecular analysis of fecal microbiota using phylogenetic microarray and quantitative PCR, Rajilic-Stojanovic and co-workers identified that, compared with healthy subjects, patients with IBS have a 2-fold increase in the ratio of *Firmicutes: Bacteroidetes*, and significant reductions in the number of *Bacteroidetes, Bifidobacterium, Faecalibacterium* and methanogens (2 fold, 1.5 fold, 1.5 fold and 4 fold) respectively. The study suggested that the microbial groups of *Firmicutes* and *Proteobacteria* had a correlation with IBS symptom score.[5]

There are no fewer that 8 articles cited within this issue of Probiotics Watch that provide state-of-the-art reviews of current understanding and management approaches to IBS. Among these, Sainsbury and Ford highlight that approaches to improving IBS symptoms now go beyond the traditional attempts to change gut behaviour and transit using antispasmodic drugs and dietary fibre, to management approaches focused on greater understanding of IBS pathophysiology.[6] While the quest to find treatments that might alter the course of IBS continues in earnest, there is growing appreciation and recognition within the medical and scientific communities that both understanding and managing gut microbiota can affect IBS symptomatology.[7, 8]

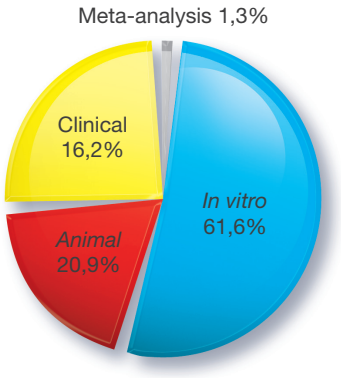
Clinical studies assessing probiotics in IBS have driven the acceptance of some probiotics within IBS treatment guidelines. The evidence-base continues to grow and help distinguish those probiotics strains with activity and effect in IBS. Current guidelines stress the need to differentiate between probiotic species or strains, and to choose probiotic products and mixtures according to strain and depending on the patient's needs and symptoms[1-3] . The abstract selection of this issue includes several clinical studies performed in IBS cohorts, including a randomized, placebo-controlled study of *Bifidobacterium bifidum* MIMBb75 which reported significant improvements in IBS symptoms and in patient-reported quality of life, and another study, with a less positive outcome, which found that effects of fermented milk containing *Lactobacillus paracasei ssp paracasei* F19, *Lactobacillus acidophilus* La5 and *Bifidobacterium lactis* Bb12 on IBS symptoms did not differ from the effects of acidified milk.[9,10] These and other studies published in recent months, contribute to our growing understanding of the nuances of how different probiotics may affect gut microbiota and impact on IBS symptoms, and exemplify the need for clinical decisions regarding the role for probiotics to be based on an established evidence per probiotic strain.

1. *Layer P, Andresen V, Pehl C, et al. [Irritable bowel syndrome: German consensus guidelines on definition, pathophysiology and management]. Z Gastroenterol 2011;49:237-93.*
2. *Map of Medicine Royal College of Physicians Accredited care map: Irritable bowel syndrome (IBS)Management (http://eng.mapofmedicine.com/evidence/map/irritable_bowel_syndrome_ibs_2.html)*
3. *National Institute for Health and Clinical Excellence (NICE). Irritable bowel syndrome: Diagnosis and management in primary care. Clinical guideline 61. London: NICE; 2008. (www.nice.org.uk/CG061)*
4. *Johnson AC, Greenwood-Van Meerveld B, McRorie J. Effects of Bifidobacterium infantis 35624 on Post-Inflammatory Visceral Hypersensitivity in the Rat. Dig Dis Sci. 2011 May 12.*
5. *Rajilic-Stojanovic M, Biagi E, Heilig HG, Kajander K, Kekkonen RA, Tims S, de Vos WM. Global and Deep Molecular Analysis of Microbiota Signatures in Fecal Samples from Patients with Irritable Bowel Syndrome. Gastroenterology. 2011 Aug 4.*
6. *Sainsbury A, Ford AC. Treatment of irritable bowel syndrome: beyond fiber and antispasmodic agents. Therap Adv Gastroenterol. 2011 Mar;4(2):115-27.*
7. *Suares NC, Ford AC. Diagnosis and treatment of irritable bowel syndrome. Discov Med. 2011 May;11(60):425-33.*
8. *Quigley EM. Therapies aimed at the gut microbiota and inflammation: antibiotics, prebiotics, probiotics, synbiotics, anti-inflammatory therapies. Gastroenterol Clin North Am. 2011 Mar;40(1):207-22.*
9. *Guglielmetti S, Mora D, Gschwender M, Popp K. Randomised clinical trial: Bifidobacterium bifidum MIMBb75 significantly alleviates irritable bowel syndrome and improves quality of life--a double-blind, placebo-controlled study. Aliment Pharmacol Ther. 2011 May;33(10):1123-32.*
10. *Søndergaard B, Olsson J, Ohlson K, Svensson U, Bytzer P, Ekesbo R. Effects of probiotic fermented milk on symptoms and intestinal flora in patients with irritable bowel syndrome: a randomized, placebo-controlled trial. Scand J Gastroenterol. 2011 Jun;46(6):663-72.*

THE MAIN POINTS OF THE QUARTER

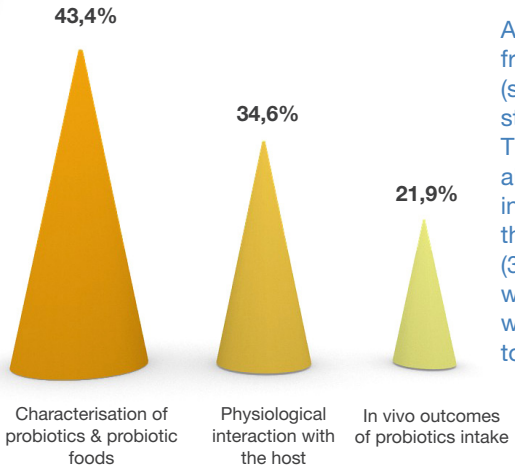
Contents

A 5-month period with many *in vitro* studies



609 publications were retrieved from Medline during this period, including 23.6% of review papers. This monitoring period (like the preceding ones) is characterised by the publication of a majority of *in vitro* studies.

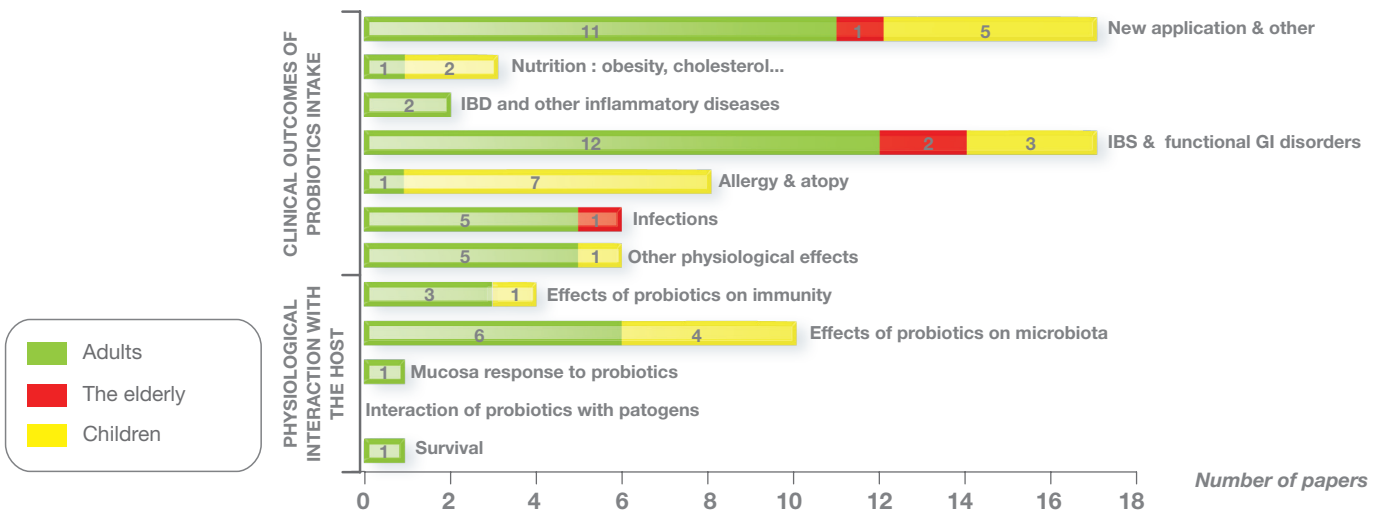
Nearly 50% of the original papers relate to the characterisation of probiotics



A significant proportion of the 465 original papers retrieved from Medline were related to probiotic characterisation (strain identification, metabolism and physiology of the strains; and technology of probiotics and probiotic foods). The papers dealing with *in vivo* and clinical effects (infections, allergy & atopy, IBS & functional GI disorders, IBD and other inflammatory diseases, nutrition, etc.) represent 21.9% of the total number of original papers. A third type of study (34.6%) assesses the physiological interaction of probiotics with the host (survival & physical interactions of probiotics with mucosa, interaction with pathogens, mucosa response to probiotics, effects on microbiota and immunity).

Probiotics in clinical trials are mainly assessed against GI disorders

A total of 75 clinical trials were published during this period; 48 of them were performed in adults, 23 in children, and 4 in elderly people. Intake of probiotics is mainly assayed for irritable bowel syndrome and other functional gastrointestinal disorders in adults. Studies on allergy and atopy are mainly carried out in children.



1. CHARACTERISATION OF PROBIOTICS AND PROBIOTIC FOODS

Contents

1.1. STRAIN METABOLISM AND PHYSIOLOGY

Evidence for molecular mechanisms involved in bifidobacteria adaptation to infant gut environment

OBJECTIVES

Bifidobacterium infantis is a common member of the infant intestinal microbiota, and it has been characterised by its foraging capacity for human milk oligosaccharides (HMO). Its genome sequence revealed an overabundance of Family 1 solute binding proteins (F1SBPs), which are part of the ABC transporters and are associated with the import of oligosaccharides. This work aimed to characterise *B. infantis* F1SBP oligosaccharide affinities, expression during growth on different prebiotics and interactions with epithelial cells in order to better understand the complex nature of oligosaccharide foraging by this bacteria.

METHODS & MEASURES

F1SBP was cloned and expressed. The relative levels of gene expression for each F1SBP gene under growth with different carbon sources were evaluated by quantitative PCR. The specific affinities of F1SBPs were determined by Mammalian Glycan Array.

RESULTS

Half of the F1SBPs in *B. infantis* were determined to bind mammalian oligosaccharides. Their affinities included different blood group structures and mucin oligosaccharides. Related to HMO, other proteins were specific for oligomers of lacto-N-biose and polylactosamines with different degrees of fucosylation. Growth on HMO induced the expression of specific binding proteins that import HMO isomers, but also bind blood group and mucin oligosaccharides, suggesting coregulated transport mechanisms. The prebiotic inulin induced other F1SBPs with affinity for intestinal glycans. Most of the host glycan F1SBPs in *B. infantis* do not have homologs in other bifidobacteria. Finally, some of these proteins were found to be adherent to human intestinal epithelial cells (Caco-2 cells) *in vitro.*

CONCLUSION

This study represents further evidence for the particular adaptations of *B. infantis* to the infant gut environment, and helps to understand the molecular mechanisms involved in this process. Defining the details of the intricate tripartite network between human milk, the developing infant intestinal epithelium and the intestinal microbiota is critical for understanding the biology of the microbial foundation process occurring in the first years of life in the infant gut, as well as for the improvement of infant formulas.

Garrido D, Kim JH, German JB, Raybould HE, Mills DA. Oligosaccharide binding proteins from Bifidobacterium longum subsp. infantis reveal a preference for host glycans. PLoS One. 2011 Mar 15;6(3):e17315.

COMMENTARY

from James Versalovic, Baylor College of Medicine, Houston, USA

"This report investigated the interactions of the probiotic *Bifidobacterium longum* subsp. *infantis* with host glycans such as human milk oligosaccharides (HMOs). The genome of *B. longum* subsp. *infantis* revealed a large number of genes encoding Family 1 of solute binding proteins (F1SBPs) and ABC transporters associated with the import of oligosaccharides. The Mammalian Gycan Array was used to determine the relative affinities of F1SBPs for mammalian oligosaccharide moieties. *B. longum* subsp. *infantis* were cultured with different carbohydrates as carbon sources, and four F1SBP genes were induced with HMOs. Different F1SBP genes were induced by commercial prebiotics, and F1SBPs were shown to faciliate interactions and binding with human intestinal epithelial cells. These findings suggest that probiotics can express oligosaccharide-binding proteins depending on the presence of prebiotics or dietary components. Probiotics may be able to bind and metabolize different carbohydrates, and the coupling of probiotics and prebiotics (including human milk components) may help to determine the adherence and sustainability of colonization by probiotics."

> The studies performed in humans are highlighted using the following symbols :

for the **GENERAL POPULATION**

for **CHILDREN**

for the **ELDERLY**

In **VITRO/ANIMALS** studies are highlighted with the symbol :

1. CHARACTERISATION OF PROBIOTICS AND PROBIOTIC FOODS

Contents

Functional probiotic features predicted by metabolic modelling and transcriptomics

OBJECTIVES

The genomes of four *Lactobacillus reuteri* strains isolated from human breast milk and the gastrointestinal tract have been recently sequenced. To explain possible mechanisms of survival in the host a study completed a detailed genomic comparison of two breast milk-derived isolates: an established probiotic strain (*L. reuteri* ATCC 55730) and a strain with promising probiotic features (*L. reuteri* ATCC PTA 6475).

METHODS & MEASURES

Transcriptomes of *L. reuteri* strains in different growth phases were monitored using strain-specific microarrays, and compared using a pan-metabolic model representing all known metabolic reactions present in these strains.

RESULTS

Both strains contained candidate genes involved in survival and persistence in the gut, such as mucus-binding proteins and enzymes scavenging reactive oxygen species. A large operon predicted to encode the synthesis of an exopolysaccharide was identified in strain 55730. Both strains were predicted to produce health-promoting factors, including antimicrobial agents and vitamins. Additionally, a complete pathway for thiamine biosynthesis was predicted in strain 55730 for the first time in this species. Candidate genes responsible for the immunomodulatory properties of each strain were identified by transcriptomic comparisons.

CONCLUSION

The production of bioactive metabolites by human-derived probiotics may be predicted using metabolic modelling and transcriptomics. Such strategies may facilitate the selection and optimization of probiotics for health promotion, disease prevention and amelioration.

Saulnier DM, Santos F, Roos S, Mistretta TA, Spinler JK, Molenaar D, Teusink B, Versalovic J. Exploring metabolic pathway reconstruction and genome-wide expression profiling in Lactobacillus reuteri to define functional probiotic features. PLoS One. 2011 Apr 29;6(4):e18783.

Contents

2.1. SURVIVAL & PHYSICAL INTERACTIONS OF PROBIOTICS WITH MUCOSA

A Bifidobacterium locus shown to be an essential host-colonization factor

OBJECTIVES

Development of the human gut microbiota commences at birth, with bifidobacteria being among the first colonizers of the sterile new-born gastrointestinal tract. To date, the genetic basis of *Bifidobacterium* colonization and persistence remains poorly understood. The objective of this work was to understand bifidobacterial genomics and elucidate key determinants of *Bifidobacterium* – host interactions in the gastrointestinal tract by determining the complete genome sequence of *B. breve* UCC2003, a nursing stool isolate.

METHODS & MEASURES

The complete genome sequence of *B. breve* UCC 2003 was determined. To investigate genome variability of *B. breve* UCC 2003, comparative genome hybridization analysis of 18 *B. breve* strains was done. To determine which *B. breve* UCC 2003 genes are differentially transcribed in the murine GIT and persist, a *B. breve* UCC 2003 transcriptome in a murine colonization model was performed.

RESULTS

Transcriptome analysis of the *B. breve* UCC 2003 2.42-Mb genome in a murine colonization model revealed differential expression of a type IVb tight adherence (Tad) pilus-encoding gene cluster designated tad_{2003}. Mutational analysis demonstrated that the tad_{2003} gene cluster is essential for efficient *in vivo* murine gut colonization, and immunogold transmission electron microscopy confirmed the presence of Tad pili at the poles of *B. breve* UCC2003 cells.

CONCLUSION

Analysis of *B. breve* genome sequence coupled with *in vivo* transcriptome studies identified a *tad* locus essential for efficient host colonization of the murine intestine. Conservation of the Tad pilus-encoding locus among other *B. breve* strains and among sequenced *Bifidobacterium* genomes supports the notion of a ubiquitous pili-mediated host colonization and persistence mechanism for bifidobacteria.

O'Connell Motherway M, Zomer A, Leahy SC, Reunanen J, Bottacini F, Claesson MJ, O'Brien F, Flynn K, Casey PG, Moreno Munoz JA, Kearney B, Houston AM, O'Mahony C, Higgins DG, Shanahan F, Palva A, de Vos WM, Fitzgerald GF, Ventura M, O'Toole PW, van Sinderen D. Functional genome analysis of Bifidobacterium breve UCC2003 reveals type IVb tight adherence (Tad) pili as an essential and conserved host-colonization factor. Proc Natl Acad Sci U S A. 2011; 108(27):11217-11222.

COMMENTARY

from Bruno Pot, Institut Pasteur de Lille, France

"This article in PNAS is interesting, both for the results obtained and for its research approach. Based on a complete genome sequence of the *B. breve* UCC2003 strain, comparative genome hybridization experiments were performed with 18 additional *B. breve* strains, using an array designed on 1,864 of the 1,985 annotated genes. Interestingly 1,393 genes were shown to be present in all *B. breve* strains tested, supporting the notion of a closed pangenome architecture for *B. breve.* In a second series of experiments the *in vivo* transcriptome (total bacterial RNA), isolated from ceca of Balb/c mice that had been stably colonized with the strain, was compared with that of an exponential-phase culture in MRS medium, supplemented with glucose. The 105 genes significantly up-regulated suggested specific adaptations of the strain to the mice gut, while down-regulated genes primarily encoded proteins for replication, transcription, and translation, suggesting that the strain divides at a slower pace in the murine GIT compared to a rich medium.

In a further functional analysis, the up-regulation of the tad_{2003} genes measured by the microarray experiments were confirmed by quantitative RT-PCR and the $tadA_{2003}$ gene (responsible for Tad pilus assembly) was knocked out. This mutant, together with immunogold transmission electron microscopy experiments, allowed the authors to demonstrate that the tad_{2003} gene cluster is essential for efficient *in vivo* murine gut colonization. Conservation of the Tad pilus-encoding locus among other *B. breve* strains and among other bifidobacteria, supports the notion of an ubiquitous pili-mediated host colonization and persistence mechanism for bifidobacteria, as described earlier for LGG (albeit mediated by sortase-dependent pili). These results not only provided a significant step towards understanding the molecular details of the interaction between bifidobacteria and their host, it also offers a nice working model for analogous studies in the search of functionality mechanisms of probiotic strains."

Contents

2.2. MUCOSA RESPONSE TO PROBIOTICS (TROPHICITY & STRUCTURE)

Probiotic-derived polyphosphate enhances intestinal barrier in mice

OBJECTIVES

It is highly probable that some common molecules secreted by probiotic and/or commensal bacteria contribute to the maintenance of intestinal homeostasis and protect the intestinal epithelium from injurious stimuli. To address this question, this study aimed to isolate the cytoprotective compound from *Lactobacillus brevis* SBC8803 which possess the ability to induce cytoprotective heat shock proteins (HSP) in mouse small intestine.

METHODS & MEASURES

Human colonic epithelial Caco2/BBE cells were treated with the culture supernatant of *L. brevis* SBC8803, and HSP27 expression was evaluated. HSP27-inducible components were separated. Finally, the HSP27-inducible fraction was identified as polyphosphate, a simple repeated structure of phosphates, which is a common product of lactobacilli and other bacteria associated with intestinal flora without any definitive physiological functions. Then, polyphosphate was synthesized and assessed *ex vivo* in mice intestinal loops and in DSS-administered mice (a model for colitis).

RESULTS

The synthesized polyphosphate significantly induced HSP27 from Caco2/BBE cells. In addition, polyphosphate suppressed the oxidant-induced intestinal permeability *ex vivo* in the mouse small intestine and pharmacological inhibitors of p38 MAPK and integrins counteract its protective effect. Daily intrarectal administration of polyphosphate improved the inflammation grade and survival rate in DSS-administered mice.

CONCLUSION

This study demonstrated, for the first time, that polyphosphate - a common product of lactobacilli and other bacteria associated with intestinal flora - is responsible for probiotic actions that protect the intestinal epithelia from oxidant stress and improve epithelial injury due to excess inflammation. This protective action on the intestinal barrier is mediated through the intestinal integrin-p38 MAPK.

Segawa S, Fujiya M, Konishi H, Ueno N, Kobayashi N, Shigyo T, Kohgo Y. Probiotic-Derived Polyphosphate Enhances the Epithelial Barrier Function and Maintains Intestinal Homeostasis through Integrin-p38 MAPK Pathway. PLoS One. 2011;6(8):e23278.

Contents

2.3. EFFECTS OF PROBIOTIC ON IMMUNITY (MUCOSAL & SYSTEMIC)

Immunomodulation of human dendritic cells by a lactobacilli is dose-dependent

OBJECTIVES

The response of the immune system to probiotics remains controversial. Some strains modulate the cytokine production of dendritic cells (DCs) *in vitro* and induce a regulatory response, while others conversely induce a pro-inflammatory response. These strain-dependent effects are thought to be linked to specific interactions between bacteria and pattern recognition receptors. This study investigated the effects of *Lactobacillus rhamnosus* Lcr35on human monocyte-derived immature dendritic cells (DCs).

METHODS & MEASURES

A variety of techniques, from global gene transcription profile to expression of membrane and soluble protein analysis, were used to characterise the DCs responses after contact with a wide range of probiotic concentrations, MOI (multiplicity of infection) from 0.01 to 100.

RESULTS

DNA microarray and qRT-PCR analysis showed that the probiotic induced a large-scale change in gene expression (nearly 1,700 modulated genes, with 3-fold changes), but only with high doses (MOI, 100). The upregulated genes were mainly involved in immune response and identified a molecular signature of inflammation. Flow cytometry analysis also revealed a dose-dependent maturation of the DCs membrane phenotype, until DCs reached a semi-mature state, with an upregulation of the membrane expression of CD86, CD83, HLA-DR and TLR4, associated with a down-regulation of DC-SIGN, MR and CD14. Measurement of the DC-secreted cytokines showed that the probiotic induced a strong dose-dependent increase of the pro-Th1/Th17 cytokine levels (TNFα, IL-1β, IL-12p70, IL-12p40 and IL-23), but only a low increase in IL-10 concentration.

CONCLUSION

The probiotic *L. rhamnosus* Lcr35 induces a dose-dependent immunomodulation of human DCs leading, at high doses, to the semi-maturation of the cells and to a strong pro-inflammatory effect. These results contribute to a fuller understanding of the mechanism of action of this probiotic, and open up broader prospects regarding the clinical indications in which this probiotic could be used to strengthen the immune defences.

Evrard B, Coudeyras S, Dosgilbert A, Charbonnel N, Alamé J, Tridon A, Forestier C. Dose-dependent immunomodulation of human dendritic cells by the probiotic Lactobacillus rhamnosus Lcr35. PLoS One. 2011 Apr 18;6(4):e18735.

COMMENTARY

from Bruno Pot, Institut Pasteur de Lille, France

"Things change quickly as we get a better understanding of the immune system's responses towards selected probiotic strains. The role of dendritic cells (DCs) for the induction of regulatory responses has been well documented and seems to be strain-specific. In this PLoS One paper, dose effects of the strain *Lb.rhamnosus* Lcr35 on human monocyte-derived immature DCs were investigated using DNA microarray and qRT-PCR analyses. Results showed that, at high doses (MOI, 100), the strain induced a large-scale change in gene expression (close to 1,700 genes with at least 3-fold change) as well as a dose-dependent maturation of the DC membrane phenotype. Maturation was towards a semi-mature state, characterized by the upregulation of CD86, CD83, HLA-DR and TLR4 expression, the down-regulation of DC-SIGN, MR and CD14, as measured by flow cytometry, as well as strong pro-inflammatory signals (increase of Th1/Th17 cytokine levels). Recent murine work showed that Lcr35 has potential to prevent asthma, as previous oral treatment with this strain prior to sensitization attenuated airway inflammation and hyperreactivity. As high concentrations of *Lb. rhamnosus* Lcr35 induced only very low increases in levels of IL-10, and did not modulate the gene expression of TGF betanor of enzymes involved in retinoic acid synthesis, it is unlikely that it can induce regulatory T-cells, even at this higher dose. Therefore it was hypothesized that possible modulation of the Th1/Th2 balance towards the Th1 (/Th17) response in DCs could counterbalance the impaired Th2-skewed cytokine profile observed in IgE-dependent allergies, creating prospects for anti-allergic activity. Clearly this needs to be confirmed in future clinical investigations."

Contents

A strain of *Lactobacillus casei* acts at the effector phase of immune inflammation

OBJECTIVES

Some probiotics were found to have protective effects in mouse models of allergic and autoimmune diseases. This study investigated whether probiotics might affect allergic and autoimmune inflammation by acting at the effector phase of adaptive immune responses.

METHODS & MEASURES

The effects of *Lactobacillus casei* were investigated: 1) *in vivo* on IgE-induced passive systemic anaphylaxis (model for acute allergic inflammation) and on IgG-induced passive arthritis (model of autoimmune inflammation) which bypass the induction phase of immune responses; 2) *in vitro* on IgE- and IgG-induced mouse mast cell activation, and 3) *ex vivo* on IgE-dependent human basophil activation.

RESULTS

L. casei protected from anaphylaxis and arthritis, and inhibited mouse mast cell and human basophil activation. Inhibition required contact between mast cells and bacteria, was reversible, and selectively affected the Lyn/Syk/linker for activation of T cells pathway induced on engagement of IgE receptors, leading to decreased MAPK activation, Ca2+ mobilization, degranulation, and cytokine secretion. Also, adoptive anaphylaxis induced on antigen challenge in mice injected with IgE-sensitized mast cells was stopped in mice injected with IgE-sensitized mast cells exposed to bacteria.

CONCLUSION

These results demonstrate that *L. casei* can influence the effector phase of adaptive immunity in allergic and autoimmune diseases. Based on these results, one can extrapolate that some probiotics may prevent symptoms in allergic or autoimmune patients who have already produced IgE anti-allergen or IgG autoantibodies.

Schiffer C, Lalanne AI, Cassard L, Mancardi DA, Malbec O, Bruhns P, Dif F, Daëron M. A Strain of Lactobacillus casei Inhibits the Effector Phase of Immune Inflammation. J Immunol. 2011 Sep 1;187(5):2646-55.

Contents

3.1. INFECTIONS (GI, RESPIRATORY INCL. FLU, GENITO-URINARY, OTHER)

A probiotic against urogenital infections in women

OBJECTIVES / BACKGROUND

Urinary tract infections (UTIs) are common among women and frequently recur. Depletion of vaginal lactobacilli is associated with UTI risk, which suggests that repletion may be beneficial. A clinical trial was conducted to verify this hypothesis using Lactin-V, an intravaginal suppository containing *Lactobacillus crispatus.*

DESIGN

Double-blind placebo-controlled trial.

SETTINGS & PARTICIPANTS

One hundred young women (18-31 years) with a history of recurrent UTI received antimicrobials for acute UTI and then were randomized to receive either Lactin-V or placebo daily for 5 days, then once weekly for 10 weeks. Participants were followed up at 1 week and 10 weeks after intervention and for UTIs. Urine samples for culture and vaginal swabs for real-time quantitative 16S ribosomal RNA gene polymerase chain reaction for *L. crispatus* were collected.

MAIN OUTCOME

The primary objective was to evaluate the ability of Lactin-V to reduce the incidence of cystitis and to produce high-level vaginal colonization with *L. crispatus* after 10 weeks. Secondary objectives were to evaluate patterns of vaginal colonization with *L. crispatus.*

RESULTS

Recurrent UTI occurred in 15% of women receiving Lactin-V compared with 27% of women receiving placebo (RR 0.5; 95% CI, 0.2-1.2). High-level vaginal colonization with *L. crispatus* throughout follow-up was associated with a significant reduction in recurrent UTI only for Lactin-V (RR 0.07 for Lactin-V; RR 1.1 for placebo; P<0.01).

CONCLUSION

Lactin-V treatment in women with recurrent UTI resulted in robust and prolonged colonization with *L. crispatus*, with a trend of reducing the incidence of UTI by 50%. The protective effects of Lactin-V were even greater in women who achieved the most robust colonization with *L.crispatus* and reflect an apparent advantage for Lactin-V treatment over the natural recovery of vaginal microbiota after an episode of UTI.

Stapleton AE, Au-Yeung M, Hooton TM, Fredricks DN, Roberts PL, Czaja CA, Yarova-Yarovaya Y, Fiedler T, Cox M, Stamm WE. Randomized, placebo-controlled phase 2 trial of a Lactobacillus crispatus probiotic given intravaginally for prevention of recurrent urinary tract infection. Clin Infect Dis. 2011 May;52(10):1212-7.

COMMENTARY

from James Versalovic, Baylor College of Medicine, Houston, USA

"This report highlights the application of a vaginal probiotic *Lactobacillus crispatus* strain for the prevention of recurrent urinary tract infections in women. The formulation, Lactin-V (Osel), includes a single, hydrogen peroxide-producing *L. crispatus* strain CTV-05 delivered as an intravaginal suppository daily for 5 days, then weekly for 10 weeks. Premenopausal women (18-40 yo) with a median age of 21 were enrolled, and they received either placebo or Lactin-V. Recurrent UTIs were documented in 7/48 (15%) of women receiving Lactin-V and 13/48 (27%) of women receiving placebo. The results translated into a relative risk of 0.5 for women in the Lactin-V group. Women who demonstrated enhanced colonization by *L. crispatus* after Lactin-V administration (presumably enriched for CTV-05 in the vagina) had a significant reduction in the risk of UTI. Interestingly, enhanced vaginal colonization by indigenous strains of *L. crispatus* did not affect the recurrence risk. Presumably the effect of this probiotic on risk of recurrent UTI is strain-specific. This trial suggests that the protective effect of probiotics may be similar to antimicrobial prophylaxis strategies. Future studies should include direct comparisons of probiotics versus antibiotics in clinical trials and larger, randomized, double-blind, placebo-controlled clinical trials with specific probiotic strains."

Contents

Enteral supplementation of probiotics prevents enterocolitis in preterm infants: a result from a meta-analysis

OBJECTIVES / BACKGROUND

An update review was conducted to compare the efficacy and safety of prophylactic enteral probiotics administration versus placebo or no treatment in the prevention of severe necrotizing enterocolitis (NEC) and/or sepsis in preterm infants.

DATA COLLECTION AND ANALYSIS

Data search was made on MEDLINE (1966 to October 2010), EMBASE (1980 to October 2010), the Cochrane Central Register of Controlled Trials (CENTRAL, The Cochrane Library, Issue 2, 2010), and abstracts of annual meetings of the Society for Paediatric Research (1995 to 2010). Only were considered randomized or quasi-randomized controlled trials that enrolled preterm infants inferior to 37 weeks gestational age and/or less than 2500 g birth weight. Trials were included if they involved enteral administration of any live microbial supplement and measured at least one pre-specified clinical outcome. Standard methods of the Cochrane Collaboration and its Neonatal Group were used to assess the methodological quality of the trials, data collection and analysis.

MAIN OUTCOME

The primary outcomes were severe NEC (stage II or more) as per Bell's criteria, and nosocomial sepsis, defined as positive blood or cerebrospinal fluid cultures taken beyond 5 days of age.

RESULTS

Sixteen eligible trials randomizing 2842 infants were included. Included trials were highly variable with regard to birth weight and gestational age, baseline risk of NEC in the control groups, timing, dose, formulation of the probiotics, and feeding regimens. Data regarding extremely low birth weight infants could not be extrapolated. In a meta-analysis of trial data, enteral probiotics supplementation significantly reduced the incidence of severe NEC (typical RR 0.35, 95% CI 0.24 to 0.52) and mortality (typical RR 0.40, 95% CI 0.27 to 0.60). There was no evidence of significant reduction of nosocomial sepsis (typical RR 0.90, 95% CI 0.76 to 1.07). The included trials reported no systemic infection with the probiotics supplemental organism. The statistical test of heterogeneity for NEC, mortality and sepsis was insignificant.

CONCLUSION

Enteral supplementation of probiotics prevents severe NEC and all mortality causes in preterm infants. According to the authors, this updated review supports a change in practice. However, more studies are needed to assess efficacy in extremely low birth weight infants and assess the most effective formulation and dose to be utilised.

Alfaleh K, Anabrees J, Bassler D, Al-Kharfi T. Probiotics for prevention of necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev. 2011 Mar 16;(3):CD005496.

COMMENTARY

from Hania Szajewska, The Medical University of Warsaw, Poland

"A number of systematic reviews, with or without a meta-analysis, have reviewed data on the effects of the enteral administration of probiotics on the risks of necrotizing enterocolitis (NEC) and mortality in preterm infants.[1 2 3 4] Overall, probiotics (as a class) prevent NEC. Not surprisingly, the conclusions of the most recent meta-analysis by *Alfaleh et al.*, which is an update of the previously published Cochrane review,[2] confirm earlier findings. Nevertheless, despite this evidence, whether probiotic supplementation should become the standard of care is under discussion.

Recently, the Committee on Nutrition of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition concluded that the presently available data does not allow the recommendation of the routine use of prebiotics or probiotics as food supplements in preterm infants to prevent NEC. The Committee also recommended that each probiotic strain and potential combinations need to be characterized separately for each product.[5] Similarly, the American Academy of Pediatrics (AAP)[6] recently stated that there is some evidence to support the use of probiotics to prevent NEC in preterm infants with a birth weight of at least 1000 g. However, the amount and specificity of which probiotic or mixture of probiotics to use is problematic. The AAP also noted that many of the probiotics used in clinical studies are not readily available.

It is worth noting that these statements, from both sides of the Atlantic, do not mean that the use of probiotics for preventing NEC should be totally abandonned. Rather that, in settings in which the incidence of NEC is high, one may consider the use of probiotics – single strains or a combination. However, care should be taken to choose those that are best studied, with the highest effect size, and the best safety profile."

[1] *Deshpande G, Rao S, Patole S. Probiotics for prevention of necrotising enterocolitis in preterm neonates with very low birthweight: a systematic review of randomised controlled trials. Lancet 2007;369:1614-20.*
[2] *Alfaleh K, Bassler D. Probiotics for prevention of necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev. 2008;(1):CD005496.*
[3] *Barclay AR, Stenson B, Simpson JH, et al. Probiotics for necrotizing enterocolitis: a systematic review. J Pediatr Gastroenterol Nutr 2007;45:569-76.*
[4] *Deshpande G, Rao S, Patole S, et al. Updated meta-analysis of probiotics for preventing necrotizing enterocolitis in preterm neonates. Pediatrics 2010;125:921-30.*
[5] *ESPGHAN Committee on Nutrition. Agostoni C, Buonocore G, Carnielli VP, et al. Enteral nutrient supply for preterm infants: commentary from the European Society of Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition. J Pediatr Gastroenterol Nutr 2010;50:85-91.*
[6] *Thomas DW, Greer FR; American Academy of Pediatrics Committee on Nutrition; American Academy of Pediatrics Section on Gastroenterology, Hepatology, and Nutrition. Probiotics and prebiotics in pediatrics. Pediatrics 2010;126:1217-31.*

Contents

A meta-analysis highlights the effectiveness of probiotics against AAD and CDAD in adults

OBJECTIVES / BACKGROUND

The purpose of this article is to report findings from a meta-analysis of available studies on adult hospitalized populations to evaluate efficacy of probiotics for the prevention of antibiotic-associated diarrhoea (AAD) and *Clostridium difficile*-associated disease (CDAD).

DESIGN

Meta-analysis.

SETTINGS & PARTICIPANTS

A search was carried out for all available randomized controlled trials evaluating the effectiveness of probiotics for the prevention of AAD and CDA. Only studies conducted after 1990 on adults in hospital-based populations were included in this review.The literature search yielded 37 abstracts; 27 of those abstracts were excluded because the studies were not conducted on hospitalized adults; 1 was excluded because the use of antibiotics was not clearly defined and another one because the full article was not published in English. A total of 8 studies met the criteria and were included in the meta-analysis; the total number of participants across all studies was 1220.

MAIN OUTCOME

Outcome measurements recorded were the incidence of diarrhoea and the presence of CDAD-positive stools.

RESULTS

AAD affects one in five people on antibiotics. Risk factors for the development of AAD include the use of broad-spectrum antibiotics and host factors such as age, health status, hospitalization status, and exposure to nosocomial pathogens. About a third of AAD cases have CDAD. Meta-analysis showed that administration of probiotics led to a statistically significant relative risk reduction of 44% (RR=0.56; 95% CI 0.44-0.71) for AAD and 71% (RR=0.29; 95%CI 0.18-0.46) for CDAD.

CONCLUSION

This meta-analysis supports that probiotics are efficacious in preventing AAD and CDAD among hospitalized adults. Thus, reducing the occurrence of AAD and CDAD by administering probiotics concurrently with antibiotics could be a relevant new strategy for prophylaxis.

Avadhani A, Miley H. Probiotics for prevention of antibiotic-associated diarrhea and Clostridium difficile-associated disease in hospitalized adults-A meta-analysis. J Am Acad Nurse Pract. 2011 Jun;23(6):269-74.

Contents

3.2. ALLERGY & ATOPY

Lactobacilli intake during pregnancy prevents atopic eczema in children: a result from a meta-analysis

OBJECTIVES / BACKGROUND

A meta-analysis was conducted to compare the development of atopic eczema in children whose mothers took probiotics during pregnancy and lactation.

SETTINGS & PARTICIPANTS

A systematic database research for randomized, controlled studies on probiotic administration during pregnancy and the risk of atopic eczema within the first years of life was conducted. A total of 7 randomized, double-blinded, placebo-controlled trials, published between 2001 and 2009 were included in the meta-analysis. These studies observed 2843 children whose mothers took probiotics or placebo during pregnancy and lactation. Of those studies, 4 used lactobacilli as probiotics, 3 used a mixture of various bacterial strains (including lactobacilli) and 1 included bifidobacteria.

MAIN OUTCOME

All studies that were included used atopic eczema as an endpoint.

RESULTS

The completed meta-analysis of the 7 studies shows a significant risk reduction for atopic eczema in children aged 2-7 years by the administration of probiotics during pregnancy and lactation (reduction 5.7%; P=0.022). However, this effect was only significant for lactobacilli (reduction 10.6 %; P=0.045), but not for a mixture of various bacterial strains as probiotics (difference 3.06 %, P=0.204).

CONCLUSION

The meta-analysis shows that the administration of lactobacilli to mothers during pregnancy prevents atopic eczema in children. However, a mixture of various bacterial strains does not affect the development of atopic eczema, independent of whether they contain lactobacilli or not.

Doege K, Grajecki D, Zyriax BC, Detinkina E, Zu Eulenburg C, Buhling KJ. Impact of maternal supplementation with probiotics during pregnancy on atopic eczema in childhood - a meta-analysis. Br J Nutr. 2011 Jul 26:1-6.

COMMENTARY

from Hania Szajewska, The Medical University of Warsaw, Poland

"Evidence suggests that gut microbiota play a critical role in the development of immune tolerance and contribute to the prevention of allergic disease. Firstly, it has been suggested that improved hygiene and the reduced exposure of the immune system to microbial stimulus early in childhood has contributed to the rising number of allergic disorders worldwide.[1] Secondly, there are differences in the neonatal gut microflora that may precede or coincide with the early development of atopy. Atopic subjects have more clostridia and tend to have fewer bifidobacteria than non-atopic subjects.[2] If so, the concept of manipulating the gut microbiota through the administration of probiotics during early life in order to prevent disease is appealing.

A number of recent meta-analyses have suggested that probiotics are effective in preventing eczema, particularly if the probiotics are administered both pre- and postnatally.[3 4 5] However, one major limitation of the previously published meta-analyses is that all of them pooled data obtained from different probiotic strains, with no analyses based on individual probiotic strain(s). It is well accepted that all probiotics are not created equal.

A recent meta-analysis by Doege et al. differs from those previously published. While it presents pooled data, it also presents data from studies that used mixtures of probiotics separately from those that only used lactobacilli. It is worth noting that only the lactobacilli proved to be effective. However, different lactobacilli were still pooled together, calling for caution when interpreting the pooled effect. While the title of the paper suggests that the probiotics were administered only during pregnancy, probiotics were administered both pre- and postnatally in all included studies. In contrast, a recent study suggests that exclusive prenatal supplementation is not effective.[6]

Should the results of this meta-analysis alter our practices? According to recommendations by the American Academy of Pediatrics[7], 'the results of some studies support the prophylactic use of probiotics during pregnancy and lactation and during the first 6 months of life in infants who are at risk of atopic disorders. However, further confirmatory evidence is necessary before a recommendation for routine use can be made'. This recommendation is hard to contest. There is a particular need to determine which microorganisms are suitable for use and in which types of population."

[1] *Prescot SL. Allergy: the price we pay for cleaner living? Ann Allergy Asthma Immunol 2003;90:64-70.*
[2] *Kalliomaki M, Kirjavainen P, Eerola E, et al. Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. J Allergy Clin Immunol 2001;107:12934.*
[3] *Osborn DA, Sinn JK. Probiotics in infants for prevention of allergic disease and food hypersensitivity. Cochrane Database of Systematic Reviews 2007, Issue 4. Art. No.: CD006475.*
[4] *Lee J, Seto D, Bielory L. Meta-analysis of clinical trials of probiotics for prevention and treatment of pediatric atopic dermatitis. J Allergy Clin Immunol 2008;121:116-121.*
[5] *Betsi GI, Papadavid E, Falagas ME. Probiotics for the treatment or prevention of atopic dermatitis: a review of the evidence from randomized controlled trials. Am J Clin Dermatol. 2008;9:93-103.*
[6] *Boyle RJ, Ismail IH, Kivivuori S, et al. Lactobacillus GG treatment during pregnancy for the prevention of eczema: a randomized controlled trial. Exclusive prenatal supplementation with probiotics was not effective in preventing infant eczema. Allergy 2011;66:509–516.*
[7] *Thomas DW, Greer FR; American Academy of Pediatrics Committee on Nutrition; American Academy of Pediatrics Section on Gastroenterology, Hepatology, and Nutrition. Probiotics and prebiotics in pediatrics. Pediatrics 2010;126:1217-31.*

Contents

Protective factors - including probiotics - against allergic diseases in high-risk children

OBJECTIVES / BACKGROUND

Environmental and lifestyle factors seem to affect atopic disease prevalence. This large prospective cohort followed infants until the age of 5 years and aimed to identify risk factors for allergic diseases. The study evaluated the effect of allergic heredity, parental smoking, exposure to pets, mode of delivery, breast-feeding duration, start of solid foods, infectious diseases, and use of antibiotics on the development of allergic diseases up to the age of 5.

DESIGN

Randomized, double-blind, placebo-controlled trial.

SETTINGS & PARTICIPANTS

A cohort of 1223 children born into allergic families (at least one parent having an allergy) was followed until the age of 5. In the probiotic group, for 4 weeks before delivery, mothers received *Lactobacillus rhamnosus* GG, *L. rhamnosus* Lc705, *Bifidobacterium breve* 99, and *Propionibacterium freudenreichii* spp. *shermanii* JS. Their new-born infants received the same probiotics and a galacto-oligosaccharide from birth once daily for 6 months. In the placebo group, mothers and infants received identical preparations without probiotics. At 3 months, 6 months, 2 years and 5 years, a paediatrician blinded to group assignment recorded the infants' history of symptoms related to allergic diseases and clinically examined each one.

MAIN OUTCOME

The primary outcome measures at 2 and 5 years were the cumulative incidence of any allergic disease (food allergy, eczema, asthma, or allergic rhinitis) and IgE associated (atopic) disease. Secondary outcome measures, also at 2 and 5 years, were eczema, atopic eczema, or sensitization; at 5 years, in addition, outcomes were IgE-associated asthma, allergic rhinitis, or IgE-associated respiratory allergy.

RESULTS

Compared to allergy in one parent only, allergy in both parents conferred an increased risk of allergic disease at the ages of 2 (OR 1.64; 95% CI 1.11-2.42, P=0.013) and 5 (OR 1.83; 95% CI 1.24-2.70, P=0.002) and at the age of 2 for eczema (OR 1.74; 95% CI 1.17-2.58, P=0.006). Exclusive breast-feeding over 2 months elevated the risk of eczema at the ages of 2 (OR 1.73; 95% CI 1.15-2.61, P=0.009) and 5 (OR 1.51; 95% CI 1.03-2.23, P=0.036). Cat or dog exposure at 0-2 years and at 0-5 years protected against IgE sensitization until 5 years of age (OR 0.60; 95% CI 0.37-1.00, P=0.048, and OR 0.61; 95% CI 0.39-0.96P=0.033), and exposure at the ages of 0-5 years protected against allergic rhinitis until the age of 5 (OR 0.46; 95% CI 0.25-0.85, P=0.013) in the probiotic group.

CONCLUSION

Allergy in both parents is an independent predictor of eczema and of allergic disease until the ages of 2 and 5. Long, exclusive breast-feeding was associated with increased eczema at the ages of 2 and 5, and cat or dog exposure was associated with decreased IgE sensitization and allergic rhinitis in the probiotic group. In addition and earlier, the authors reported less atopic disease until the age of 2 years from perinatal use of pre- and probiotics in the same cohort (Kukkonen *et al.* 2007).

Sandini U, Kukkonen AK, Poussa T, Sandini L, Savilahti E, Kuitunen M. Protective and Risk Factors for Allergic Diseases in High-Risk Children at the Ages of Two and Five Years. Int Arch Allergy Immunol. 2011 Jun 29;156(3):339-348.

Kukkonen K, Savilahti E, Haahtela T, Juntunen-Backman K, Korpela R, Poussa T, Tuure T, Kuitunen M.Probiotics and prebiotic galacto-oligosaccharides in the prevention of allergic diseases: a randomized, double-blind, placebo-controlled trial.J Allergy Clin Immunol. 2007 Jan;119(1):192-8.

Contents

3.3. IBS & FUNCTIONAL GI DISORDERS

A *bifidobacterium* prevents functional gastrointestinal symptoms in adults

OBJECTIVES / BACKGROUND

Functional gastrointestinal symptoms - as nonspecific conditions with no identifiable structural or biochemical cause - are often associated with disturbed intestinal transit. This study was designed to determine if dietary supplementation with *Bifidobacterium lactis* HN019 can shorten whole gut transit time (WGTT) and reduce the frequency of functional gastrointestinal symptoms in adults.

DESIGN

Triple-blind, randomized, placebo-controlled trial.

SETTINGS & PARTICIPANTS

100 adults (64% female) with functional GI symptoms were randomized to consume capsules containing *B. lactis* HN019, at daily doses of $1.7x10^{10}$ CFU (high dose; n=33), $1.8 x10^{9}$CFU (low dose; n=33), or placebo (n=34) for 14 days.

MAIN OUTCOME

The primary endpoint of WGTT was assessed by X-ray on days 0 and 14 and was preceded by consumption of radiopaque markers once a day for 6 days. The secondary endpoint of functional GI symptom frequency was recorded with a subject-reported numeric (1-100) scale before and after supplementation.

RESULTS

Decreases in mean WGTT over the 14-day study period were statistically significant in the high dose group (49 ± 30 to 21 ± 32 h, P<0.001) and the low dose group (60 ± 33 to 41 ± 39 h, P=0.01), but not in the placebo group (43 ± 31 to 44 ± 33 h). Time to excretion of all ingested markers was significantly shorter in the treatment groups versus placebo. Of the nine functional GI symptoms investigated, eight significantly decreased in frequency in the high dose group and seven decreased with low dose, while two decreased in the placebo group. No adverse events were reported in any group.

CONCLUSION

Daily consumption of capsules of *B. lactis* HN019 is well tolerated; shortens whole gut transit time in a dose-dependent manner, and reduces the frequency of functional GI symptoms in adults.

Waller PA, Gopal PK, Leyer GJ, Ouwehand AC, Reifer C, Stewart ME, Miller LE. Dose-response effect of Bifidobacterium lactis HN019 on whole gut transit time and functional gastrointestinal symptoms in adults. Scand J Gastroenterol. 2011 Jun 13.

Contents

Modest but clinically significant effect of a probiotic against functional gastrointestinal disorders in children – As concludes a meta-analysis

OBJECTIVES / BACKGROUND

This systematic review aimed to update and summarize the available randomised controlled trial (RCT) evidence of the likely effects of *Lactobacillus rhamnosus* GG (LGG) in children affected by abdominal pain-related functional gastrointestinal disorders.

DESIGN

Meta-analysis.

SETTINGS & PARTICIPANTS

Medline, Embase, CINAHL, the Cochrane Library, trial registries and proceedings of major meetings were searched for RCTs evaluating LGG supplementation in children with abdominal pain-related functional gastrointestinal disorders based on the Rome II or Rome III criteria. Risk of bias was assessed for generation of the allocation sequence, allocation concealment, blinding and follow-up. Three trials were eligible; two RCTs enrolled patients with functional abdominal pain, and one RCT also enrolled children with functional dyspepsia. In all studies, the sample size ranged from 64 to 141 participants; LGG was compared with placebo; doses of LGG ranged from $1x10^9$ to $3x10^9$ CFU twice daily and supplementation lasted for 4 to 8 weeks.

MAIN OUTCOME

The primary outcome is the rate of responders to the treatment or treatment success defined as no pain or a decrease in pain intensity.

RESULTS

Compared with placebo, LGG supplementation was associated with a significantly higher rate of treatment responders (defined as no pain or a decrease in pain intensity) in the overall population with abdominal pain-related functional gastrointestinal disorders (3 RCTs, n=290; risk ratio, RR 1.31, 95% CI 1.08-1.59, NNT 7, 95% CI 4-22) and in the irritable bowel syndrome (IBS) subgroup (3 RCTs, n=167; RR 1.70, 95% CI 1.27-2.27, NNT 4, 95% CI 3-8). However, no difference was found in the rate of treatment responders between children with functional abdominal pain or functional dyspepsia who received placebo or LGG. The intensity of pain was significantly reduced in the overall study population and in the IBS subgroup. The frequency of pain was significantly reduced in the IBS subgroup only.

CONCLUSION

The use of *L. rhamnosus* GG increased the proportion of responders to the treatment (defined as no pain or a decrease in pain intensity) in children with abdominal pain-related functional gastrointestinal disorders, particularly among children with IBS. Additionally, the probiotic reduced the frequency and intensity of pain, again particularly among children with IBS. It is worth noting that, although positive and statistically significant, the effects were clinically modest.

Horvath A, Dziechciarz P, Szajewska H. Meta-analysis: Lactobacillus rhamnosus GG for abdominal pain-related functional gastrointestinal disorders in childhood. Aliment Pharmacol Ther. 2011 Jun;33(12):1302-10.

Contents

3.4. IBD AND OTHER INFLAMMATORY DISEASES

LTA-deficient lactobacilli regulates colonic inflammation in mice

OBJECTIVES

Imbalance in the regulatory immune mechanisms that control intestinal cellular and bacterial homeostasis may lead to induction of the detrimental inflammatory signals characterized in humans as inflammatory bowel disease. Recent studies show the ability of probiotics to treat and prevent inflammatory bowel disease. The purpose of this paper was to study the molecular mechanisms of *Lactobacillus acidophilus* NCFM involved in the induction and repression of intestinal inflammation.

METHODS & MEASURES

The phosphoglycerol transferase gene that plays a key role in lipoteichoic acid (LTA) biosynthesis in *L. acidophilus* was deleted. Analysis of the regulation of dendritic cells (DCs, producers of pro-inflammatory cytokines i.e.IL12) by the LTA-deleted *L. acidophilus* and determination of the immunomodulatory properties of the LTA-deleted *L. acidophilus* in dextran sulfate sodium (DSS)-induced colitis in mice were performed.

RESULTS

The data shows that the LTA-deleted *L. acidophilus* not only down-regulated IL12 and TNFα but also significantly enhanced IL10 in DCs and controlled the regulation of co-stimulatory DC functions, resulting in their inability to induce $CD4^+$ T-cell activation. Moreover, treatment of mice with LTA-deleted *L. acidophilus*, compared with native *L. acidophilus*, significantly mitigated DSS and $CD4^+CD45RB^{high}$T cell-induced colitis and effectively ameliorated DSS-established colitis through a mechanism that involves IL10 and $CD4^+FoxP3^+$ T regulatory cells to dampen exaggerated mucosal inflammation.

CONCLUSION

Deletion of the entire gene involved in the cell surface component LTA synthesis in *L. acidophilus* NCFM significantly impacted the intestinal microenvironment, inducing regulatory signals (i.e., IL10, T regulatory cells, and regulatory DCs). Moreover, alteration of LTA in *L. acidophilus* makes this probiotic able to alleviate colitis in mice. Thus, directed alteration of cell surface components of *L. acidophilus* NCFM establishes a potential strategy for the treatment of inflammatory intestinal disorders.

Mohamadzadeh M, Pfeiler EA, Brown JB, Zadeh M, Gramarossa M, Managlia E, Bere P, Sarraj B, Khan MW, Pakanati KC, Ansari MJ, O'Flaherty S, Barrett T, Klaenhammer TR. Regulation of induced colonic inflammation by Lactobacillus acidophilus deficient in lipoteichoic acid. Proc Natl Acad Sci U S A. 2011 Mar 15;108 Suppl 1:4623-30.

COMMENTARY

from James Versalovic, Baylor College of Medicine, Houston, USA

"This manuscript describes potential mechanisms of probiosis via studies of the probiotic species, *Lactobacillus acidophilus*. Prior studies had indicated that the D-alanine component of lipoteichoic acid is important for stimulating the production of pro-inflammatory cytokines. Lipoteichoic acid (LTA) is present in the cell walls of many gram-positive bacteria, and the composition of LTA varies among different bacterial species. These investigators identified a gene complex including the bacterial gene encoding phosphoglycerol transferase. This gene and the entire operon have a putative role in LTA biosynthesis, and the phosphoglyceroltransferase gene was inactivated in *L. acidophilus* NCFM (NCK56) to create the mutant strain, NCK2025, purportedly deficient in LTA.

L. acidophilus strain NCK2025 downregulated MHC II and costimulatory molecules CD40, CD80 and CD86 on the surfaces of murine bone marrow-derived dendritic cells. This mutant strain NCK2025 had a diminished ability to induce TNF, but this strain induced production of the immunoregulatory cytokine IL-10. In *vivo* studies also demonstrated the importance of cell wall LTA for this probiotic strain to exert its immunostimulatory effects. Strain NCK2025 diminished the disease phenotype in a DSS chemical colitis model and an adoptive transfer colitis model. In addition to diminishing disease onset, strain NCK2025 attenuated established intestinal inflammation in the DSS colitis model, and this LTA-deficient strain stimulated proliferation of CD4-positive, Foxp3-positive regulatory T cells. IL-10 appears to be essential for this anti-inflammatory effect because IL-10 deficient mice did not respond to NCK2025. In summary, cell wall composition of probiotics may be important for selecting natural strains and engineering probiotic strains for suppression of mucosal inflammation."

Contents

3.5. NEW APPLICATIONS & OTHER

Variations in the composition of infant gut microbiota in relation to age and to sensitization state

OBJECTIVES / BACKGROUND

At birth the human infant gut is sterile, and becomes fully colonized within a few days. This initial colonization process has a major impact on immune development. The aim of the present work was to develop the GA-map™ (Genetic Analysis - microbiota analysis platform) infant array, and to use it to compare the temporal development of the gut microbiota in IgE sensitized and non-sensitized children during the two first years of life.

DESIGN *Ex vivo* trial.

SETTINGS & PARTICIPANTS

A total of 16 sensitized and 31 non sensitized children (aged between 1 day and 2 years) were selected from the Prevention of Allergy Among Children in Trondheim (PACT) study which is a large population-based intervention study in Norway focused on childhood allergy. From these 47 children, 216 faecal samples were collected and analysed by GA-map.The GA-map infant array is composed of highly specific 16S rRNA gene-targeted single nucleotide primer extension (SNuPE) probes, which were designed on extensive infant 16S rRNA gene sequence libraries. The GA-map assay is based on the SNuPE in combination with microarray hybridization.

MAIN OUTCOME

The main outcome of the trial was to prospectively compare the development of the dominant microbiota in IgE-sensitized children and non-sensitized children during the first 2 years of life.

RESULTS

The results showed that at a high taxonomic level, *Actinobacteria* was significantly over-represented at 4 months, while *Firmicutes* was significantly over-represented at 1 year for the sensitized children. At a lower taxonomic level for the sensitized group, *Bifidobacterium longum* were significantly over-represented at 1 year, and *Enterococcus* at 4 months. For most phyla, however, there were consistent differences in composition between age groups, irrespective of the sensitization state. The main age patterns were a rapid decrease in staphylococci from 10 days to 4 months, and a peak of bifidobacteria and bacteroides at 4 months.

CONCLUSION

These results showed consistent microbiota colonization and IgE sensitization patterns that can be important for understanding both normal and diseased immunological development in infants.The study highlighted the usefulness of the GA-map infant assay in determining variations in the composition of infant gut microbiota.

Vebø HC, Sekelja M, Nestestog R, Storrø O, Johnsen R, Oien T, Rudi K. Temporal development of the infant gut microbiota in IgE sensitized and non-sensitized children determined by the GA-map infant array. Clin Vaccine Immunol. 2011; 18: 1326-1335.

COMMENTARY

from Bruno Pot, Institut Pasteur de Lille, France

"In this work, an infant high-throughput 16S rRNA gene microarray, called Genetic Analysis microbiota-analysis-platform (GA-map™) is described and used to compare the development of the gut microbiota during the two first years of life of IgE-sensitized and non-sensitized children.
The technique differs from the traditional 16S rRNA gene array approach by the use of 'single nucleotide primer extension' (SNuPE)-probes, highly specific for target/non-target discrimination and obtained by combining a DNA polymerase-based incorporation of a fluorescently labeled dideoxynucleotide with a targeted hybridization: if the target bacterium is present, a labeled dideoxynucleotide is incorporated by the polymerase.
The target population was a selected subset of children (16 sensitized and 31 non-sensitized infants) of the IM-PACT (Prevention of Allergy Among Children in Trondheim) study cohort, a cohort in which the effects of the temporal development of 12 selected bacteria on allergy development was previously inventoried.
The paper discusses some of the disadvantages of the GA-map, as well as the advantages in terms of specificity and sensitivity. The microbiota analysis shows consistent differences in composition between age groups, irrespective of the sensitization state. At 4 months, Actinobacteria are significantly over-represented, while Firmicutes were significantly over-represented in the IgE-sensitized group at one year. On a lower taxonomic level, *Bifidobacterium longum* was significantly over-represented in the sensitized group at the age of one year. This is surprising as most previous work has actually suggested that this species is protective with respect to sensitization.
Consistent microbiota colonization and IgE sensitization patterns can be important for understanding both normal and diseased immunological developments in infants."

Contents

Does probiotics intake impact growth of ELBW infants?

OBJECTIVES / BACKGROUND

This trial was designed to test whether probiotic-supplemented feeding to extremely low-birth-weight infants (ELBW) will improve growth, feeding tolerance and reduce the number of days of antimicrobial treatment.

DESIGN

Multicentric, randomized, controlled, double-blinded trial.

SETTINGS & PARTICIPANTS

A total of 101 premature infants (birth weight 501-1000g; age at the time of feeding initiation ≤14 days) were enrolled. The tested group received *Lactobacillus rhamnosus* GG and *Bifidobacterium infantis* added to the first enteral feeding and continued once daily with feedings thereafter until discharge or until 34 weeks postmenstrual age. The control group received no probiotics. Infant weight and feeding volumes were recorded daily during the first 28 days of the study period. Weights were also recorded at 34 weeks postmenstrual age.

MAIN OUTCOME

The main outcome was growth improvement as determined by decreasing the percentage of infants with weight below the 10th percentile at 34 weeks postmenstrual age. Other important outcome measures were: improved feeding tolerance determined by toleration of larger volumes of food per day and reduced antimicrobial treatment days during the first 28 days following the initiation of feeding supplementation.

RESULTS

There was no difference between the two groups in the percentage of infants with weight below the 10th percentile at 34 weeks postmenstrual age (58% tested vs. 60% control; P=0.83) or in the average volume of food during 28 days after study entry (59 ml/kg tested vs. 71 ml/kg control; P=0.11). Calculated growth velocity was higher in the tested group compared with the control group (14.9 vs. 12.6 g per day; P=0.05). Incidences of necrotizing enterocolitis, as well as mortality were similar between the two groups.

CONCLUSION

Although probiotic-supplemented feedings improve growth velocity in ELBW infants, there was no improvement in the percentage of infants with growth delay at 34 weeks postmenstrual age. There were no probiotic-related adverse events reported.

Al-Hosni M, Duenas M, Hawk M, Stewart LA, Borghese RA, Cahoon M, Atwood L, Howard D, Ferrelli K, Soll R. Probiotics-supplemented feeding in extremely low-birth-weight infants. J Perinatol. 2011 May 5.

Contents

Temporal and dynamic analysis of intestinal flora in healthy adults

OBJECTIVES / BACKGROUND

While our knowledge of the intestinal microbiota during disease is accumulating, basic information of the microbiota in healthy subjects is still scarce. An observational study expands the present concept of temporal dynamics of the overall microbiota and its individual members, and analysed - for the first time - the associations between the intestinal microbiota and intestinal symptoms in healthy adults.

DESIGN

Longitudinal study. The study subjects were a subset of healthy Finnish adults from a larger randomized, double-blinded, placebo-controlled intervention trial.

SETTINGS & PARTICIPANTS

A set of 15 healthy adults were followed for 7 weeks and regularly assessed for their intestinal bacteria and *Archaea* with the Human Intestinal Tract Chip, a phylogenetic microarray, in conjunction with qPCR analyses. At the time of each faecal sampling (faecal samples were collected at six time points) the subjects filled a Health-Related Quality of Life questionnaire, which was supplemented with questions assessing gastrointestinal symptoms. The questions covered general and emotional health with focus on the intestinal symptoms whose occurrence and impact on daily activities were assessed with 14 questions using a 5-step Likert scale (e.g. 1=no symptoms, 5=very severe disturbance). The recall period was one or two weeks according to the sampling intervals.

MAIN OUTCOME

Main outcomes were analysis of compositional microbiota and temporal variation of the healthy microbiota and the individual core, and the correlation between intestinal symptoms and faecal microbiota.

RESULTS

The largest differences in the microbiota profiles were the inter-individual differences (mean inter-individual Pearson correlation; r=0.78, SD ±0.04). All samples clustered in a subject-wise manner, and a high overall stability of the individuals' microbiota was observed (mean intra-individual r=0.96,±0.02 over all time points). Five subjects showed transient microbiota destabilization, which correlated not only with the intake of antibiotics but also with overseas travelling and temporary illness, expanding the hitherto known factors affecting the intestinal microbiota. Significant correlations were identified between the microbiota and common intestinal symptoms, including abdominal pain and bloating. The most striking finding related to abdominal pain was its negative correlation with the abundance of bifidobacteria (r=20.45, ±0.03). The majority of the *Bifidobacterium* phylotypes that were above the detection limit (14/21) had statistically significant (P≤0.02) negative correlation with abdominal pain. The subjects who experienced pain had over five times less bifidobacteria compared to the subjects without recorded pain. Finally, a novel computational approach was used to define the common core microbiota, highlighting the role of analysis depth in finding the phylogenetic core and estimating its size. The in-depth analysis suggested that we share a substantial number of our intestinal phylotypes but as they represent highly variable proportions of the total community, many of them often remain undetected.

CONCLUSION

This overall and high-resolution microbiota analysis revealed, in healthy adults, the temporal stability, the individual and common core microbiota, and its relationship between several gastrointestinal symptoms. These results could suggest that repletion with probiotic bifidobacteria may be beneficial against intestinal symptoms.

Jalanka-Tuovinen J, Salonen A, Nikkilä J, Immonen O, Kekkonen R, Lahti L, Palva A, de Vos WM. Intestinal microbiota in healthy adults: temporal analysis reveals individual and common core and relation to intestinal symptoms. PLoS One. 2011;6(7):e23035.

COMMENTARY

from James Versalovic, Baylor College of Medicine, Houston, USA

"This manuscript describes the temporal stability and variation of the intestinal microbiota in a small group of 15 healthy Finnish adult subjects. In general, a high degree of overall temporal stability was noted using a phylogenetic microarray, HITChip, that detects bacterial and archaeal taxa. HITChip detected an average of 470 phylotypes per subject, and members of the phylum Firmicutes accounted for more than 80 percent of the taxa. A fundamental observation of this study is the presence of definable core microbiome among nine healthy adults. The definable core contains 288 phylotypes containing mosty members of Clostridium clusters XIVa and IV if one considers low abundance taxa in the group. The coefficients of variation at the genus level averaged 6.3% with a range of 0.83% to 46.1%. The most common genus-level groups in this population were *Ruminococcus obeum* and relatives (*et rel.*), *Blautia,* and *Clostridium symbiosium et rel*. As expected, antimicrobial intake was associated with dramatic changes in the gut microbiome, but interestingly overseas travel across time zones was also associated with notable variation of the gut microbiome. Disease features such as abdominal pain and bloating were correlated with changes in the gut microbiome, and one example was the association of abdominal pain with a 5-fold reduction of the genus *Bifidobacterium*. In summary, novel computational approaches were used to define a phyogenetic core and the variation among individuals. Future considerations include the evaluation of the presence of core components, both stable and variable, in individuals in health and disease, including different well-defined gastrointestinal disease states."

Contents

Composition of the human intestinal flora is determined by host genotype

OBJECTIVES / BACKGROUND

Intestinal microbiota plays an important role in human health, and its composition is determined by several factors, such as host genotype. Although growing evidence indicates that host genetic background has a significant impact on the microbiota composition in the intestine, no specific genetic factors determining intestinal microbiota composition have been established to date. This study explores this new avenue of research.

DESIGN

Observational, *ex vivo* study.

SETTINGS & PARTICIPANTS

71 healthy adults (64 females and 7 males aged 31-61 years) were recruited. The individuals had not received antibiotic therapy within 2 months of the faecal sampling time. Probiotic consumption was restricted 1 week before sampling. In this cohort, 14 were non-secretor individuals and the remainders were secretors. The secretor status is defined by the expression of the ABH and Lewis histo-blood group antigens in the intestinal mucus and other secretions. It is determined by the presence of mucosal α1,2-fucosylated glycan structures (such as ABH and Lewis b histo-blood group antigens) linked to fucosyltransferase 2 enzyme activity, which is encoded by the *FUT2* gene. A non-functional enzyme resulting from a nonsense mutation in the *FUT2* gene leads to the non-secretor phenotype. Both faecal and blood samples were collected. PCR-DGGE and qPCR methods were applied for the intestinal microbiota analysis.

MAIN OUTCOME

Analysis of the diversity and abundance of dominant faecal bacteria and bifidobacteria.

RESULTS

Principal component analysis of bifidobacterial profiles showed that the samples of non-secretor individuals formed a separate cluster within the secretor samples. Moreover, bifidobacterial diversity ($P<0.0001$), richness ($P<0.0003$), and abundance ($P<0.05$) were significantly reduced in the samples from the non-secretor individuals as compared with those from the secretor individuals. The non-secretor individuals lacked, or were rarely colonized by, several genotypes related to *Bifidobacterium bifidum*, *B. adolescentis* and *B. catenulatum/pseudocatenulatum*. In contrast to bifidobacteria, several bacterial genotypes were more common and the richness ($P<0.04$) of dominant bacteria was higher in the non-secretor individuals than in the secretor individuals.

CONCLUSION

The study reports that the genetic variation in the *FUT2* gene is strongly associated with the microbiota composition, in particular that of bifidobacteria, in the human intestinal tract. This association can be explained by the difference between the secretor and non-secretor individuals in their expression of ABH and Lewis glycan epitopes in the mucosa. Consequently, secretor status determining the expression of the ABH and Lewis b glycan epitopes in the human intestine seems to be one of the host features significantly shaping the composition of bifidobacteria in the intestine.

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TECHNOLOGY OF PROBIOTIC AND PROBIOTIC FOODS

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KEY DATES

UEGW STOCKHOLM 2011 — 19th United European Gastroenterology Week — October 22 – 26	**October 22-26**	Stockholm, Sweden *http://uegw11.uegf.org*
DANONE RESEARCH Institut Pasteur INRA — microbes for health — 2nd International Symposium — December 1-2, 2011 - Paris	**December 1-2**	France, Paris *http://www.microbes-for-health.com/diaporamas/edition2011*
Canadian Digestive Diseases Week	**February 24-27**	Montreal, Quebec *http://www.cag-acg.org/program-and-registration*

METHOD

> **Monitoring period:** 01/03/2011 to 15/08/2011
> **Database:** Medline
> **Result:** 609 publications

> **Keywords:** probiotic / lactic acid bacteria / streptococcus thermophilus / lactobacilli / fermented milk / bifidobacteria

This probiotics watch is designed as a time-saving tool for scientists and clinicians interested in probiotic research. In an interactive format, the quarterly report provides timely, quasi-exhaustive lists of the scientific publications of the previous three months. It sorts them by topic and highlights some of the most relevant results. Readers can also check out upcoming scientific events and regular bibliometric analyses.

Objectivity is a strong commitment, that's why the articles are selected by an editorial committee, composed of renowned scientists in the field. Editorial committee members also comment on what they believe are the quarter's most relevant publications.
Last but not least each issue features an editorial by a probiotic expert, which offers special insight into this fascinating field of science.

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- Selecting the articles of interest in the probiotics field.
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