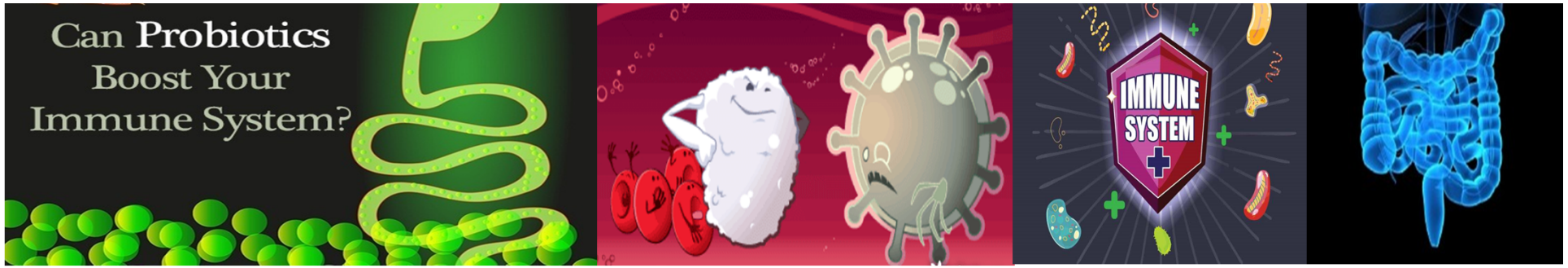

Emerging Applications of Probiotics: Immunity

Dr. V Deepak Bamola and Prof. Rama Chaudhry

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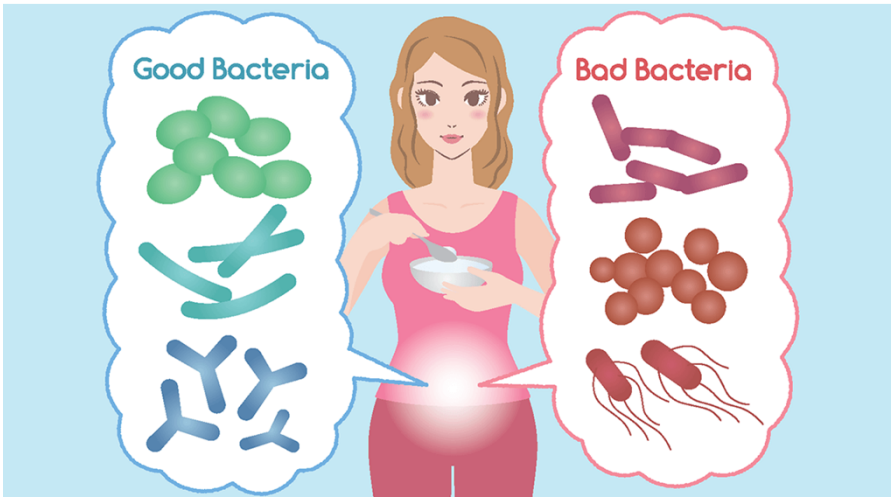
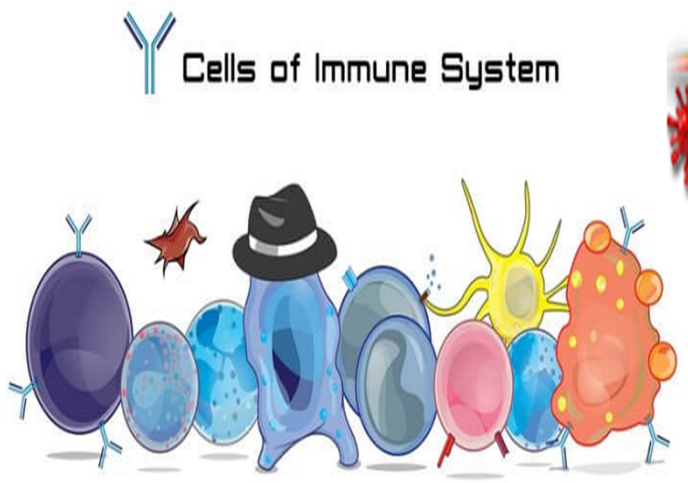
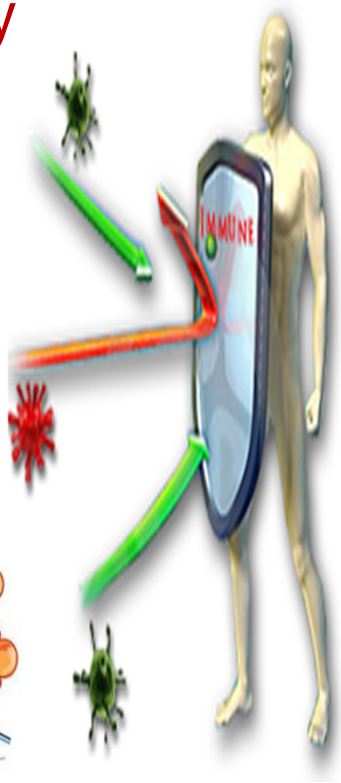
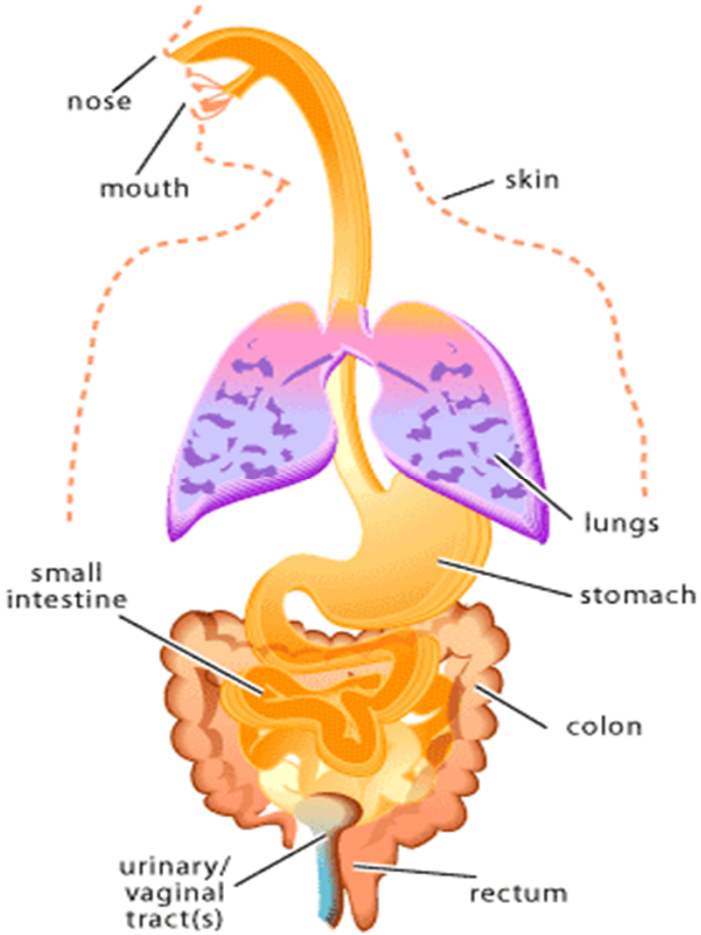
All India Institute of Medical Sciences (AIIMS),
New Delhi, India

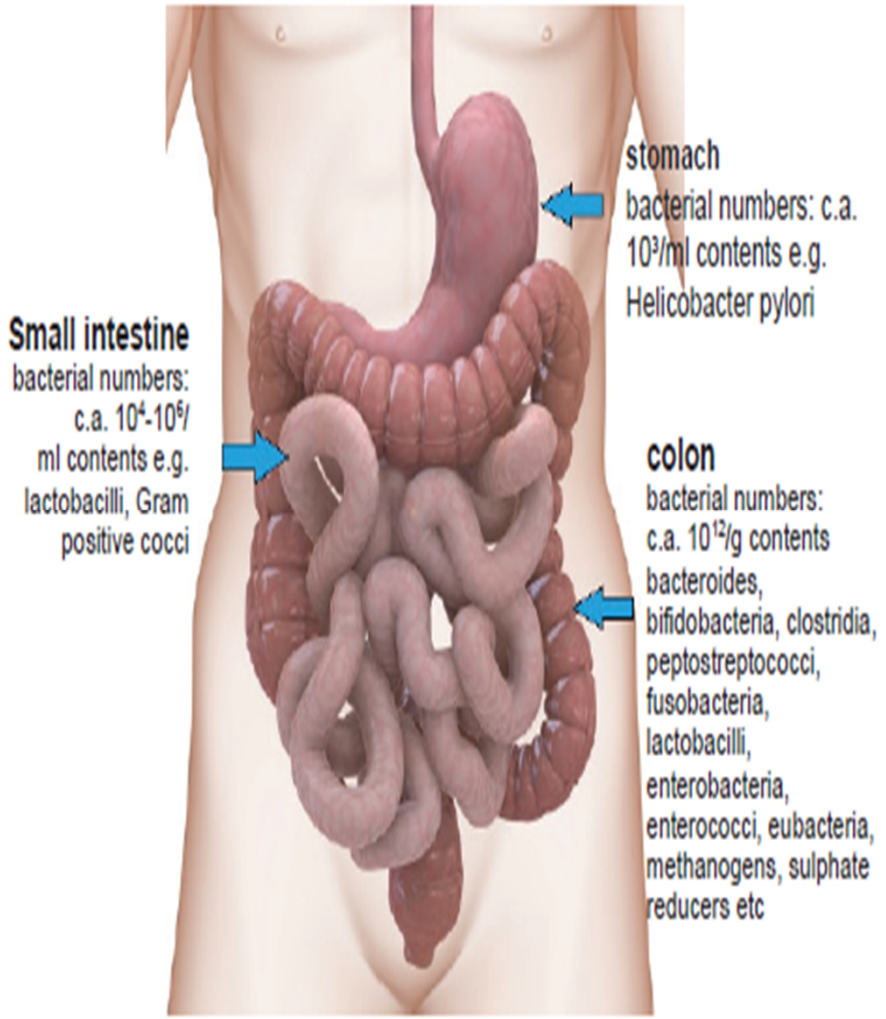






Microbiota in human body

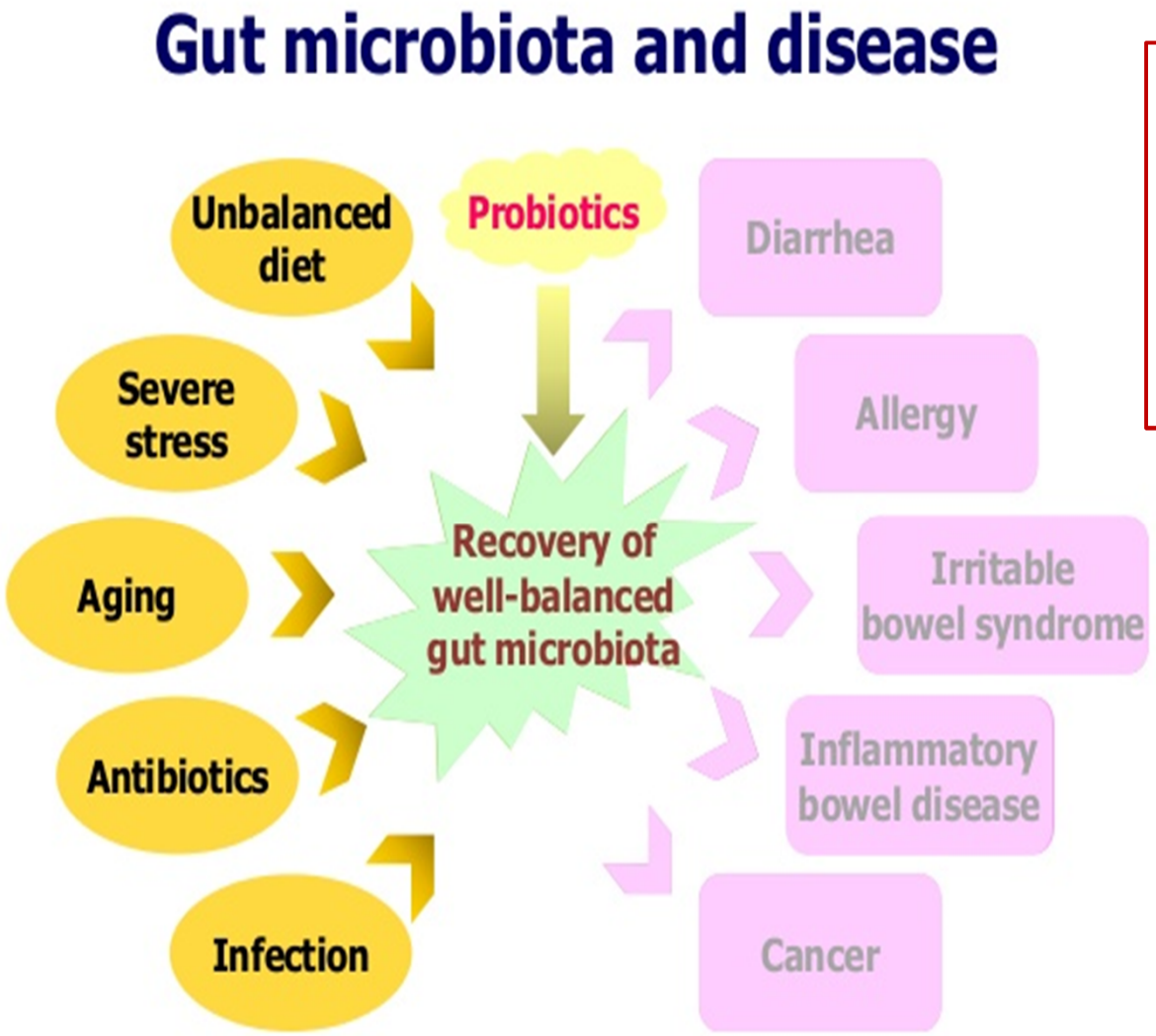




The collective genome- "microbiome" contains at least 100 times as many genes as our own genome

Sharma & Bamola 2014; in Probiotics edited by Chaudhry R

Dysbiosis – causes & effects

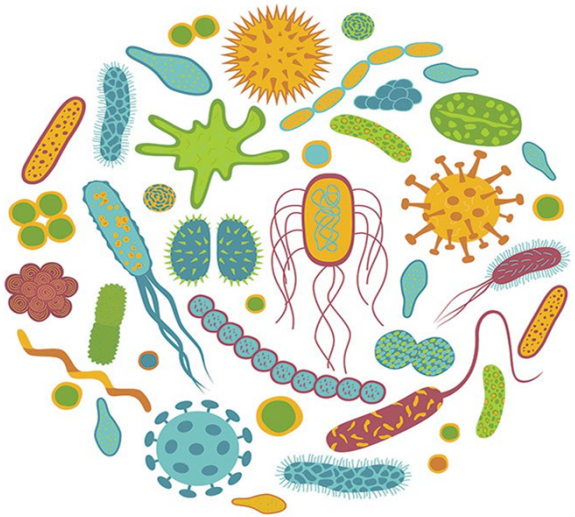


- Poor nutritional response
- Physiological dysfunction
- Accelerated aging.
- Deficient immune response
- Susceptibility to infection
- Diseases

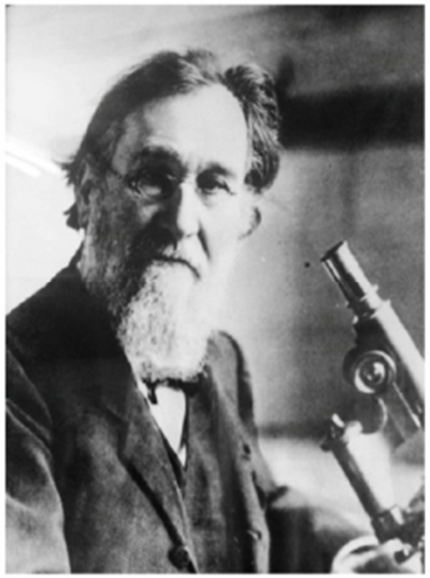
Probiotics

- Probiotics are live microbial feed supplements when administered in adequate amount, confers health benefits.

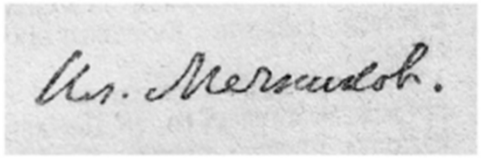
- Probiotics suppress the harmful bacteria and exert many beneficial physiological effects.



History of Probiotics

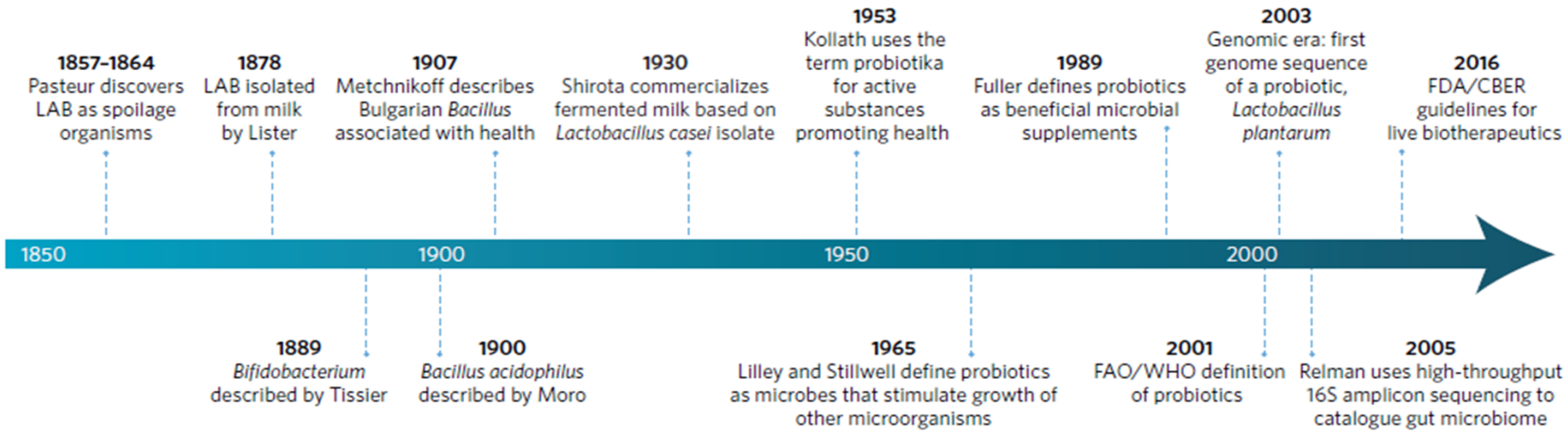


"Our aging
is a disease
that must be treated
like any other
desease."

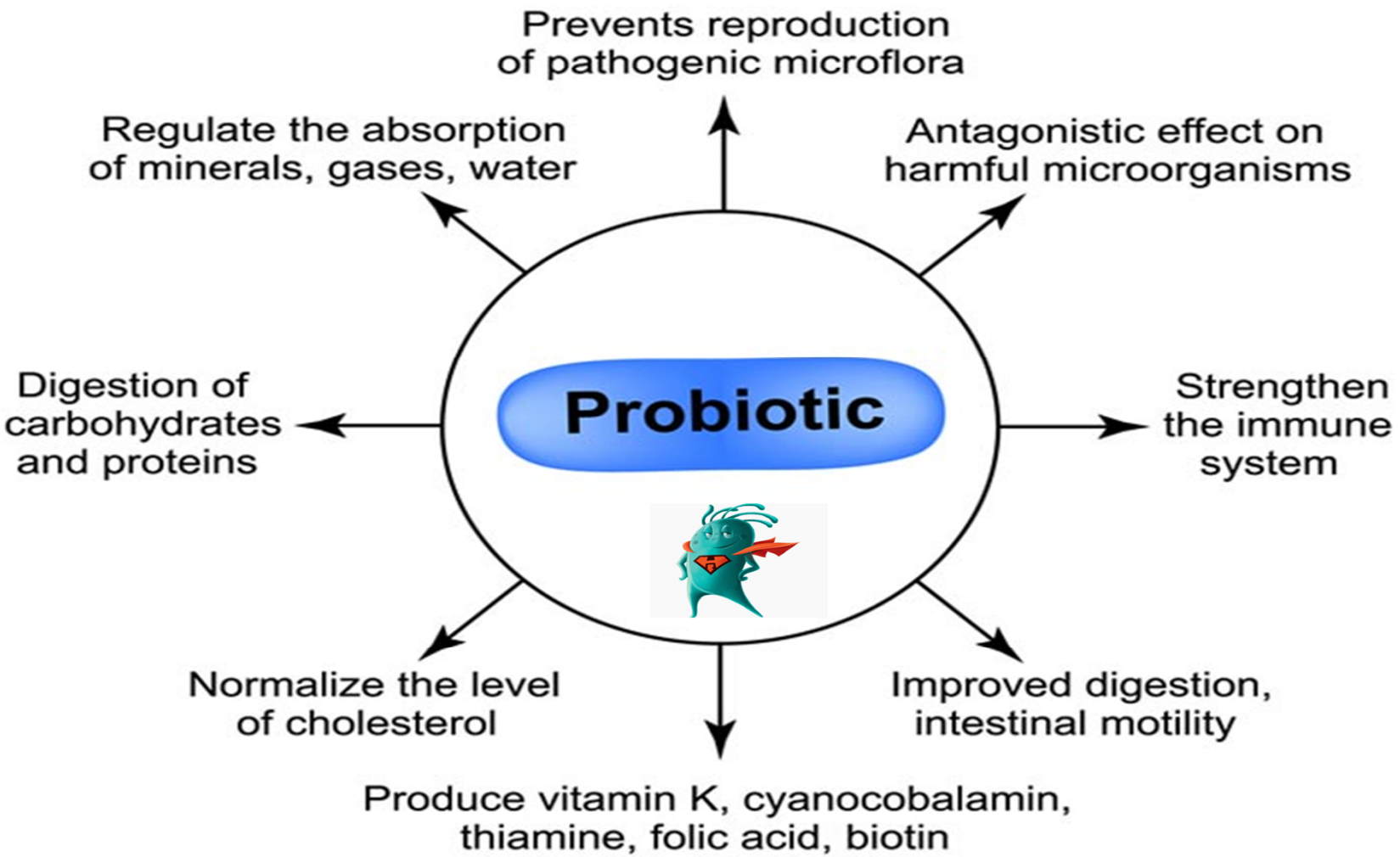


I. Mechnikov
1845-1916

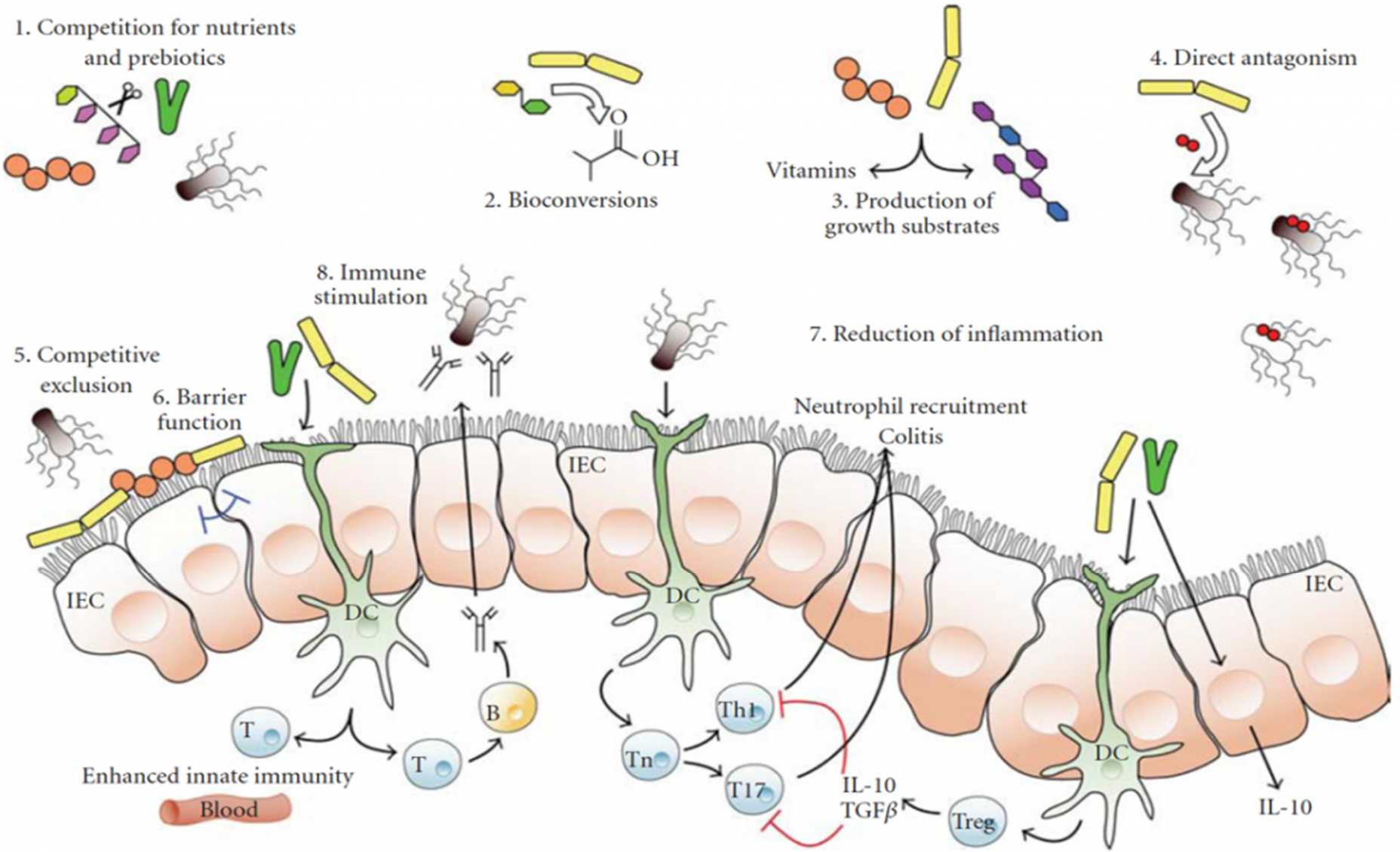
- In 1907, Russian noble prize winner and father of modern immunology, Elie Metchnikoff, was the first conceptualize "**PROBIOTICS**".
- Metchnokoff proposed fermented milk products could prevent "fouling" in the intestine if consumed regularly, lead to a healthy life.



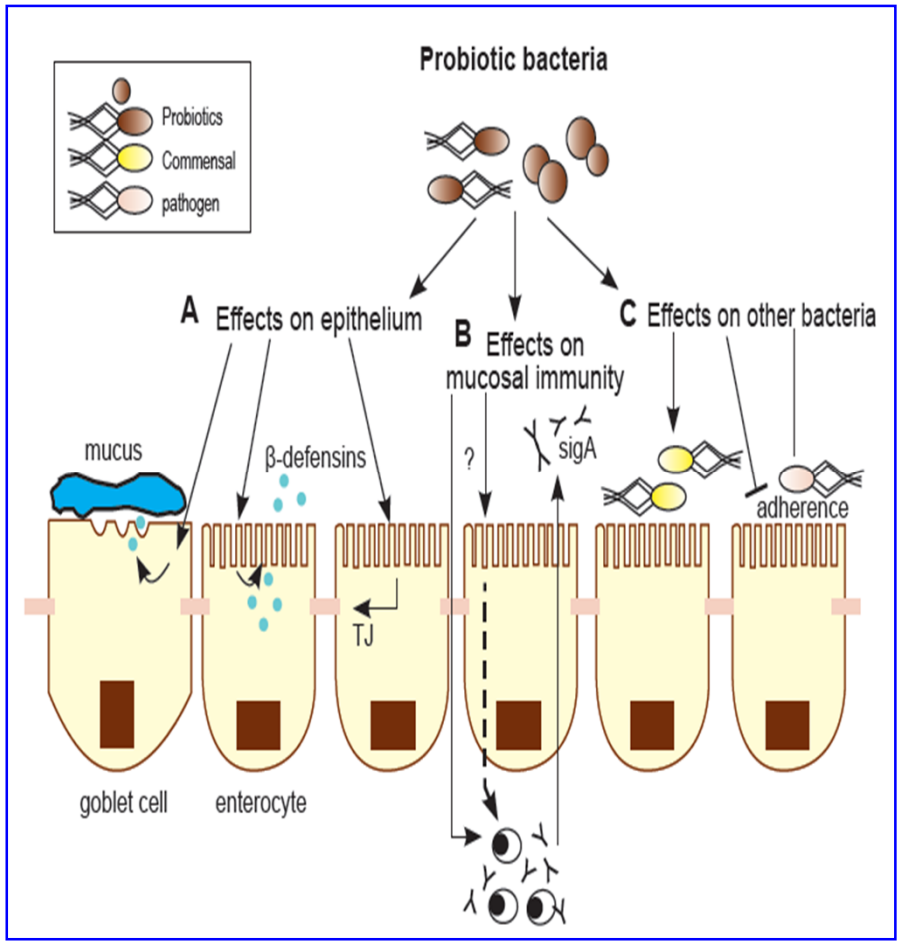
FUNCTIONS OF PROBIOTICS



Mechanisms of action-Probiotics

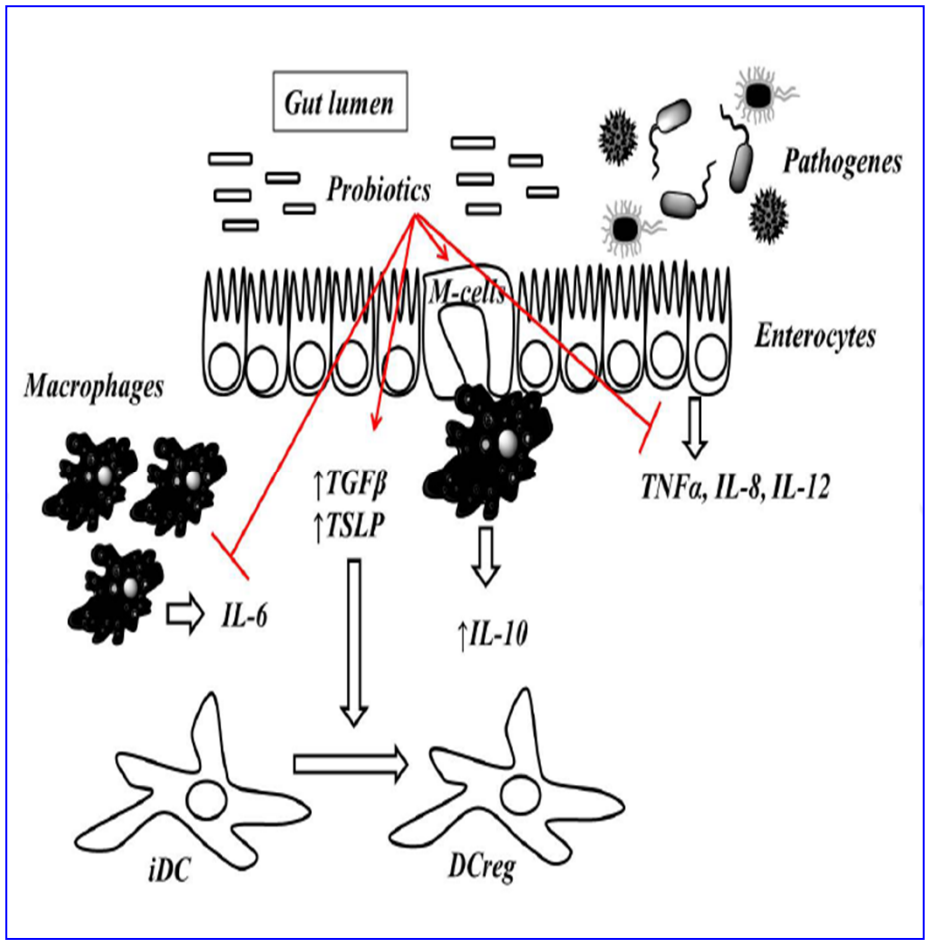


Effects of probiotc on intestinal epithelial barrier function



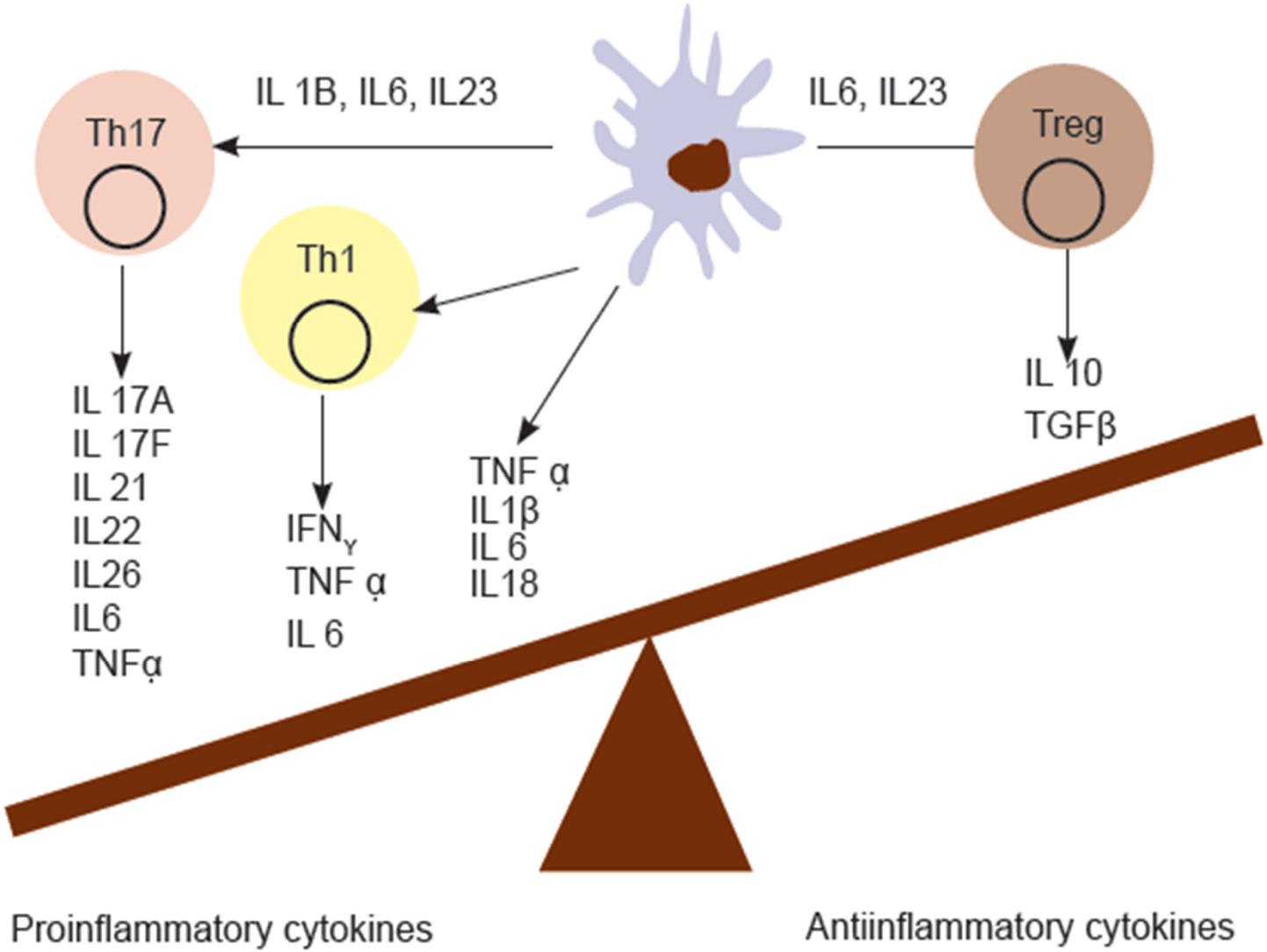
Bamola, Sharma and Chaudhry 2014

Probiotic modulation of the mucosal immune system



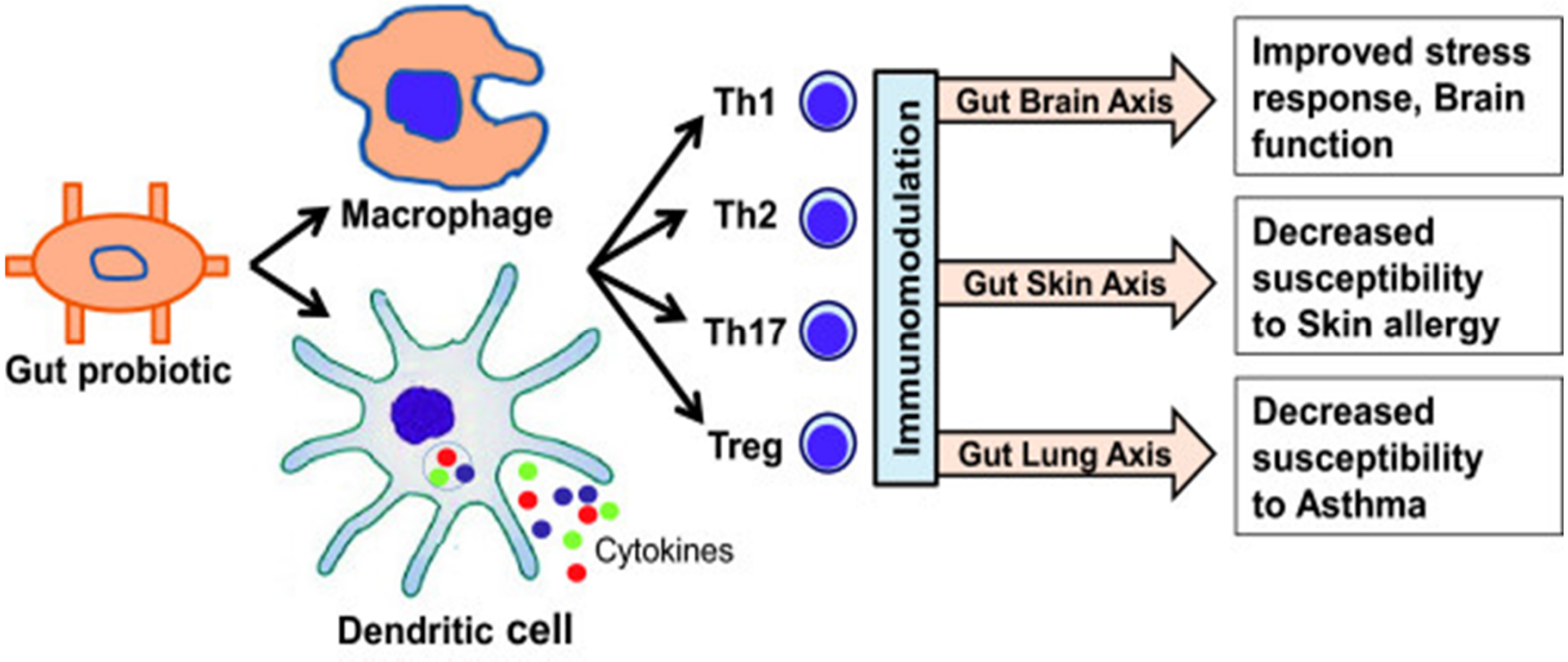
Georgieva et al 2015

Pro & Anti-inflammatory cytokines balance

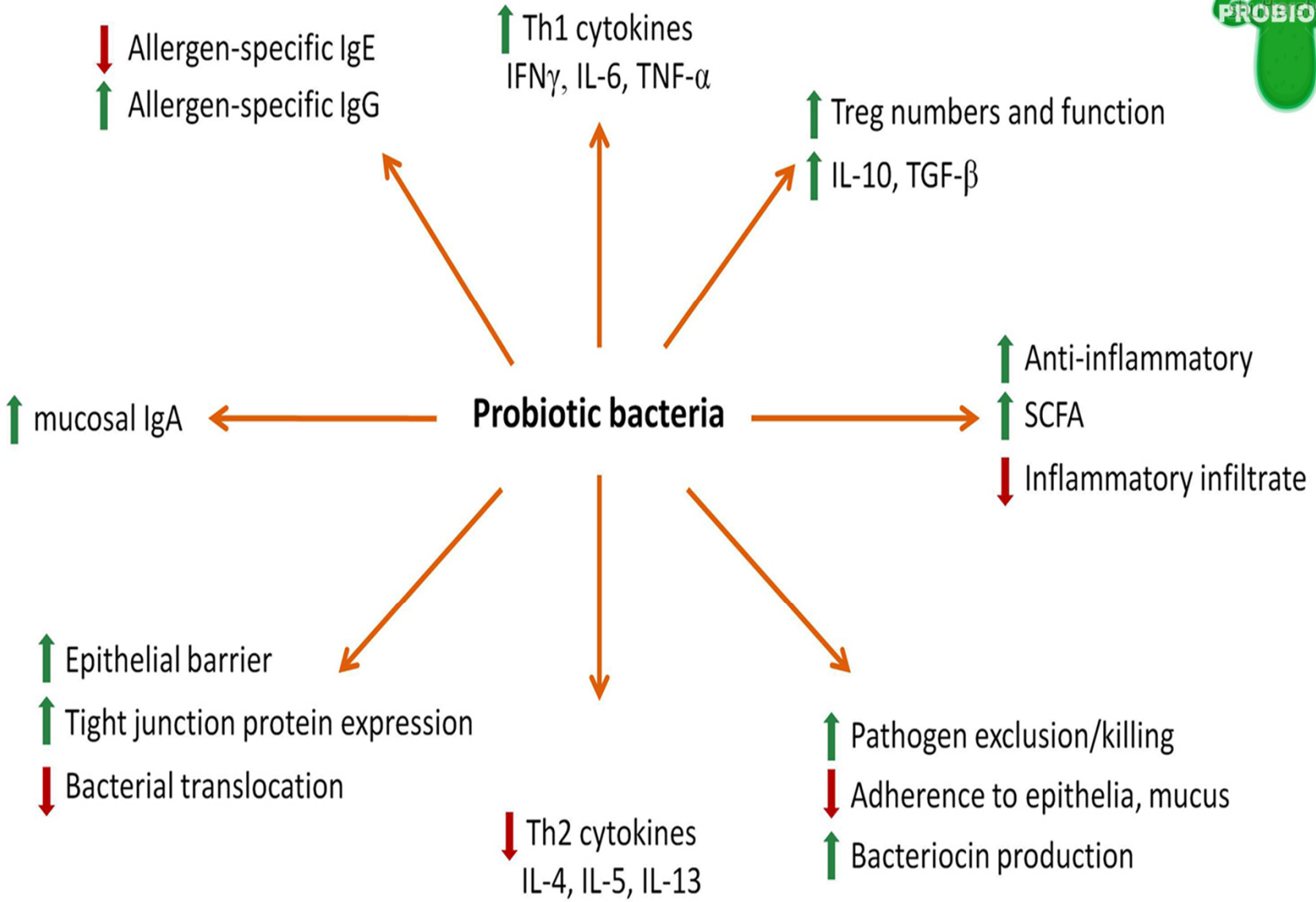


Bamola, Sharma and Chaudhry 2014

Role of probiotics in systemic immunity

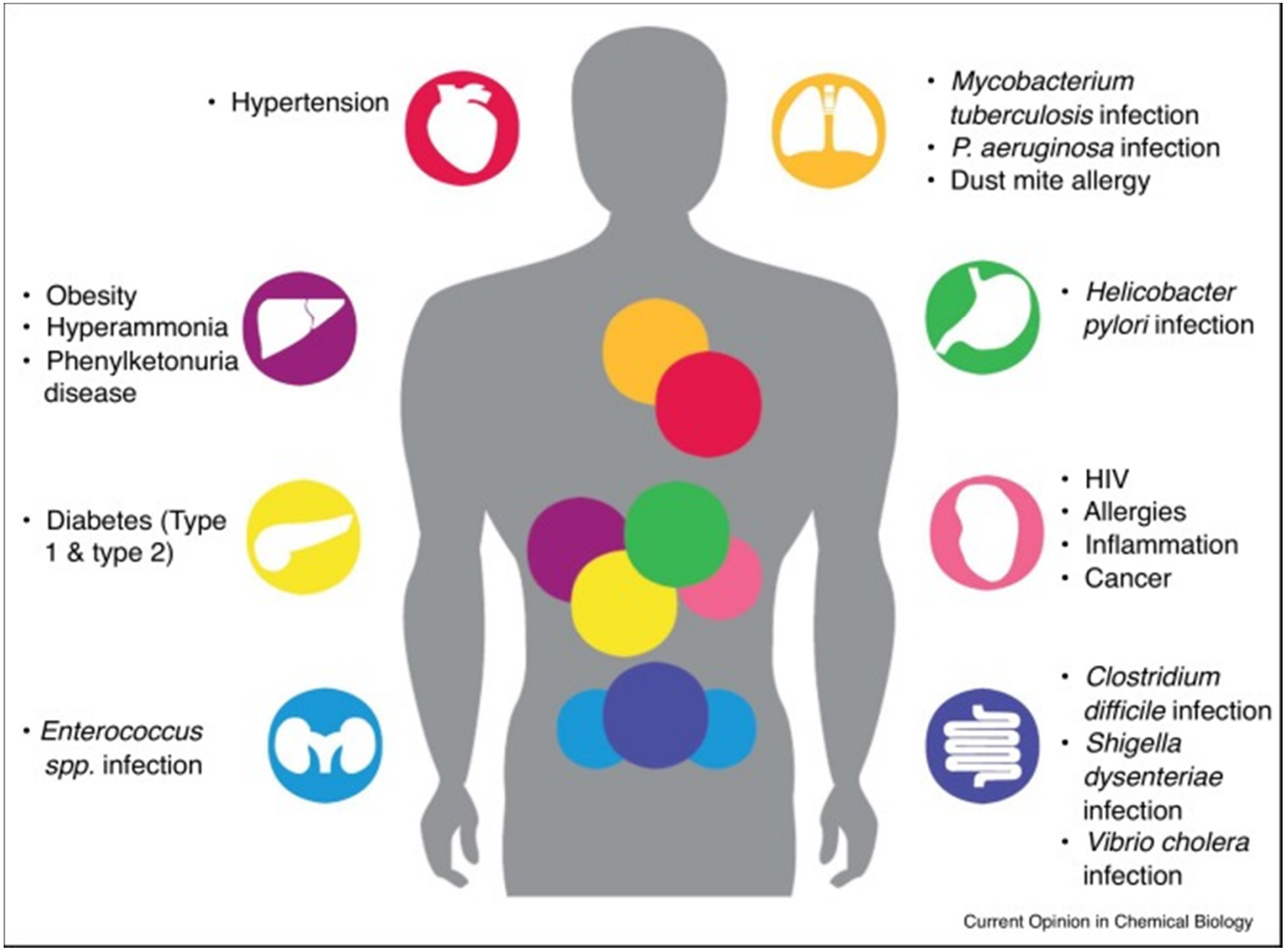


Summary of Immune effects of Probiotics



Heal the gut, improve immunity and heal the body ------> Probiotics is a cure

Health targets for probiotic intervention



Probiotic strains modulate T cell differentiation and effectors cytokines

Cytokines (Immune Response)	Cell system	Response	Probiotic strain
IFN-γ & IL-12 (Th_1-associated, CMI and NK cell activity)	PBMCs	Increase	*L. rhamnosus*
			L. plantarum
			L. lactis
			L. casei
			L. rhamnosus GG
			L. lactis W58
			L. casei Shirota
			L. casei Shirota
			L. paracasei
			L. salivarius
			B. longum W11
			L. rhamnosus
			L. gasseri
			B. bifidum
			E. coli (TG1)
			L. casei Shirota
			L. plantarum strains
	PBMC-Mo	Increase	*S. aureus*
			L. johnsonii
	PBMC-DCs	Increase	*L. salivarius*
			L. rhamnosus Lcr35
	PBMC-NK cells	Increase	*L. acidophilus*
			L. reuteri
	Myeloid DCs	Increase	*L. gasseri*
			L. johnsonii
			L. reuteri
	PBMC-NK cells	Decrease	*B. bifidum*
IL-23 & IL-17 (Th_{17}-associated, pro-inflammatory)	Mo-DCs	Increase	*L. rhamnosus* Lcr35
	PBMCs	Decrease	*B. breve*
			LGG
	Caco-2 cell line	Decrease	*L. plantarum*

Probiotic strains modulate pro- and anti-inflammatory cytokines

Cytokines (Immune Response)	Cell system	Response	Probiotic strain
TNF-α and IL-1β (Pro-inflammatory)	PBMCs	Increase	*L. rhamnosus*
			L. bulgaricus
			S. pyogenes
			Bifidobacteria
			L. casei Shirota
			L. salivarius
			L. fermentum
			L. plantarum strains
	PBMC-DCs	Increase	*L. rhamnosus* Lcr35
	Myeloid DCs	Increase	*L. reuteri*
	Epithelial cells	Increase	*L. sakei*
	Macrophage subset cell line	Increase and decrease (subset-specific)	*L. casei Shirota*
	THP-1 cell line	Decrease	*L. reuteri*
IL-6 (Pro-inflammatory)	PBMCs	Increase	*L.rhamnosus*
			L. bulgaricus
			S. pyogenes
	Epithelial cells	Increase	*B. lactis* Bb12
			L. casei CRL431
			L. helveticus R389
	PBMCs	Decrease	*L. casei* Shirota
IL-10 (Anti-inflammatory)	PBMCs	Increase	Bifidobacteria DNA
			Bifidobacteria
			B. longum W11
			L. fermentum
			L. acidophilus
			L. plantarum strains
			L. acidophilus
			L. reuteri
	PBMC-NK cells	Increase	*B. bifidum*
			VSL#3
			L. reuteri
	Blood-DCs	Increase	*L. plantarum*
	Mo-DCs	Increase	*L. casei*
			L. rhamnosus
			Bifidobacteria

Hardy et al 2017

Studies on immune responses by probiotics

Probiotic studied	Administering protocol	Population tested	Immune response	References
B. lactis Bi-07, *B. lactis* Bl-04, *L. acidophilus* La-14, *L. acidophilus* NCFM, *L. plantarum* Lp-115, *L. paracasei* Lpc-37, *L. salivarius* Ls-33	Individually in the form of two capsules/day for 21 days containing 1×10^{10} cfu /capsule of bacteria	Humans (healthy volunteers aged 18–62 years)	During early response (day 0–21), serum IgG significantly increased in subjects consuming *Bifidobacterium lactis* Bl-04 and *L. acidophilus* La-14 ($P = 0.01$) compared with controls. During late response (day 21–28) serum IgA and IgM increased in subjects consuming *L. acidophilus* NCFMs. The overall vaccination titer was not influenced by the administration of the probiotics during the oral preparation of cholera vaccination protocol	(Paineau et al., 2008)
L. acidophilus, *B. infantis*, *B. bifidium*	In combination with yogurt starter, i.e., *L. bulgaricus* & *S. thermophilus*	Mice (female B6C3F1, eight weeks old)	Yoghurt supplemented with *L. acidophilus* and *Bifidobacterium* spp. stimulated enhanced mucosal and systemic anticholera toxin IgA	(Tejada-Simon et al., 1999; Sanders and Klaenhammer, 2001)
L. acidophilus, *L. reuteri*, *B. infantis*, *L. casei* GG	Individually via orally and anally with 1 mL of 10^7 cfu/mL probiotic and *Candida albicans*	Mice (C57BL/6 *bg/bg-nu/nu* and *bg/bg-nu/+*)	Increased the levels of IgG, IgA, and IgM in euthymic immunocompromised mice. Antibody and cell-mediated responses to *Candida albicans* in immunodeficient mice could decrease the incidence and severity of candidiasis	(Wagner et al., 1997)
L. acidophilus, *Bacillus subtilis*	Individually and in combination to get 1×10^7 cfu/g of bacteria supplemented to diet & fed 8% of body weight for eight weeks	Fish-Nile tilapia (*Oreochromis niloticus*)	Increase in hematocrit values and serum bactericidal activity in group given mixture of bacteria. A significant increase in the values of the nitroblue tetrazolium (NBT) assay, neutrophil adherence, and lysozyme activity in all probiotic-treated groups after one and two months of feeding compared with untreated control group	(Aly et al., 2008)
L. acidophilus (LAVRI/DSM)	Administered 1×10^9 bacteria in 0.2 m LPBS/day for two weeks to 5 mice/group and orally postchallenged with 1×10^8 *Candida albicans* blastoconidia	Mice (male BALB/c; H-2 d and DBA/2; H-2d, six–eight weeks old)	Stimulation of IL-4–nitric oxide paracrine loop. Enhancement of both IL-4 and IFN-γ production in the regional lymph nodes, and secretion of IFN-γ and nitric oxide in saliva	(Clancy, 2003; Elahi et al., 2003)

Gut Microbiota and Probiotics- *Our Experience*

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https://doi.org/10.1080/16512235.2017.1322447

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RESEARCH ARTICLE

OPEN ACCESS Check for updates

Gut microbial diversity in health and disease: experience of healthy Indian subjects, and colon carcinoma and inflammatory bowel disease patients

V. Deepak Bamola[a], Arnab Ghosh[a], Raj Kishor Kapardar[b], Banwari Lal[b], Simrita Cheema[b], Priyangshu Sarma[b] and Rama Chaudhry[a]

[a]Department of Microbiology, All India Institute of Medical Sciences, New Delhi, India; [b]Microbial Biotechnology Division, The Energy and Resources Institute, New Delhi, India

ABSTRACT

Background: The intestinal microbiota, through complex interactions with the gut mucosa, play a key role in the pathogenesis of colon carcinoma and inflammatory bowel disease (IBD). The disease condition and dietary habits both influence gut microbial diversity.

Objective: The aim of this study was to assess the gut microbial profile of healthy subjects and patients with colon carcinoma and IBD. Healthy subjects included 'Indian vegetarians/lactovegetarians', who eat plant produce, milk and milk products, and 'Indian non-vegetarians', who eat plant produce, milk and milk products, certain meats and fish, and the eggs of certain birds and fish. 'Indian vegetarians' are different from 'vegans', who do not eat any foods derived wholly or partly from animals, including milk products.

Design: Stool samples were collected from healthy Indian vegetarians/lactovegetarians and non-vegetarians, and colon cancer and IBD patients. Clonal libraries of 16S ribosomal DNA (rDNA) of bacteria were created from each sample. Clones were sequenced from one representative sample of each group. Approximately 500 white colonies were picked at random from each sample and 100 colonies were sequenced after amplified rDNA restriction analysis.

Results: The dominant phylum from the healthy vegetarian was Firmicutes (34%), followed by Bacteroidetes (15%). The balance was reversed in the healthy non-vegetarian (Bacteroidetes 84%, Firmicutes 4%; ratio 21:1). The colon cancer and IBD patients had higher percentages of Bacteroidetes (55% in both) than Firmicutes (26% and 12%, respectively) but lower Bacteroidetes:Firmicutes ratios (3.8:1 and 2.4:1, respectively) than the healthy non-vegetarian. Bacterial phyla of Verrucomicrobiota and Actinobacteria were detected in 23% and 5% of IBD and colon patients, respectively.

Conclusions: Ribosomal Database Project profiling of gut flora in this study population showed remarkable differences, with unique diversity attributed to different diets and disease conditions.

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KEYWORDS
Indian vegetarian; colon cancer; IBD; gut microbiota; India

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Microbial & Biochemical Technology

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Research Article Open Access

A Non Invasive Technique to Assess Mucosal Immunity in Healthy Population by Measuring Immunoglobulin Receptor Expression on Viable Colonocytes

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[1]*Department of Microbiology, All India Institute of Medical Sciences, New Delhi, India*
[2]*University of Nebraska Medical Center and Director, Center for Global Health and Development College of Public Health, Nebraska Medical Center, Omaha, NE, USA*

Abstract

Human gut plays a vital role in the regulation of immune function, mucosal defense and homeostasis. Gut epithelial cells function as an immune cell and express receptors for microbial-associated molecular patterns. The gut epithelium undergoes constant and rapid renewal and some of these cells are exfoliated into the fecal stream. These cells are an important source of macromolecules, which provides a patho-physiological profile of the colonic epithelium. Most of the methods to harvest colonic epithelial cells are highly invasive and involve endoscopy and biopsy. Researchers suggest that studies of gastrointestinal pathophysiology are not feasible by biopsies in neonates and paediatric population. Therefore, isolation of theses exfoliated viable colonocytes from human stool is a non-invasive as well as a highly convenient approach that can be used for diagnostic and research purposes. A very few studies are available across the globe and no study is available from India of using this non-invasive techniques to recovered viable colonocytes in healthy population. For the first time we are reporting the results of the study on healthy Indian population where we recovered viable colonocytes from the stool samples using this non-invasive approach (Cell Sampling Recovery Method) and assessed immunoglobulins (IgA & IgG) receptors expression by Flowcytometry using specific florochrom conjugated antibodies. No study is available which provides the normal reference range of IgA and IgG receptor concentration on viable colonocytes in healthy Indian population. In this study we recruited 25 healthy children and 25 healthy adults from North India and provided the range of IgA and IgG receptor concentration on viable colonocytes for both groups. Results indicated that the difference in the mean IgA and IgG receptor concentration was statistically significant in both groups.

	Children		Adult	
	% IgA	% IgG	% IgA	% IgG
Average ± SE	71.30 ± 0.97	65.73 ± 1.13	66.82 ± 1.26	62.71 ± 1.32
Range (Min –Max)	64.2 – 86.5	56.5 – 76.5	50.5 – 78.2	45.5 – 70.4
Median	70.2	66.5	66.8	64.5

Table 1: IgA and IgG receptor concentration on viable colonocytes isolated from stool samples of children and adult group.

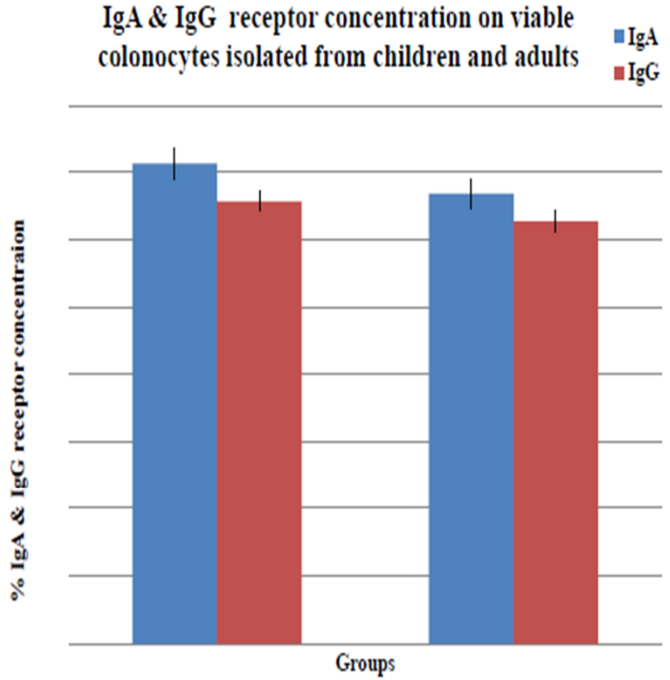

Figure 1: IgA & IgG receptor concentration on viable colonocytes isolated from stool samples of children and adult subjects.

ARTICLE

doi:10.1038/nature23480

A randomized synbiotic trial to prevent sepsis among infants in rural India

Pinaki Panigrahi[1,2], Sailajanandan Parida[3], Nimai C. Nanda[4], Radhanath Satpathy[5], Lingaraj Pradhan[6], Dinesh S. Chandel[7], Lorena Baccaglini[1], Arjit Mohapatra[5], Subhranshu S. Mohapatra[5], Pravas R. Misra[5], Rama Chaudhry[8], Hegang H. Chen[9], Judith A. Johnson[10], J. Glenn Morris Jr[10], Nigel Paneth[11] & Ira H. Gewolb[12]

Sepsis in early infancy results in one million annual deaths worldwide, most of them in developing countries. No efficient means of prevention is currently available. Here we report on a randomized, double-blind, placebo-controlled trial of an oral synbiotic preparation (*Lactobacillus plantarum* plus fructooligosaccharide) in rural Indian newborns. We enrolled 4,556 infants that were at least 2,000 g at birth, at least 35 weeks of gestation, and with no signs of sepsis or other morbidity, and monitored them for 60 days. We show a significant reduction in the primary outcome (combination of sepsis and death) in the treatment arm (risk ratio 0.60, 95% confidence interval 0.48–0.74), with few deaths (4 placebo, 6 synbiotic). Significant reductions were also observed for culture-positive and culture-negative sepsis and lower respiratory tract infections. These findings suggest that a large proportion of neonatal sepsis in developing countries could be effectively prevented using a synbiotic containing *L. plantarum* ATCC-202195.

Nature : 2017 Vol 548

GLOBAL HEALTH

Probiotic prevents infections in newborns

A major cause of death and disease in babies is the failure of their developing immune systems to block life-threatening infections. A clinical trial reports that the use of a probiotic can help to prevent such infections.

DANIEL J. TANCREDI

Infections continue to be a considerable cause of death and disease among infants in low- and middle-income countries[1]. Newborns are susceptible to infection because key parts of their immune systems are still developing and not fully functional, particularly in premature babies (born at less than 37 weeks of gestation) and those with a low birth weight[2,3]. Also of concern is a response to infection that results in a condition known as sepsis, in which widespread inflammation and a compromised blood circulation can result in devastating organ and tissue injuries[3] and impairments to growth and development. In a paper online in *Nature*, Panigrahi *et al.*[4] report the results of a clinical trial conducted in rural India to assess whether feeding newborn infants with preparations of health-promoting bacteria can prevent serious bacterial infections and sepsis.

Intensive care and antibiotic treatment are usually, but not always, effective in treating severe sepsis due to bacteria. However, timely antibiotic treatment might not be available in some locations, and antibiotic use can have adverse effects, including decimation of health-promoting gut bacteria and selection for antibiotic-resistant bacteria. There is a continuing need to develop and implement effective sepsis-prevention strategies.

Panigrahi and colleagues tested whether sepsis could be prevented in newborns by orally administering probiotics — live microorganisms that can provide a health benefit — and prebiotics, which are molecules,

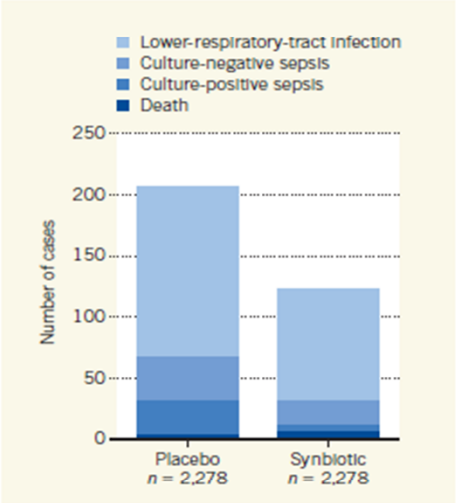

Figure 1 | A clinical trial to prevent sepsis. Panigrahi *et al.*[4] conducted a randomized clinical trial in rural India to assess whether feeding newborns a daily dose of a probiotic strain of the gut bacterium *Lactobacillus plantarum* and a carbohydrate that promotes healthy bacteria — a combination known as a synbiotic — for one week affected the incidence of a serious inflammatory condition called sepsis. Outcomes, including death and the occurrence of three types of sepsis, were monitored for 2 months in 4,556 infants who were randomly assigned to either a group receiving placebo or one receiving the synbiotic preparation. Sepsis or death occurred in 9.0% of the placebo group compared with 5.4% of the infants in the synbiotic group — a reduction of 40%.

developed in the Indian state of Odisha, a region classified as in the low to middle tier of sociodemographic development[8]. This setting was well suited for evaluating the benefit of a probiotic-based strategy in a context in which other health-promoting strategies are being used in newborns. Although the study was specifically designed to include infants born in the community, around 85% of the study subjects were born in hospitals, reflecting the increasing use of hospitals for deliveries. In addition, to be eligible for the study, mothers had to have started breastfeeding within the first 24 hours of the infant's life, a practical and effective strategy for reducing the risk of infection. A mother's milk contains prebiotics, as well as other molecules that strengthen gut barriers and immunological defences against pathogens[9].

The authors evaluated a synbiotic preparation given daily for one week to full-term and late-preterm infants, beginning around postnatal day 3. The oral preparation contained the bacterium *Lactobacillus plantarum* — selected from other probiotic candidates because it had previously been shown[10] to have favourable gut-colonizing properties in newborns in this setting — along with fructooligosaccharide, a plant-derived prebiotic[5]. This well-conducted double-blind trial, with a placebo control, began to enrol infants in 2008 and is the first to examine whether a probiotic-based preparation can prevent sepsis in a large sample consisting mainly of full-term newborns.

There is no consensus definition of sepsis. To measure the incidence of the condition, community health workers checked the infants daily for the presence of one of seven signs of possible severe bacterial infection recommended by the World Health Organization as criteria[11] to facilitate early referral, diagnosis and treatment of young infants[12,13]. For an infant to be counted as having sepsis, a physician had to confirm that one of the seven signs was present and conclude that the infant required hospitalization and antibiotic treatment for five days or more. Because such cases occurred later than postnatal day 3, they are termed late-onset cases[3]. The

Changes in the Gut Microbiota After Early Administration of Oral Synbiotics to Young Infants in India

*†Dinesh S. Chandel, ‡§Maria E. Perez-Munoz, ||Fang Yu, ¶Robert Boissy, #Radhanath Satpathy, #Pravas R. Misra, **Nidhi Sharma, **Rama Chaudhry, ††Sailajanandan Parida, ‡‡‡Daniel A. Peterson, §§Ira H. Gewolb, and *||||Pinaki Panigrahi

ABSTRACT

Objectives: The authors examined the changes in the developing gut microbiota of Indian infants enrolled in a colonization study of an oral synbiotic (*Lactobacillus plantarum* and fructo-oligosaccharides) preparation.

Methods: Frozen stool samples were available from a previously published clinical study of the synbiotic preparation administered daily for 7 days to full-term Indian infants delivered by C-section. 16S rRNA gene sequencing of fecal bacterial community-DNA was done in 11 infants sampled on day 7 and day 60 of life.

Results: All infants showed changes in bacterial diversity with age. While Firmicutes and Proteobacteria were predominant in all, Actinobacteria and Bacteroidetes were initially low on day 7. In control infants, we observed a significant increase ($P = 0.012$) in the proportions of Actinobacteria on day 60. In the treated group, during the 60-day period, there was a 10-fold increase in Bacteroidetes, a somewhat smaller increase in Firmicutes, and a reduction in Proteobacteria. Compared to controls, treated infants were increasingly colonized by different Gram-positive genera including *Enterococcus*, *Lactobacillus*, and *Bifidobacterium*. Relatively less known taxa and some unassigned sequence reads added to enriched diversity observed in the treated group.

Conclusions: There was a high level of bacterial diversity among infants examined in the present study. Synbiotic treatment induced an increase in overall taxa and Gram-positive diversity, especially in the first week of life. Changes in the microbiota during early infancy should be used as a rationale for selecting probiotics in diverse clinical settings.

Key Words: 16S rRNA gene sequencing, gut microbiota, infant, *Lactobacillus plantarum*, probiotics, synbiotics

(*JPGN* 2017;65: 218–224)

What Is Known

- Enterobacteriaceae, followed by microaerophiles, and finally strict anaerobes colonize the human gut during early infancy.
- Limited number of sequence-based analyses in Western countries show predominance of Firmicutes, Proteobacteria, and subsequent rise in Bacteroidetes when solid food is introduced.

What Is New

- Utilizing 16S rRNA gene sequence analyses, the present study shows low proportion of Proteobacteria and high abundance of Bacteroidetes and Firmicutes in 2-month-old Indian infants.
- The present study also demonstrates a high level of microbiota diversity among infants in early infancy.
- Synbiotic treatment for 1 week induces and maintains diversity for 2 months in life.

Although the nascent microbiome acquired during or after birth may influence infant gut development and immunity, disrupted colonization patterns early in infancy can lead to morbidity later in life (1). It is conceivable that interventions during this early infancy period could have a long-lasting effect on human health and disease. Although investigators in the Western world have engaged in

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Changes in the Gut Microbiota After Early Administration of Oral Synbiotics to Young Infants in India

Figure 1. Synbiotic treated vs. Controls: Phylum proportions

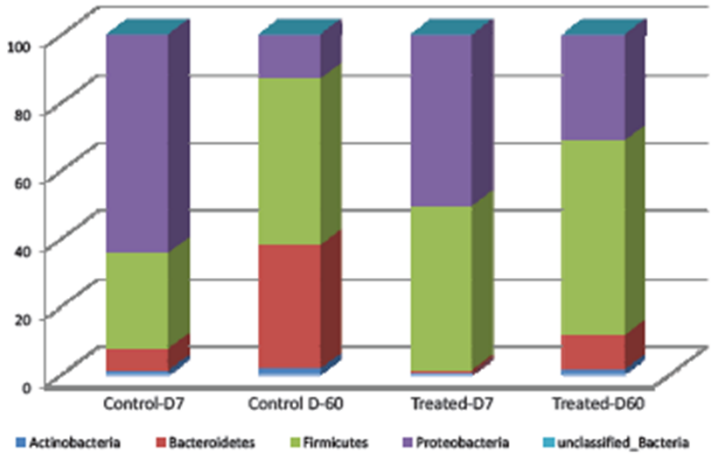
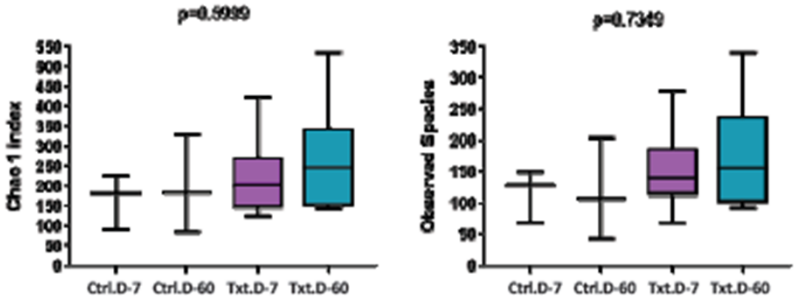


Figure 3. Alpha Diversity in Control and Synbiotic treated subjects

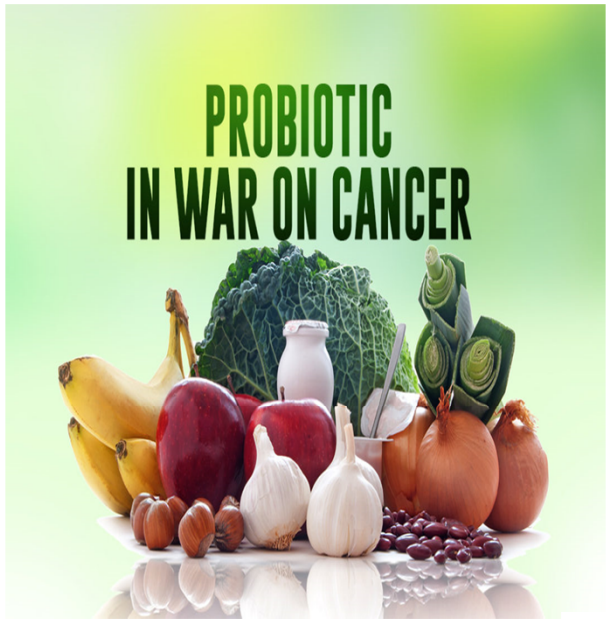


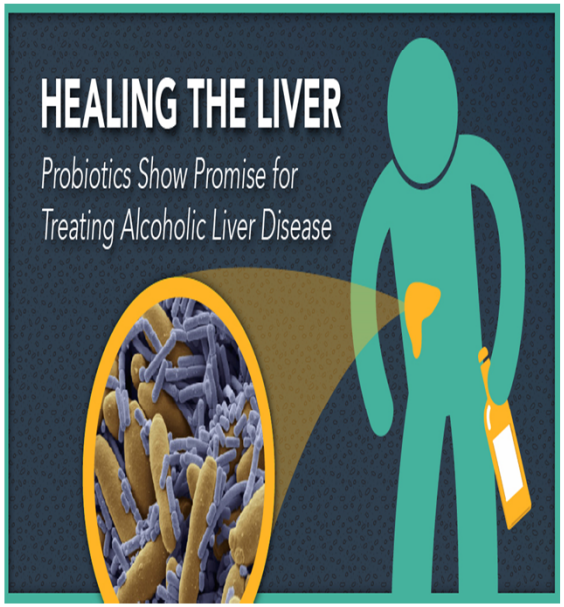
Conclusion : There was a high level of bacterial diversity among infants examined in this study. Synbiotic treatment induced an increase in overall taxa and Gram positive diversity, especially in the first week of life. Changes in the microbiota during early infancy should be used as a rationale for selecting probiotics in diverse clinical settings.

Conclusions and directions

- **Probiotics are good immuno-modulator and may have an important role in the prevention & treatment of diseases.**
- **The clinical benefit of probiotic are strain specific and depends on numerous factors including, dosing, duration and host factors**
- **Need for the development of high quality probiotics and probiotic products**
- **Identification & use of molecular components from probiotics, may be a novel approach for targeted treatment of various diseases**
- **Well designed RCTs and Observational studies to establish disease / health condition based safety and efficacy of probiotic strains and products.**

Thank you





Probiotics are wonder immunomodulatory agent to promote a good health as well as for prevention & treatment of various clinical conditions.