BIOTASCOPE

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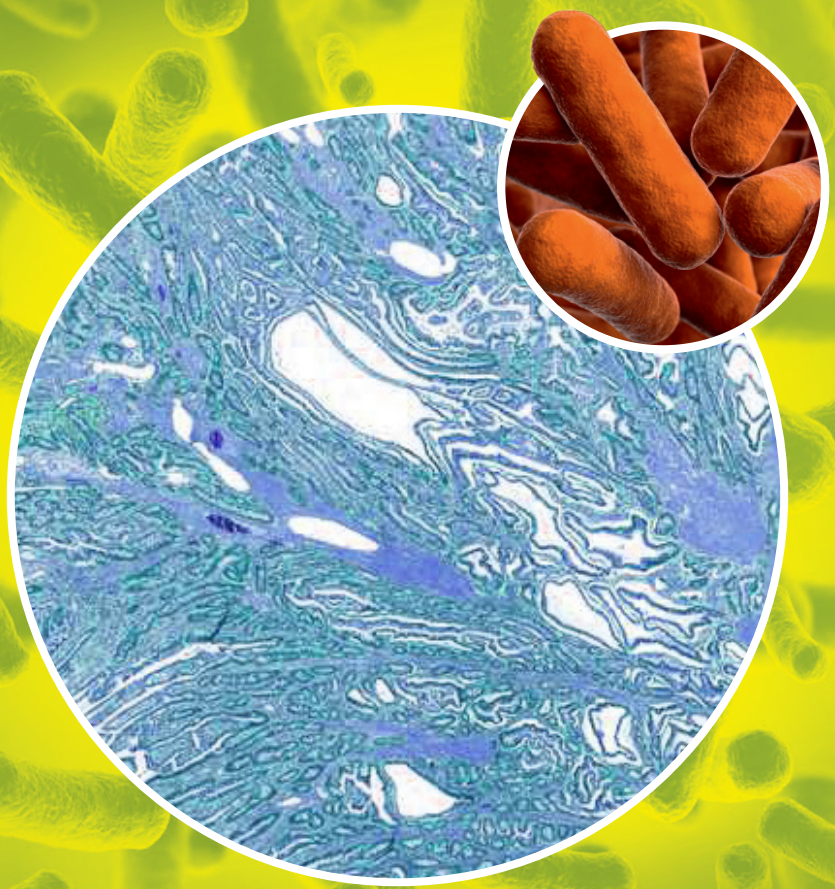
Translational Science in Microbiota



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Cover: Fotolia.com - Microscope picture of intestinal polyp.

Editorial

Dear Colleagues,

It was a great pleasure for us to hold the first issue of Biotascope in our hands. This new journal was distributed to various countries around the world and attracted great attention by our readers. We appreciate all of your feedback that we have received by e-mail.

Each issue of Biotascope will be divided into sections reporting clinical (adult and pediatric) data, in basic translational review articles, as well as providing summaries of the latest publications in the field (Essence From the Literature) and from important international meetings (Whispers From Conferences). These sections will allow readers from various backgrounds to advance their knowledge in the field.

Here we present the second issue of Biotascope, with interesting articles from world-wide authors known in the field.

Irritable bowel syndrome (IBS) is a common disease affecting approximately 10–15% of the population worldwide resulting in significant global morbidity. Its relationship with gastrointestinal infectious disease was defined more than 40 years ago. This association opened new therapeutic areas such as controlled manipulation of gut flora with medications (probiotics, antibiotics, etc) as well as the newer therapeutic option **Fecal Microbiota Transplantation.** Three of the articles in this issue focused on this interesting new therapeutic option; **Dr Satish Rao** (Augusta, GA, USA) wrote an article on post-infectious IBS, covering from basic science to clinical approach, **Dr Giovanni Barbara** and colleagues from Bologna University, Italy communicated their experience in a basic science (and translational) article entitled **"Gut Microbiota and Permeability in Irritable Bowel Syndrome"** and **Dr Eamonn Quigley** from Houston Methodist Hospital, Houston, TX, USA, summarized the latest achievement in the field of **Fecal Microbiota Transplantation.** For our readers who are interested in pediatric age groups, another article by a well-known scientific group from UZ Brussels, Department of Neonatology, Brussels, Belgium lead by **Dr. Vandenplas,** discussed a totally different topic **"Probiotic-Fortified Formula in the Prevention of NEC"** (necrotizing enterocolitis).

Annalisa Passariello, a member of ISGoP who works in the Department of Translational Medical Science at the University of Naples Neonatology Unit, in Italy summarized the latest data from the **48th Annual Meeting of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN).** This meeting was held in Amsterdam from May 6 to 9, 2015. If you did not attend the meeting do not worry, Annalisa covered nearly all presentations on microflora. Another conference summary which we call **"Whispers From Conferences"** was written by **Henry Cohen** from one of the biggest conference in the field of Gastroenterology, **Digestive Diseases Week,** which was held in Washington, May 16–19, 2015.

Biotascope covers the latest data and science in each issue with the summaries of the literature. In this issue, **Dr Tarkan Karakan** from the Gastroenterology Department of the Gazi University School of Medicine, Ankara, Turkey, reviewed the latest literature including publications entitled **"The Oral and Gut Microbiomes are Perturbed in Rheumatoid Arthritis and Partly Normalized After Treatment", "Effect of Probiotics on Glycemic Control: A Systematic Review and Meta-Analysis of Randomized, Controlled Trials",** and **"Gut-Microbiota-Metabolite Axis in Early Renal Function Decline".** He also looked into some aspects of hygiene theory in children with an interesting publication about whether older siblings have an effect on gut microbiota development in younger siblings during early childhood.

The next issue will cover the relationship between diabetes-obesity and microflora and some dermatological problems related to gut microbiota disturbances.

Please send us your opinions and feedback as well as the topics you would like to see in upcoming journal issues to our e-mail seen below.

Best wishes from International Study Group of Probiotics (ISGoP).

Sincerely,

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FECAL MICROBIOTA TRANSPLANTATION (FMT)

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INTRODUCTION

Although fecal microbiota transplantation (FMT), or stool transplantation, as it was formerly known, has been used for decades, for a variety of intestinal and systemic ills it has only been recently, in the wake of the microbiota revolution, that it has attracted serious scientific interest. Furthermore, up until very recently clinical data were almost entirely composed of anecdotal reports and case series based on unstandardized protocols. Within the past few years, the status of FMT has changed dramatically. The first high-quality randomized clinical trials have emerged, and considerable efforts are being made to standardize protocols and investigate the biological basis for the apparent success of FMT.

CLOSTRIDIUM DIFFICILE INFECTION

For the most part, the impetus for the exponential increase in the interest in FMT has related to its use in recurrent *Clostridium difficile* infection. Uncontrolled case series, as well as a limited amount of data from randomized controlled trials, attest to its dramatic and long-lasting impact. *Pseudomembranous colitis*, due to *C. difficile* infection, is the most severe manifestation of antibiotic-related diarrhea and should serve as a constant reminder of the potential impact of disrupting the commensal microbiome and its symbiotic/mutually beneficial relationship with the host. *C. difficile* has emerged as a major clinical issue across the globe; in the US almost half a million cases were reported in 2011[1]. Indeed, *C. difficile* is now the most common cause of healthcare-associated infection in the US, and an ever increasing proportion of cases are community acquired[1,2]. Furthermore, it has been estimated that approximately 21% of healthcare-acquired infections will recur and that 9% of affected individuals will die within 30 days of acquiring their first infection[1]. In various case series and meta-analyses, risk factors for recurrence have variably included: older age, and persistent use of antibiotics (e.g. fluoroquinolones), antacids and proton pump inhibitors[3-5]. Of these, older age has been the most consistent risk factor[3-5]. There is some evidence that newer generation antibiotics, such as fidaxomycin, may be associated with a lower recurrence rate[2]. Nevertheless, as many as 60% of patients with recurrent infection will develop further episodes, despite further courses of standard antibiotic therapy (typically, metronidazole followed by vancomycin)[2].

The microbiome of the individual who develops recurrent *C. difficile* infection displays a marked reduction in bacterial diversity, is depleted in normal constituents, such as *Bacteroidetes* and *Firmicutes*, replete with aerotolerant organisms, such as *Proteobacteria* and *Bacilli* and, at a metabolic level, devoid of butyrate producers[6-9]. FMT seems to effect a persisting "therapeutic reset" of the microbiome of the recipient which comes to resemble that of the donor with restoration of butyrate producers, *Clostridia* and *Bacteroidia*[7-11]. Interestingly, FMT has also been shown to normalize the composition of bile acids in feces[12].

Overall, the response rate from FMT in recurrent *C. difficile* infection ranges from 83% to 94%[13-15]. In a long-term (mean 17 months) follow-up of 77 patients treated with FMT at various centers in the US, the primary cure rate was 91% and the secondary cure rate (i.e., after further courses of FMT) was 98%[16]. It was notable that in this series 74% of patients had resolution of diarrhea within 3 days, and all late recurrences were attributable to further use of antibiotics for reasons unrelated to *C. difficile* infection[16]. The findings from open, uncontrolled, studies have now been confirmed by a randomized, controlled trial of nasoduodenal infusion of donor stool[17] and by an open-label, randomized trial of colonoscopically delivered FMT[18]. FMT has even been reported to be effective in treating toxic megacolon related to *C. difficile* infection[19].

While this is a rapidly evolving field, FMT is currently recommended exclusively for individuals who have had at least three recurrent episodes despite appropriate antibiotic therapy. For colonoscopically-delivered FMT a standardized approach to donor screening and selection has been developed (Table 1 and 2)[20]. While there is some evidence to suggest that results are better with transplants from immediate family members, the availability of effective frozen transplants[21], stool substitutes[22] or even encapsulated frozen transplants[23] from universal donors may greatly simplify donation.

At his time however, data on these alternative approaches is limited. Several other issues remain to be resolved and include such important issues as optimal patient preparation, route of administration, and volume and site of infusion[20,24,25].

Table 1. **Donor selection criteria for fecal microbiota transplantation[20]**
1 - No known communicable disease.
2 - No recent (3 months) antibiotic use.
3 - No history of chronic diarrhea.
4 - No history of an immune disorder including atopic diseases including eczema, asthma, or eosinophilic disorders of the gastrointestinal tract.
5 - No concurrent immunosuppressive agents.
6 - No history of inflammatory bowel disease, chronic constipation, or irritable bowel syndrome.
7 - No history of malignancy (except non-melanoma skin cancer)
8 - No recent (6 months) travel to endemic diarrhea areas.
9 - No current anti-neoplastic agent therapy.
10 - No current gastrointestinal symptoms.
11 - No risk factors—intravenous drug use, high-risk sexual behaviors, tattoos, current or historical incarceration, or body piercing (6 months).
12 - No diabetes mellitus type II or metabolic syndrome.
13 - No chronic pain syndromes.

Table 2. **Screening protocol prior to fecal microbiota transplantation[20]**

Screen	Blood	Stool
Recipient	Hepatitis A IgM	
	Hepatitis B core IgM and IgG	
	Hepatitis B surface antigen	
	Hepatitis B surface antibody	
	Hepatitis C IgG	
	HIV types 1, 2	
	RPR test for syphilis	
Donor	Hepatitis A IgM	*Clostridium difficile* toxin B by PCR
	Hepatitis B core IgM and IgG	Giardia, norovirus antigen
	Hepatitis B surface antigen	Cyclospora, crytosporidia, isospora
	Hepatitis B surface antibody	Ova and parasites
	Hepatitis C IgG	Shiga toxin, *Escherichia coli*, Salmonella, Shigella, Yersinia, Campylobacter, Non-cholera Vibrio
	HIV types 1, 2	
	RPR test for syphilis	

HIV = Human Immunodeficiency Virus; Ig = immunoglobulin; PCR = polymerase chain reaction; RPR = Rapid Plasma Reagin.

INFLAMMATORY BOWEL DISEASE

A considerable body of evidence suggests that the gut microbiome and its interaction with the host are fundamental to the pathogenesis of inflammatory bowel disease (IBD), irrespective of whether it is Crohn's disease (CD) or ulcerative colitis (UC)[26]. However, it remains unknown whether a specific microbial signature (or signatures) predisposes to IBD. To date, fully published studies of FMT in IBD have been limited to case reports and case series[26-29]. While there is good evidence that FMT is effective in addressing recurrent *C. difficile* infection complicating IBD[30,31], results in the management of IBD, per se, have been mixed[28]. Therefore, pending the publication of well-designed clinical trials, FMT cannot be recommended as a management strategy in patients with IBD.

FMT IN FUNCTIONAL GASTROINTESTINAL DISORDERS

Changes in the small intestine and colonic microbiome have been described in both irritable bowel syndrome (IBS) and chronic constipation. In these populations, anecdotal reports and small case series suggest that FMT is effective[32,33]. Again a lack of high-quality data precludes a therapeutic recommendation for the use of FMT in these patients. As with IBD, IBS and other functional disorders are phenotypically heterogeneous and may well harbor entities of varying pathophysiologies. It is very likely that the role of the microbiome may vary considerably between such phenotypes, and that the "reset" approach which seems to work so well in recurrent *C. difficile* infection may be too simplistic in IBD and IBS where a much more tailored approach may needed.

OTHER DISORDERS

Changes in the microbiome have been reported in a host of systemic diseases. However, the significance of such associations is generally unclear due to small study populations, lack of accountability for confounding factors, and failure to report the results of interventions. Nevertheless, based on such observations, as well as on a purely empirical basis, FMT has been advocated and sometimes performed across a variety of disorders, ranging from eosinophilic enteritis through to neurological disorders, such as autism, multiple sclerosis and Parkinson's disease, and metabolic disorders[34-36]. Of these, the best evidence relates to the metabolic syndrome where improved insulin sensitivity has been documented following FMT[37]. In other areas, data is confined to case reports, small series or remains in the realm of speculation.

SAFETY

Apart from the procedural-related risks associated with the transplant, FMT could, in theory, be associated with a variety of adverse events. Of greatest concern, is the possibility of transmission of infection; thus rigorous screening protocols have been recommended and instituted at many centers. To date, there have been reports of transmission of norovirus[38], bacteremia (in a patient with IBD)[39], and the development of diverticulitis[40] following FMT. Conversely, FMT has been safely and effectively performed to treat recurrent *C. difficile* infection in children[41] and immunocompromised individuals[42]. In the longer term, there is also the theoretical possibility that one could transfer a microbial signature that is truly associated with the development of a disease state in susceptible individuals[43]. Therefore some centers have insisted that obese individuals are excluded from donation. Only time will tell.

CONCLUSION

At the present time, the only established indication for FMT is recurrent *C. difficile* infection. Rapid progress in the microbiological aspects of the procedure may lead to the development of more readily available and easily delivered preparations, as well as microbial cocktails containing the bacteria essential for a given indication. From a technique that historically owed more to anecdotal evidence and speculation than science a novel therapeutic intervention has emerged that may well have an enormous impact on medicine and medical science.

References

(1) Lessa FC, Mu Y, Bamberg WM, Beldavs ZG, Dumyati GK, Dunn JR, et al. Burden of Clostridium difficile infection in the United States. N Engl J Med. 2015;372:825–34.

(2) Bagdasarian N, Rao K, Malani PN. Diagnosis and treatment of Clostridium difficile in adults: a systematic review. JAMA. 2015;313:398–408.

(3) Garey KW, Sethi S, Yadav Y, DuPont HL. Meta-analysis to assess risk factors for recurrent Clostridium difficile infection. J Hosp Infect. 2008;70:298–304.

(4) Shivashankar R, Khanna S, Kammer PP, Scott Harmsen W, Zinsmeister AR, Baddour LM, et al. Clinical predictors of recurrent Clostridium difficile infection in out-patients. Aliment Pharmacol Ther. 2014;40:518–22.

(5) Zilberberg MD, Reske K, Olsen M, Yan Y, Dubberke ER. Risk factors for recurrent Clostridium difficile infection (CDI) hospitalization among hospitalized patients with an initial CDI episode: a retrospective cohort study. BMC Infect Dis. 2014;14:306.

(6) Chang, JY, Antonopoulos DA, Kalra A, Tonelli A, Khalife WT, Schmidt TM, et al. Decreased Diversity of the Fecal Microbiome in Recurrent Clostridium difficile—Associated Diarrhea, J Infect Dis;2008;197:435–8.

(7) Song Y, Garg S, Girotra M, Maddox C, von Rosenvinge EC, Dutta A, et al. Microbiota dynamics in patients treated with fecal microbiota transplantation for recurrent Clostridium difficile infection. PLoS One. 2013;8:e81330.

(8) Seekatz AM, Aas J, Gessert CE, Rubin TA, Saman DM, Bakken JS, et al. Recovery of the gut microbiome following fecal microbiota transplantation. Mol Bio. 2014;5:e00893–14.

(9) Shankar V, Hamilton MJ, Khoruts A, Kilburn A, Unno T, Paliy O, et al. Species and genus level resolution analysis of gut microbiota in Clostridium difficile patients following fecal microbiota transplantation. Microbiome. 2014;2:13.

(10) Hamilton MJ, Weingarden AR, Unno T, Khoruts A, Sadowsky MJ. High-throughput DNA sequence analysis reveals stable engraftment of gut microbiota following transplantation of previously frozen fecal bacteria. Gut Microbes. 2013;4:125-35.

(11) Fuentes S, van Nood E, Tims S, Heikamp-de Jong I, ter Braak CJ, Keller JJ, et al. Reset of a critically disturbed microbial ecosystem: faecal transplant in recurrent Clostridium difficile infection. ISME J. 2014;8:1621–33.

(12) Weingarden AR, Chen C, Bobr A, Yao D, Lu Y, Nelson VM, et al. Microbiota transplantation restores normal fecal bile acid composition in recurrent Clostridium difficile infection. Am J Physiol Gastrointest Liver Physiol. 2014; 306:G310–9.

(13) Gough, E, Shaikh H, Manges AR. Systematic review of intestinal microbiota transplantation (fecal bacteriotherapy) for recurrent Clostridium difficile infection. Clin Infect Dis. 2011;53:994–1002.

(14) Brandt LJ. American Journal of Gastroenterology Lecture: Intestinal Microbiota and the Role of Fecal Microbiota Transplant (FMT) in treatment of C difficile infection. Am J Gastroenterol. 2013;108:177–85.

(15) Cammarota G, Ianiro G, Gasbarrini A. Fecal microbiota transplantation for the treatment of Clostridium difficile infection: a systematic review. J Clin Gastroenterol. 2014; 48: 693–702.

(16) Brandt LJ, Aroniadis OC, Mellow M, Kanatzar A, Kelly C, Park T, et al. Long-term follow-up of colonoscopic fecal microbiota transplant for recurrent Clostridium difficile infection. Am J Gastroenterol. 2012;107:1079–87.

(17) van Nood, E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, et al. Duodenal infusion of donor feces for recurrent Clostridium difficile. N Engl J Med. 2013;368:407–15.

(18) Cammarota G, Masucci L, Ianiro G, Bibbò S, Dinoi G, Costamagna G, et al. Randomised clinical trial: faecal microbiota transplantation by colonoscopy vs. vancomycin for the treatment of recurrent Clostridium difficile infection. Aliment Pharmacol Ther. 2015;41(9):835–43.

(19) Gweon TG, Lee KJ, Kang DH, Park SS, Kim KH, Seong HJ, at al. A case of toxic megacolon caused by Clostridium difficile infection and treated with fecal microbiota transplantation. Gut Liver. 2015;15:247–50.

(20) Owens, C, Broussard E, Surawicz C. Fecal microbiota transplantation and donor standardization. Trends Microbiol. 2013;21:443–5.

(21) Satokari R, Mattila E, Kainulainen V, Arkkila PE. Simple faecal preparation and efficacy of frozen inoculum in faecal microbiota transplantation for recurrent Clostridium difficile infection--an observational cohort study. Aliment Pharmacol Ther. 2015;41:46–53.

(22) Petrof EO, Gloor GB, Vanner SJ, Weese SJ, Carter D, Daigneault MC, et al. Stool substitute transplant therapy for the eradication of Clostridium difficile infection: 'RePOOPulating' the gut. Microbiome. 2013;1:3.

(23) Youngster I, Russell GH, Pindar C, Ziv-Baran T, Sauk J, Hohmann EL. Oral, capsulized, frozen fecal microbiota transplantation for relapsing Clostridium difficile infection. JAMA. 2014;312:1772–8.

(24) Bourlioux P; the workgroup of the French Academy of Pharmacy. Faecal microbiota transplantation: Key points to consider. Ann Pharm Fr. 2015;73(3):163–8.

(25) Vyas D, Aekka A, Vyas A. Fecal transplant policy and legislation. World J Gastroenterol. 2015;21:6–11.

(26) Wang ZK, Yang YS, Chen Y, Yuan J, Sun G, Peng LH. Intestinal microbiota pathogenesis and fecal microbiota transplantation for inflammatory bowel disease. World J Gastroenterol. 2014;20:14805–20.

(27) Bennet JD, Brinkman M. Treatment of ulcerative colitis by implantation of normal colonic flora. Lancet. 1989;1:164.

(28) Colman RJ, Rubin DT. Fecal microbiota transplantation as therapy for inflammatory bowel disease: a systematic review and meta-analysis. J Crohns Colitis. 2014;8:1569–81.

(29) Cui B, Feng Q, Wang H, Wang M, Peng Z, Li P, et al. Fecal microbiota transplantation through mid-gut for refractory Crohn's disease: safety, feasibility, and efficacy trial results. J Gastroenterol Hepatol. 2015;30:51–8.

(30) Smith S. Intestinal microbiota transplantation: a case of Crohn's colitis with superimposed Clostridium difficile infection. West Indian Med J.2013;62:675–7.

(31) Aratari A, Cammarota G, Papi C. Fecal microbiota transplantation for recurrent C. difficile infection in a patient with chronic refractory ulcerative colitis. J Crohns Colitis. 2015;9(4):367.

(32) Pinn DM, Aroniadis OC, Brandt LJ. Is fecal microbiota transplantation (FMT) an effective treatment for patients with functional gastrointestinal disorders (FGID)? Neurogastroenterol Motil. 2015;27:19–29.

(33) Pinn DM, Aroniadis OC, Brandt LJ. Is fecal microbiota transplantation the answer for irritable bowel syndrome? A single-center experience. Am J Gastroenterol. 2014;109:1831–2.

(34) Borody TJ, Warren EF, Leis SM, Surace R, Ashman O, Siarakas S. Bacteriotherapy using fecal flora: toying with human motions. J Clin Gastroenterol. 2004;38:475–83.

(35) Sha S, Liang J, Chen M, Xu B, Liang C, Wei N, et al. Systematic review: faecal microbiota transplantation therapy for digestive and nondigestive disorders in adults and children. Aliment Pharmacol Ther. 2014;39:1003–32

(36) Dai YX, Shi CB, Cui BT, Wang M, Ji GZ, Zhang FM. Fecal microbiota transplantation and prednisone for severe eosinophilic gastroenteritis. World J Gastroenterol. 2014;20:16368–71.

(37) Vrieze A, Van Nood E, Holleman F, Salojärvi J, Kootte RS, Bartelsman JF, et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. Gastroenterology. 2012;143:913–6.

(38) Schwartz M, Gluck M, Koon S. Norovirus gastroenteritis after fecal microbiota transplantation for treatment of Clostridium difficile infection despite asymptomatic donors and lack of sick contacts. Am J Gastroenterol. 2013;108:1367.

(39) Quera R, Espinoza R, Estay C, Rivera D. Bacteremia as an adverse event of fecal microbiota transplantation in a patient with Crohn's disease and recurrent Clostridium difficile infection. J Crohns Colitis. 2014;8:252–3.

(40) Mandalia A, Kraft CS, Dhere T. Diverticulitis after fecal microbiota transplant for C. difficile infection. Am J Gastroenterol. 2014;109:1956–7.

(41) Kronman MP, Nielson HJ, Adler AL, Giefer MJ, Wahbeh G, Singh N, et al. Fecal microbiota transplantation via nasogastric tube for recurrent clostridium difficile infection in pediatric patients. J Pediatr Gastroenterol Nutr. 2015;60:23–6.

(42) Kelly CR, Ihunnah C, Fischer M, Khoruts A, Surawicz C, Afzali A, et al. Fecal microbiota transplant for treatment of Clostridium difficile infection in immunocompromised patients. Am J Gastroenterol. 2014;109:1065–71.

(43) Gregory JC, Buffa JA, Org E, Wang Z, Levison BS, Zhu W, et al. Transmission of atherosclerosis susceptibility with gut microbial transplantation. J Biol Chem. 2015;290:5647–60.

PROBIOTIC-FORTIFIED FORMULA IN THE PREVENTION OF NEC

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None related to this article. YVDP is consultant for Biocodex and United Pharmaceuticals.
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ABSTRACT

Necrotizing enterocolitis (NEC) is a common cause of neonatal morbidity and mortality, and in many centers it is the most common gastrointestinal emergency in newborns. The exact pathogenesis is unknown but colonization by pathologic microorganisms might be one of the risk factors for NEC in preterm newborns. Human breast milk seems to be protective in the prevention of NEC and breastfeeding should be strongly advised in all newborns of all gestational ages. In cases where breastfeeding is not possible, (pre-term) formula is the second choice. In this review, we summarized the importance of the addition of probiotics to (pre-term) formula to avoid bacterial overgrowth and prevent NEC. We performed an intensive literature search using databases to find relevant original papers and reviews. Although there are some contradictions within the literature, in general our research showed a positive effect of probiotics in decreasing the incidence and severity of NEC, lowering the NEC-related mortality, improving feeding tolerance and improving weight gain. In conclusion, given the positive effect of probiotics in NEC further research is needed to find the best strain, dosing protocol and time to start probiotics. Since there is significant variability in the incidence of NEC between hospitals, each individual physician has to consider the necessity of the use of probiotics in their clinic, taking the incidence, morbidity and mortality of NEC in their specific population into account.

INTRODUCTION

Necrotizing enterocolitis (NEC) is a multifactorial disease that results from the interaction between the loss of integrity of the intestinal mucosa and the host response to this damage. It is determined by intestinal ischemia, mucosal damage, edema, ulceration, and passage of air or (gas producing) bacteria through the wall, resulting in necrosis of the mucosa and intestinal wall[1].

NEC is a common cause of neonatal morbidity and mortality and in many centers it is the most common gastrointestinal emergency in newborns[2, 3], affecting one out of ten newborns born before 29 weeks gestation[4]. Mortality rates reach up to 20% to 30% of very-low birth weight (VLBW) newborns (birth weight ≤1500 grams) affected with NEC[5]. Newborns who survive the disease are at risk for long-term complications, including neurodevelopmental impairment, short bowel syndrome, and impaired growth[6, 7].

The exact pathogenesis of NEC is unknown but prematurity, rapid full enteral feeding and colonization by pathologic microorganisms increase the risk of NEC. Compared with preterm formula, human breast milk appears to be protective. Premature newborns are more likely to develop an overgrowth of pathologic microorganisms due to delayed acquisition of commensal microorganisms[8], exposition to antibiotic therapy[7], an increased rate of delivery by cesarean, whereby they are less likely to acquire commensal flora from the birth canal, and delayed enteral feeding where they do not acquire commensal flora from human breast milk[9].

Since pathologic bacterial overgrowth plays an important role in the pathogenesis of NEC, prevention of this overgrowth is likely to decrease the incidence and the severity of NEC in premature newborns. One of the possible ways to prevent overgrowth by pathologic microorganisms might be the administration of probiotics to (preterm) newborns. Probiotics are defined as: live microorganisms that, when administered in adequate amounts confer a health benefit on the host[10, 11]. Probiotics can improve and restore the microbial flora in two ways: by occupying functional niches, left open by the endogenous community, preventing pathogens from occupying that niche (competitive exclusion), and by actively reducing the invasion and development of opportunistic pathogens into the ecosystem[12].

It is hypothesized that probiotics may lead to a decrease in neonatal sepsis, increased tolerance of enteral food intake and consequently a positive influence on neonatal growth.

PROBIOTICS

Since the introduction of probiotics, many studies have been published evaluating their effect in the prevention of NEC. Studies have used different strains of probiotics, patient populations and dosing protocols, leading to contradictory results and varying advice regarding the use probiotics in preterm newborns.

Reuman and Millar in 1986 and 1993 respectively, investigated the potential for probiotics to expel pathogens from the (premature) newborn bowel by introducing lactobacilli in preterm formula[13, 14]. Although stool samples showed colonization with *lactobacillus*, there was no reduction of potential pathogens in the stools. In 2004, Li and colleagues showed that after a course with Bifidobacteria breve, there was significantly earlier colonization with bifidobacteria if probiotics were started within several hours after birth compared with administration after 24 hours[15]. In 2006, Mohan and colleagues showed a reduction of enterobacteria and clostridia after a course of bifidobacteria, but there was no effect on other pathogens[16].

NECROTIZING ENTEROCOLITIS

One of the first publications researching the effect of probiotics on the incidence of NEC was published in 1999 when Hoyos showed that *Lactobacillus acidophilus* and *B. infantis* in newborns (term and preterm) significantly decreased the incidence of NEC and NEC-related mortality[17]. This paper was followed by a double-blind, multicenter study in 2002 by Dani *et al.*, showing that seven days administration of *L. rhamnosus GG* was not effective in reducing the incidence of NEC in newborns born with a gestational age <33 weeks or a birth weight <1,500 g[18]. Three studies evaluated a mixture of multiple probiotics (*B. infantis/Streptococcus thermophiles/B. bifidus* and *B. infantis/L. acidophilus*) and showed a significant reduction in the incidence of NEC as well as a decrease in the severity of NEC[19, 20, 21].

Several systematic reviews and a Cochrane review on probiotics concluded that there is a significant reduction in the incidence of NEC and amelioration of its severity[22, 23, 24], and multiple studies have shown a significant decrease in NEC-related mortality[19, 20, 21, 25].

Sepsis

Two studies investigating probiotics in infants showed a significant decrease in culture proven neonatal sepsis and sepsis-related mortality[26, 27], while two others could not confirm these findings[18, 28].

Feeding tolerance and growth

Colonization with pathologic microorganisms not only leads to NEC but may also have a negative influence on feeding tolerance. This leads to a prolonged delay in the start of enteral feeding and consequently a delayed exposure to commensal microorganisms, resulting in a vicious negative cycle. Rojas *et al.*, noted a significant reduction in food intolerance in newborns weighing <2,000 g receiving *L. reuteri* compared with newborns without probiotic supplements[29]. Rouge and colleagues reported the same outcome after a combined course of *B. longum BB536* and *L. rhamnosus GG* (ATCC53103), but only in newborns with a birth weight of more than 1000 g[30].

Available evidence indicates that newborns receiving probiotics might have a better weight gain[31] and increased head circumference[32] due to improved commensal intestinal flora and better tolerance to enteral feeding.

Complications

None of the aforementioned studies reported any short-term adverse events related to the use of probiotics in newborns. Although there are at least three published reports showing lactobacillus- and bifidobacteria-related sepsis in newborns after probiotic supplementation[33, 34, 35], the use of probiotics in newborns is considered safe. The best of our knowledge, there are no published studies regarding the long-term adverse events of probiotics in new borns.

DISCUSSION

Despite numerous studies investigating the efficacy of probiotics in the prevention of NEC in newborns, there remains some controversy over the use of probiotics. Some authors state that the advantage of probiotics given to preterm newborns to prevent NEC is unequivocal, concluding that there should not be any delay in giving probiotics to all newborns[36, 37]. Although evidence shows a positive contribution of probiotics to the prevention of NEC, there should be some reservations and therefore caution in the routine use of probiotics in newborns, and especially VLBW preterm infants. Most studies included VLBW preterm infants but they also included

full-term neonates, making it difficult to extrapolate results to the VLBW preterm infants, which are the most susceptible to NEC.

Most studies were either randomized placebo-controlled trials or prospective studies with a historical control group showing probiotics to be better than no treatment. No studies have been published comparing two different strains of probiotics to determine which strain provides the best benefit, including protection against NEC. There are no recent studies investigating preterm formula fortified with microorganisms isolated from human breast milk. Furthermore, there has never been a direct comparison between formula and human milk, nor between human breast milk compared with milk from a human milk bank. Because the mechanism of action of different probiotics may vary it is not possible to extrapolate results from one strain to the other.

There is a lack of evidence from which to determine the best strain of probiotics, the ideal dosing protocol and the best time to initiate postnatal probiotics[38].

Another point of discussion is the necessity for the promotion of extra prevention for NEC, as many Western hospitals have a lower incidence of NEC and have almost no mortality due to NEC. Therefore individual physicians must determine the overall benefit of using probiotics in their own clinic[39].

CONCLUSION

Human breast milk seems to be protective in the prevention of NEC and should be strongly advised in all newborns of all gestational ages. In cases where human milk is not given, (pre-term) formula is the second choice.

Today, it remains difficult to make a straight forward recommendation about the necessity of administering probiotics in the prevention of NEC. Currently there is a lack of evidence regarding the pathogenesis of NEC and the mechanism of action of probiotics in the prevention of NEC. However, studies show a positive effect of probiotics including: i) a decrease in the incidence and severity of NEC; ii) reduced NEC-related mortality; iii) improved feeding tolerance, and iv) improved weight gain. Improved feeding tolerance leads to an earlier exposure to environmental microbes, which indirectly may also have a positive effect in the prevention of NEC. There seems to be no evidence that probiotics reduce the incidence of sepsis. Available data indicates that there are few short-term adverse events, and so probiotics can be considered safe for use in preterm newborns.

At present, it is unclear which probiotic strain should be used, what is the ideal dosing protocol and the best time after birth to start probiotics. Further research is necessary and should be focused on answering these questions. Since it is known that human milk prevents NEC it seems logical to expand research with microorganisms which are present in human milk or at least compare probiotic-fortified formula with breast milk and milk from a human milk bank.

After determining which strain and dose of probiotics has the best effect in the prevention of NEC, individual physicians have to consider the necessity of the use of probiotics in their clinic, taking the incidence and mortality of NEC in their population into account.

References

(1) Bernardo WM, Aires FT, Carneiro RM, Sá FP, Rullo VE, Burns DA. Effectiveness of probiotics in the prophylaxis of necrotizing enterocolitis in preterm neonates: a systematic review and meta-analysis. J Pediatr (Rio J). 2013;89:18-24.

(2) Henry MC, Moss LR. Necrotizing enterocolitis. Ann Rev Med. 2009; 60:111–24.

(3) Lin PW, Stoll BJ. Necrotising enterocolitis. Lancet. 2006; 368:1271–83.

(4) Stoll BJ, Hansen NI, Bell EF, Shankaran S, Laptook AR, Walsh MC, et al. Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network. Pediatrics. 2010; 126:443–56.

(5) Fitzgibbons SC, Ching Y, Yu D, Carpenter J, Kenny M, Weldon C, et al. Mortality of necrotizing enterocolitis expressed by birth weight categories. J Pediatr Surg. 2009; 44:1072–5. discussion 5–6.

(6) Hintz SR, Kendrick DE, Stoll BJ, Vohr BR, Fanaroff AA, Donovan EF, et al. Neurodevelopmental and growth outcomes of extremely low birth weight infants after necrotizing enterocolitis. Pediatrics. 2005;115:696–703.

(7) Patel RM, Denning PW. Therapeutic Use of Prebiotics, Probiotics, and Postbiotics to Prevent Necrotizing Enterocolitis: What is the Current Evidence? Clin Perinatol. 2013;40(1):11–25.

(8) Gewolb IH, Schwalbe RS, Taciak VL, Harrison TS, Panigrahi P. Stool microflora in extremely low birthweight infants. Arch Dis Child Fetal Neonatal Ed. 1999;80:F167–73.

(9) Penders J, Thijs C, Vink C, Stelma FF, Snijders B, Kummeling I, et al. Factors influencing the composition of the intestinal microbiota in early infancy. Pediatrics. 2006;118:511–21.

(10) Food and Agriculture Organization and World Health Organization. 1–4 October 2001. Food and Agriculture Organization.

(11) Hill C, Guarner F, Reid G, Gibson G, Merenstein D, Pot B, et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014;11506–14.

(12) Vandenplas Y, Huys G, Daube G. Probiotics: an update. J Pediatr (Rio J). 2015;91:6-21.

(13) Reuman PD, Duckworth DH, Smith KL, Kagan R, Bucciarelli RL et al. Lack of effect of Lactobacillus on gastrointestinal bacterial colonization in premature infants. Pediatr Infect Dis. 1986;5(6):663-8.

(14) Millar MR, Bacon C, Smith SL, Walker V, Hall MA. Enteral feeding of premature infants with Lactobacillus GG. Arch Dis Child. 1993;69:483–7.

(15) Li Y, Shimizu T, Hosaka A, Kaneko N, Ohtsuka Y, Yamashiro Y. Effects of bifidobacterium breve supplementation on intestinal flora of low birth weight infants. Pediatr Int. 2004;46(5):509–15.

(16) Mohan R, Koebnick C, Schildt J, Schmidt S, Mueller M, Possner M, et al. Effects of Bifidobacterium lactis Bb12 supplementation on intestinal microbiota of preterm infants: a double-blind, placebo-controlled, randomized study. J Clin Microbiol. 2006;44(11):4025-31.

(17) Hoyos AB. Reduced incidence of necrotizing enterocolitis associated with enteral administration of Lactobacillus aci-dophilus and Bifidobacterium infantis to neonates in an intensive care unit. Int J Infect Dis. 1999;3(4):197-202.

(18) Dani C, Biadaioli R, Bertini G, Martelli E, Rubaltelli FF. Probiotics feeding in prevention of urinary tract infection, bacterial sepsis and necrotizing enterocolitis in preterm infants. A prospective double-blind study. Biol Neonate. 2002;82(2):103–8.

(19) Bin-Nun A, Bromiker R, Wilschanski M, Kaplan M, Rudensky B, Caplan M, et al. Oral probiotics prevent necrotizing enterocolitis in very low birth weight neonates. J Pediatr. 2005;147(2):192–6.

(20) Lin HC, Su BH, Chen AC, Lin TW, Tsai CH, Yeh TF, et al. Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birth weight infants. Pediatrics. 2005;115(1):1–4.

(21) Lin HC, Hsu CH, Chen HL, Chung MY, Hsu JF, Lien RI, et al. Oral probiotics prevent necrotizing enterocolitis in very low birth weight preterm infants: a multicenter, randomized, controlled trial. Pediatrics. 2008;122(4):693-700.

(22) Chen AC, Chung MY, Chang JH, Lin HC. Pathogenesis Implication for Necrotizing Enterocolitis Prevention in Preterm Very-Low-Birth-Weight Infants. JPGN. 2014;58:7–11.

(23) Wang Q, Dong J, Zhu Y. Probiotic supplement reduces risk of necrotizing enterocolitis and mortality in preterm very low birth weight infants: an updated meta-analysis of 20 randomized, controlled trials. J Pediatr Surg. 2012;47(1):241–8.

(24) AlFaleh K, Anabrees J. Probiotics for prevention of necrotizing enterocolitis in preterm infants. Cochrane Database of Syst Rev. 2014;4: CD005496.

(25) Samanta M, Sarkar M, Ghosh P, Ghosh JK, Sinha MK, Chatterjee S. Prophylactic probiotics for prevention of necrotizing enterocolitis in very low birth weight newborns. J Trop Pediatr. 2009;55(2):128-31.

(26) Manzoni P, Rinaldi M, Cattani S, Pugni L, Romeo MG, Messner H, et al. Bovine lactoferrin supplementation for prevention of late-onset sepsis in very low-birth-weight neonates: a randomized trial. JAMA. 2009;302(13):1421-8.

(27) Romeo MG, Romeo DM, Trovato L, Oliveri S, Palermo F, Cota F, et al. Role of probiotics in the prevention of the enteric colonization by Candida in preterm newborns: incidence of late-onset sepsis and neurological outcome. J Perinatol. 2011;31(1):63-9.

(28) Mihatsch WA, Vossbeck S, Eikmanns B, Hoegel J, Pohlandt F. Effect of Bifidobacterium lactis on the incidence of nosocomial infections in very-low-birth-weight infants: a randomized controlled trial. Neonatology. 2010;98(2):156-63.

(29) Rojas MA, Lozano JM, Rojas MX, Rodriguez VA, Rondon MA, Bastidas JA, et al. Prophylactic probiotics to prevent death and nosocomial infection in preterm infants. Pediatrics. 2012;130(5):e1113-20.

(30) Rougé C, Piloquet H, Butel MJ, Berger B, Rochat F, Ferraris L, et al. Oral supplementation with probiotics in very-low-birth-weight preterm infants: a randomized, double-blind, placebo-controlled trial. Am J Clin Nutr. 2009;89(6):1828-35.

(31) Kitajima H, Sumida Y, Tanaka R, Yuki N, Takayama H, Fujimura M. Early administration of Bifidobacterium breve to preterm infants: randomised controlled trial. Arch Dis Child Fetal Neonatal Ed. 1997;76(2):F101-7.

(32) Stratiki Z, Costalos C, Sevastiadou S, Kastanidou O, Skouroliakou M, Giakoumatou A, et al. The effect of a bifidobacter supplemented bovine milk on intestinal permeability of preterm infants. Early Hum Dev. 2007;83(9):575-9.

(33) Ohishi A, Takahashi S, Ito Y, Ohishi Y, Tsukamoto K, Nanba Y, et al. Bifidobacterium septicemia associated with postoperative probiotic therapy in a neonate with omphalocele. J Pediatr. 2010;156(4):679-81.

(34) Land MH, Rouster-Stevens K, Woods CR, Cannon ML, Cnota J, Shetty AK. Lactobacillus sepsis associated with probiotic therapy. Pediatrics. 2005;115(1):178-81.

(35) Guenther K, Straube E, Pfister W, Guenther A, Huebler A. Sever sepsis after probiotic treatment with Escherichia coli NISSLE 1917. Pediatr Infect Dis J. 2010;29(2):188-9.

(36) Ofek Shlomai N, Deshpande G, Rao S, Patole S. Probiotics for preterm neonates: what will it take to change clinical practice? Neonatology. 2014;105(1):64-70.

(37) Deshpande G, Rao S, Patole S, Bulsara M. Updated meta-analysis of probiotics for preventing necrotizing enterocolitis in preterm neonates. Pediatrics. 2010 ;125(5):921-30.

(38) Athalye-Jape G, Deshpande G, Rao S, Patole S. Benefits of probiotics on enteral nutrition in preterm neonates: a systematic review. Am J Clin Nutr. 2014;100(6):1508-19.

(39) Szajewska H, van Goudoever JB. To give or not to give probiotics to preterm infants. Am J Clin Nutr. 2014;100(6):1411-2.

POST-INFECTIOUS IRRITABLE BOWEL SYNDROME

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ABSTRACT

Post-infectious irritable bowel syndrome (PI-IBS) is part of a spectrum of post-infectious gastrointestinal disorders that includes gastroparesis, functional dyspepsia, and gas/bloating. Symptomatically, it is similar to diarrhea-predominant IBS but presents either with an acute onset following an infectious gastroenteritis or with chronic symptoms that can be dated back to an infectious event. The risk is approximately seven-fold higher for younger patients, those with prolonged duration of symptoms after gastroenteritis and those with pre-existing psychological disturbances, and these factors also serve as important predictors. Both adults and children are equally affected but the role of female gender remains unclear. Bacterial gastroenteritis is the most common form of infectious event precipitating PI-IBS, although viral and protozoan infection can also cause PI-IBS. Post-inflammatory immune reactions and cytokine release are important mechanisms but there are also complex interactions with the microbiota-gut-brain axis and dysbiosis. More recently, there is evidence for a genetic predisposition. Diagnosis is symptom-based but requires exclusion of organic diseases and other conditions that mimic IBS. A history of gastroenteritis, and/or the presence of antiviral antibodies may provide clues but are not essential for diagnosis. Prognosis is generally good with a gradual recovery, but symptoms can persist in some patients. Treatment objectives are to provide symptomatic relief with antispasmodics, low-dose antidepressants, prokinetics, dietary modifications, judicious use of probiotics, and relaxation therapies, although there is limited evidence to support these approaches.

Keywords: irritable bowel syndrome, gastroenteritis, infection, pathophysiology, post-infectious management.

INTRODUCTION

Irritable bowel syndrome (IBS) is a common and challenging gastrointestinal (GI) disorder characterized by abdominal discomfort or pain and altered bowel habits but without definable biochemical or histological markers. Post-infectious IBS (PI-IBS) is a form of IBS, similar to diarrhea-predominant IBS, but which develops acutely following an epidose of infectious gastroenteritis (IGE). Earlier descriptions of this condition indicate a better prognosis and less psychiatric illnesses compared with other forms of IBS[1,2], and as such, it is relatively easier to treat. Its importance really lies in the pathophysiology which allows researchers to better understand the complex mechanisms of IBS. Sometimes PI-IBS may present with other bowel symptoms including gas and bloating or constipation, or alternating diarrhea and constipation, and may overlap with upper gastrointestinal dysfunction including gastroparesis.

EPIDEMIOLOGY

The link between IGE and chronic abdominal symptoms was first recognized decades ago[1,2] but the risks and prognosis had not been systematically studied until recently. A meta-analysis by Halvorson *et al.* in 2006 of eight studies reported a 7-fold increase in the risk of IBS following IGE[3]. This risk estimate is consistent with a subsequent meta-analysis of eighteen studies by Thabane *et al.*[4]. In addition, Thabane *et al.* observed that young age, prolonged fever, anxiety and depression were significant risk factors for developing PI-IBS. This analysis also found that PI-IBS was less likely to develop following viral rather than bacterial gastroenteritis, and the duration of IBS symptoms was also shorter following viral gastroenteritis. However, this finding has been challenged by

a more recent study by Zanini *et al.* from Italy where 13% of patients developed PI-IBS in 12 months following a norovirus outbreak[5].

Chronic abdominal symptoms have been reported in between 3.7% and 36% of adults following intestinal infection and a single organism is usually responsible in outbreaks[6,7]. The highest incidence of 36% at 24 months was reported after an outbreak of dual infection of *Escherichia coli* O157:H7 and *Campylobacter jejuni* in Walkerton, Canada[7]. Travelers'diarrhea is also associated with PI-IBS; five studies reported an incidence of PI-IBS of between 1.5% and 7.2% after traveler's diarrhea[8]. The varying percentages across studies probably reflect differences in the severity of bowel dysfunction and inflammation. Although reports from Asia are scarce, available data from China and Korea indicate a PI-IBS rate of between 8.1% and 20.8%. Children seem to have the same risk as adults for developing PI-IBS following acute bacterial gastroenteritis, and childhood infection is a possible risk factor for IBS later in life.

Although these large outbreaks provide a consistent association between the development of this disorder and an infectious etiology, it is common in clinical practice for patients to present with longstanding symptoms, making it hard to identify an infectious event either because it was too subtle or the patient is unable to recall. Hence the prevalence of this disorder is likley to be much higher than the literature reports suggest, and not fully understood.

PATHOPHYSIOLOGY

Intestinal infection results in inflammation and the subsequent gut dysfunction in the hosts arises from the products of inflammation, which include immune reactions and cytokine release. There are also a complex interactions with the microbiota-gut-brain axis. A pathophysiological framework is shown in Figure 1. However, it is unclear why gastrointestinal infectious illness clears without residual problems in the vast majority of patients (approximately 90%), but in a few it leads to a chronic low-grade inflammatory state and persistent symptoms.

Post-inflammatory immune reactions

The severity of insults may have a differential impact on immune reactions and subequent development of PI-IBS, and the types of pathogen may be important in determining the severity. For example, norovirus infection may only cause acute villous loss and lymphocytic infiltration and therefore IBS is less severe, whereas bacterial enteritis caused by *Salmonella* and *Shigellosis* may result in colonic ulcerations that are usually associated with a more severe IBS later on. The predominant IBS symptoms probably reflect the sites of initial gut inflammation and damage. For example, upper gastrointestinal symptoms including dyspepsia, early satiety and anorexia are more common following giardiasis and viral gastroenteritis, but diarrhea is more frequent following bacillary dysentery.

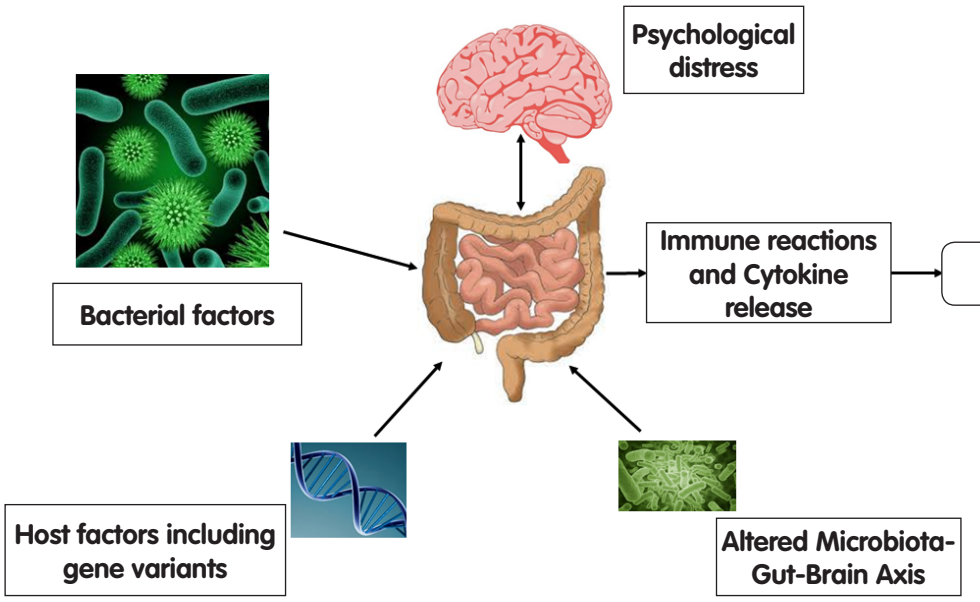

Figure 1: A pathophysiological framework for post-infectious irritable bowel syndrome. Note the two-way processes between psychological distress and alteration in microbiota-gut-brain axis with the resultant inflammatory changes.

The relationship between GI symptoms and *Helicobacter pylori* infection is still controversial, with conflicting data between the East and the West[9]. An increase in serotonin-containing enterochromaffin cells (ECs) is commonly observed from intestinal biopsies of patients with PI-IBS compared with sporadic IBS[10], although this is not always the case. Likewise, significant increases in postprandial plasma serotonin levels have been observed in PI-IBS more than in constipation-predominant IBS. ECs may also play a role in mediating visceral hypersensitivity in PI-IBS[11]. Similar to ECs, the number of mast cells is frequently elevated, although this is not a sole feature of PI-IBS[12]. The close proximity of mast cells to enteric nerves suggests that mast cell activation may be the reason why patients continue to experience visceral hypersensitivity and pyschological symptoms.

Post-inflammatory release of cytokines

Infiltration of T-lymphocytes and macrophages in PI-IBS is accompanied by the release of a multitude of inflammatory cytokines. Gwee *et al.* found increased expression of Interleukin-1β (IL-1β) in rectal biopsies from patients with PI-IBS compared with those who had infectious enteritis but did not develop PI-IBS[13]. Wang *et al.* likewise observed an increase in IL-1β in patients with PI-IBS after bacillary dysentery when compared with patients with sporadic IBS[12]. Other inflammatory cytokines that may be involved in IBS include IL-2, IL-6, IL-10, tumor necrosis factor (TGF)-β and interferon (IFN)-γ[14]. Whether the release of cytokines is an indirect cause or direct effect of psychological distress is not entirely clear, but it reflects a complex interaction between the two. Patients with PI-IBS also demonstrate increased gut permeability compared with non-IBS controls, suggesting a defect in epithelial integrity, and this may be a direct cause or indirect effect of inflammatory cytokine release[10].

Alterations in gut microbiota and gut-brain axis

Following IGE, there is profound depletion of commensal colonic flora and normal fermentation products, especially short-chain fatty acids (SCFA), as a result of intestinal inflammation[15]. This inadvertently increases overgrowth of organisms normally inhibited by SCFA not just in the colon but also in the small bowel. The role of small intestinal bacterial overgrowth (SIBO) in IBS remains controversial. Lactulose hydrogen breath test has a high positive rate for SIBO. Glucose hydrogen breath test may have lower diagnostic yield for SIBO but the agreement with duodenal culture is better[16].

There is also altered fecal microbiota composition in IBS using molecular-based technique, but this has not been well-characterized in PI-IBS until recently. An Index of Microbial Dysbiosis (IMD) that consists of twenty-seven discriminant bacterial groups was found to separate PI-IBS from healthy controls and this index correlated with a variety of host-microbe associations initiated by IGE[17].

Although there are significant changes to the enteric nerves in PI-IBS, these changes are also observed in non PI-IBS subjects[12]. These peripheral alterations, which may be the cause of visceral hypersensivity, include upregulation of tachykinins and other neuropeptides, e.g. substance P and TRPV-1 positive neuronal fibers. However, gut-brain interactions may be more complex. At the central level, corticotrophin releasing factor (CRF) is an important mediator of the stress response in animal models of PI-IBS[18]. It appears that CRF is pro-inflammatory and that stress results in an enhanced local inflammatory response to infection.

RISK FACTORS AND OTHER SPECIAL CONSIDERATIONS

Risk factors

There is a familial tendency to IBS, although social conditioning is also important since having a parent with IBS is a stronger predictor than having a dizygotic twin with IBS. More recent evidence indicates a contribution of genetic variations or single nucleotide polymorphisms (SNPs) to this condition (Figure 1). In the Walkerton study, Villani *et al.* identified three candidate gene variants, namely Toll-like receptor-9 (TLR-9), Cadherin 1 (CDH 1) and IL-6, which were associated with the development of PI-IBS[19]. This suggest that PI-IBS might result from abnormalities in genes encoding epithelial barrier functions and innate immune responses to enteric bacteria. Other studies have indicated a role of TNF-α SNPs in PI-IBS[20].

Psychosocial factors play a central role in the development of PI-IBS and these factors include high stress and anxiety levels, hypochondriasis, adverse life events in the preceding 3 months and depression[21] (Figure 1). Females are particularly susceptible although in outbreaks[7] and in Asian populations[22], this gender preponderance is less marked. Older age appears to be protective from PI-IBS, presumably because of immunity from previous exposure.

Besides the host, bacterial factors are also important. The risk of PI-IBS appears to correlate with the severity of IGE, increasing at least twofold if diarrhea lasts more than 1 week and over threefold if diarrhea lasts more than 3 weeks[7]. Pathogens that secrete toxin may be particularly potent and these include *Clostridium jejuni, enterotoxigenic E. coli* and *shiga-toxin producing E. coli* O147.

Special considerations

Epidemic tropical sprue or post-infective tropical malabsorption is a malabsorption syndrome developed following acute gastroenteritis and it shares certain similarities with PI-IBS. Diarrhea is the predominant symptom, as it is in PI-IBS, and is often accompanied by overgrowth of bacteria in the small bowel[23]. Epidemic tropical sprue is also characterized by abnormal urinary excretion of D-xylose, steatorrhea, and increased intestinal permeability. Recently, a high prevalence of idiopathic bile acid diarrhea has been reported among patients with IBS-D[24], and while this has not been documented in PI-IBS, both are closely related conditions, and further study is warranted. Likewise, in celiac disease and diverticulitis or diverticulosis, IBS-type symptoms occur more frequently than healthy controls. These conditions should be considered or excluded during the work-up for PI-IBS (Figure 2). Another consideration in PI-IBS would be parasite and helminth infestations, which can be especially prevalent among Asian populations. Protozoans including *Giardia lamblia, Entamoeba histolytica* and *Blastocystis hominis* have been shown to be associated with IBS, but helminth infestation may be protective[25].

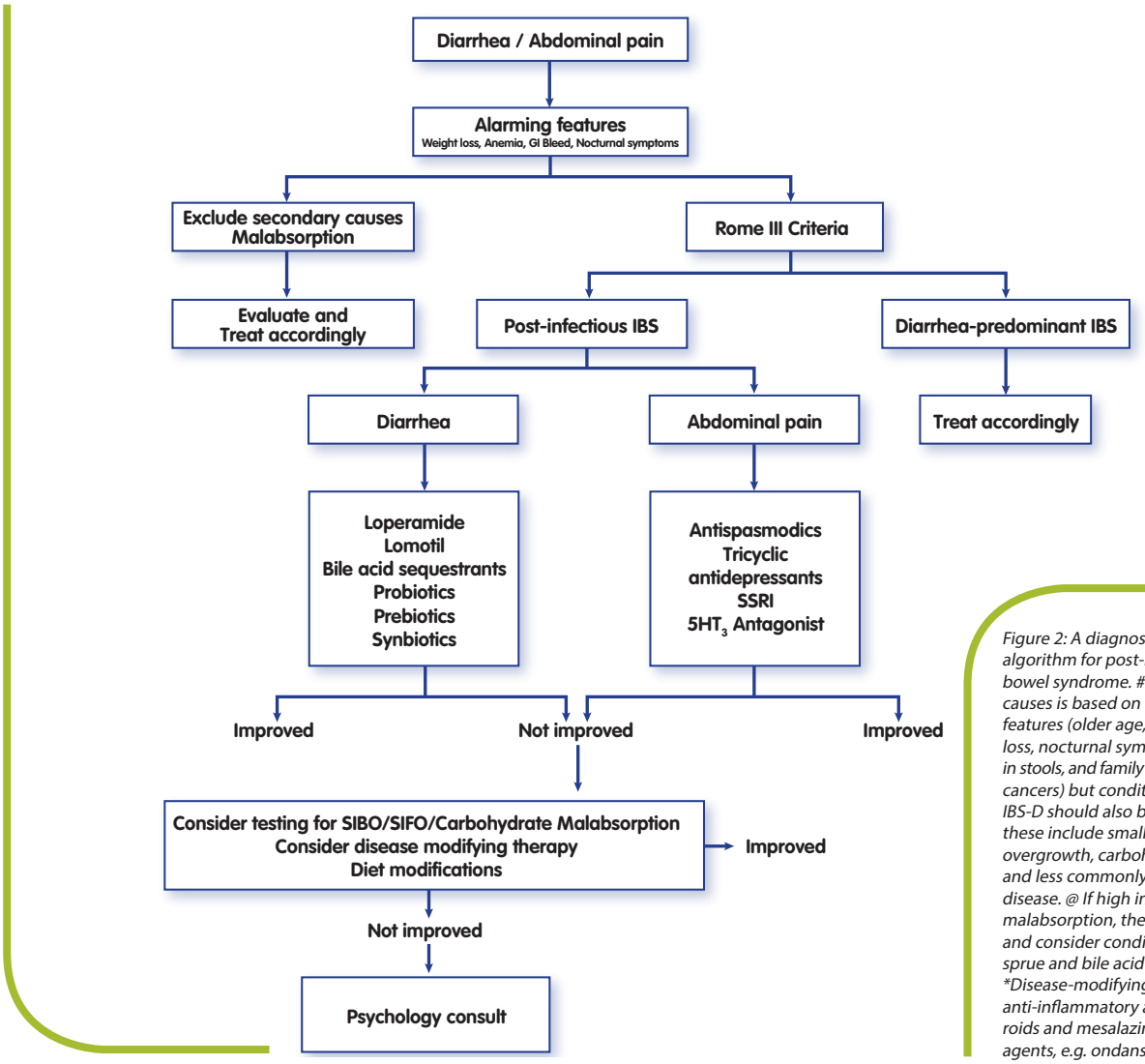

*Figure 2: A diagnostic and management algorithm for post-infectious irritable bowel syndrome. #Exclusion of secondary causes is based on the presence of alarm features (older age, unintended weight loss, nocturnal symptoms, anemia, blood in stools, and family history of intestinal cancers) but conditions that mimic IBS-D should also be considered and these include small intestinal bacterial overgrowth, carbohydrate malabsoprtion and less commonly inflammatory bowel disease. @ If high index of suspicion for malabsorption, then this should be tested and consider conditions including tropical sprue and bile acid diarrhea. *Disease-modifying therapies may include anti-inflammatory agents, e.g. corticosteroids and mesalazine, and anti-serotonergic agents, e.g. ondansetron.*

CLINICAL FEATURES, DIAGNOSIS AND PROGNOSIS

The majority of patients with PI-IBS meet the Rome symptom criteria for IBS-D, but up to a third may present with constipation, bloating, and passing mucus per rectum, or mixed bowel disturbance comprising of diarrhea and constipation. PI-IBS can be defined as an acute onset of new IBS symptoms in an individual who otherwise does not meet the Rome criteria for IBS, immediately following an episode acute gastroenteritis characterized by 2 or more of the following: fever, vomiting, diarrhea, or a positive stool culture[26]. Whilst this presentation is the classical scenario, there may be a group of patients who have had a less severe illness or sporadic gastroenteritis and develop IBS symptoms weeks or months later. It is often hard to connect the symptoms with an infectious etiology in these patients, but they also require PI-IBS diagnosis and treatment. Further research is needed to better characterize this form of late-onset PI-IBS.

Diagnosis is based on symptoms but there may be a role for biomarkers in the future[27]. It is especially important to differentiate PI-IBS from organic diseases, especially in the presence of alarm features (older age, unintended weight loss, nocturnal symptoms, anemia, blood in stools, family history of intestinal cancers), and from other related conditions including SIBO, carbohydrate malabsorption (e.g. lactose intolerance) and bile acid malabsorption (Figure 2). Inflammatory bowel disease, especially ulcerative colitis, should also be considered since it can begin acutely. Unfortunately, enteric infections are often detected in inflammatory bowel disease, which is another similarity to PI-IBS.

The prognosis of PI-IBS is generally good, with gradual recovery in many patients. However, data are limited. During long-term follow-up in the Walkerton study, there was a decline in the prevalence of PI-IBS from 28% to 15.4% after 8 years[28]. The meta-analysis by Thabane *et al.* also showed a decline in the pooled odds ratios of PI-IBS from 7.58 at 3 months to 3.85 at 24–36 months after the infectious episode[4].

MANAGEMENT

The goals of effective management of PI-IBS are early recognition, identifying the key symptoms and problems, excluding any known GI disorder(s) that masquerades as this illness, and then embarking on a symptomatic treatment approach. To date, no therapies have proven to be specifically effective for the management of PI-IBS. However, a relatively good prognosis in this condition suggests a more conservative approach to management can be used compared with standard IBS. Symptomatic relief is the main target and the use of antidiarrheal agents, e.g. loperamide or diphenoxylate/atropine, would be effective. Patients with pain may benefit from a mild tranquilizer, e.g. low-dose amitriptyline or a combination of benzodiazepine with smooth muscle relaxant such as chlordiazepoxide with clidinium[29] (Figure 2).

A better understanding of PI-IBS pathogenesis provides a reasonable rationale for some specific approaches that may target key mechanisms. These approaches may be important for some difficult-to-treat situations or in more severe forms of PI-IBS (Figure 2). Many of the reported studies, however, are limited by their small sample sizes and heterogeneous methodology and are therefore less conclusive. Our review suggests that larger clinical trials of various specific therapies are warranted.

Acute IGE can substantially alter the colonic flora, which may in turn induce or promote many of the changes in the colonic physiology described above. Hence, restoration of gut flora with probiotics or prebiotics can down-regulate inflammation, improve barrier function, and reduce visceral sensitivity[30]. Probiotics have been shown to be effective in protecting the human intestinal epithelial cells against effects of invasive organisms in vitro[31]. However, no human studies have yet assessed the efficacy of interventions that modulate the gut flora for preventing or treating PI-IBS, although probiotics have been assessed in mouse models. Generally, probiotics containing a combination of strains such as *Bifidobacterium, Lactobacillus* and *Streptococcus thermophillus* (for example VSL#3) are recommended. Sometimes a yeast preparation containing *Saccharomyces species* may also be useful, but such preparations have not been clinically evaluated[29]. While there is strong evidence for the role of intestinal dysbiosis in PI-IBS, the exact microbial dysbiosis is unclear. In general, IBS-D patients are deficient of *Bifidobacterium* and *Lactobacilli* and have higher numbers of *Firmicutes*[32]. Thus replacing the gut flora with appropriate probiotic strains may be logical in PI-IBS, but there is no evidence about which strains to use.

The observed increases in EC number and altered serotonin release in PI-IBS suggest that serotonergic-based therapies may be effective for this condition. While the therapeutic gains associated with 5-HT3 antagonists e.g. alosteron, ramosetron and more recently, ondansetron[33] in IBS-D may not be great, patients with PI-IBS may derive greater benefit from these therapies due to their homogeneous symptomatology and good prognosis. Newer drugs such as eluxadoline (a mixed μ-opioid receptor agonist/δ-opioid receptor antagonist)[34] may prove to be useful, although have not been tested in these patients. Some patients may develop idiopathic bile

salt malabsorption and, if so, bile acid-binding agents such as cholestyramine, colesevelam or colestipol may be effective. Abdominal discomfort or pain is an important component of this illness, so patients who have have no response to the aforementioned therapies may benefit from a trial of low-dose antidepressants, such as a tricyclic antidepressant (e.g. amitriptyline, nortriptyline, desipramine), trazodone, or a selective serotonin reuptake inhibitor (SSRI) such as citalopram, escitalopram, or duloxetine[29].

Although there is little doubt that PI-IBS is associated with persistent intestinal inflammation, a randomized trial of 3 weeks of prednisolone 30 mg/day in PI-IBS failed to show a significant improvement in symptoms, despite a reduction in intestinal lymphocyte counts[35]. The study was, however, underpowered and locally active steroids with reduced systemic toxicity, such as budesonide, are probably more preferable in PI-IBS clinical trials. Mesalazine, another anti-inflammatory agent, has been shown to be effective in a number of small and uncontrolled studies; however, a recent multicenter randomized controlled study did not support any clinically meaningful benefit or harm of mesalazine in patients with IBS-D[36].

Alternatively, patients with constipation-predominant symptoms may benefit with a generous trial of fiber supplements or laxatives. If these are ineffective, newer prosecretory agents including linaclotide or lubiprostone or the 5-HT4 agonist prucalopride may be helpful[29].

CONCLUSION

PI-IBS represents an acute form of IBS-D following an infectious gastroenteritis. Post-inflammatory immune reactions and cytokine release are the main mechanisms underlying PI-IBS but there are also complex interactions along the microbiota-gut-brain axis. Recent evidence also points to a role of genetic variations. It is important to exclude organic and other conditions that mimic IBS-D before making a diagnosis of PI-IBS (Figure 1). The prognosis is generally of a gradual improvement and therefore the current treatment strategy targets symptomatic relief. Difficult-to-treat or severe PI-IBS may require a trial of disease modifying therapies (Figure 2), although this merits further rigorous clinical trials.

References

[1] Chaudhary NA, Truelove SC. The irritable colon syndrome. A study of the clinical features, predisposing causes, and prognosis in 130 cases. Q J Med 1962;31:307–22.

[2] Stewart GT. Post-dysenteric colitis. Br Med J 1950;1:405–9. doi:10.1136/bmj.1.4650.405.

[3] Halvorson HA, Schlett CD, Riddle MS. Postinfectious irritable bowel syndrome--a meta-analysis. Am J Gastroenterol 2006;101:1894–9; quiz 1942. doi:10.1111/j.1572-0241.2006.00654.x.

[4] Thabane M, Kottachchi DT, Marshall JK. Systematic review and meta-analysis: The incidence and prognosis of post-infectious irritable bowel syndrome. Aliment Pharmacol Ther 2007;26:535–44. doi:10.1111/j.1365-2036.2007.03399.x.

[5] Zanini B, Caselani F, Magni A, La Roga AM, Bandera F, Ricci C, et al. Incidence of functional intestinal disorders and postinfectious irritable bowel syndrome following a waterborne viral gastroenteritis outbreak. Gut 2011;60:A161–A161. doi:10.1136/gut.2011.239301.341.

[6] Borgaonkar MR, Ford DC, Marshall JK, Churchill E, Collins SM. The incidence of irritable bowel syndrome among community subjects with previous acute enteric infection. Dig Dis Sci 2006;51:1026–32. doi:10.1007/s10620-006-9348-1.

[7] Marshall JK, Thabane M, Garg AX, Clark WF, Salvadori M, Collins SM. Incidence and Epidemiology of Irritable Bowel Syndrome After a Large Waterborne Outbreak of Bacterial Dysentery. Gastroenterology 2006;131:445–50. doi:10.1053/j.gastro.2006.05.053.

[8] Mutsch M, Pitzurra R, Hatz C, Steffen R. Post-Infectious Sequelae of Travelers ' Diarrhea : Irritable Bowel Syndrome 2014;21:141–3.

[9] Lee YY, Chua ASB. Role of Helicobacter pylori in Functional Dyspepsia: More Controversies Than Answers. J Neurogastroenterol Motil 2013;19:417. doi:10.5056/jnm.2013.19.3.417.

[10] Spiller RC, Jenkins D, Thornley JP, Hebden JM, Wright T, Skinner M, et al. Increased rectal mucosal enteroendocrine cells, T lymphocytes, and increased gut permeability following acute Campylobacter enteritis and in post-dysenteric irritable bowel syndrome. Gut 2000;47:804–11. doi:10.1136/gut.47.6.804.

[11] Park JH, Rhee PL, Kim G, Lee JH, Kim YH, Kim JJ, et al. Enteroendocrine cell counts correlate with visceral hypersensitivity in patients with diarrhoea-predominant irritable bowel syndrome. Neurogastroenterol Motil 2006;18:539–46. doi:10.1111/j.1365-2982.2006.00771.x.

[12] Wang L-H, Fang X-C, Pan G-Z. Bacillary dysentery as a causative factor of irritable bowel syndrome and its pathogenesis. Gut 2004;53:1096–101. doi:10.1136/gut.2003.021154.

[13] Gwee K-A, Collins SM, Read NW, Rajnakova A, Deng Y, Graham JC, et al. Increased rectal mucosal expression of interleukin 1beta in recently acquired post-infectious irritable bowel syndrome. Gut 2003;52:523–6. doi:10.1136/gut.52.4.523.

[14] Ghoshal UC, Shukla R, Ghoshal U, Gwee K-A, Ng SC, Quigley EMM. The gut microbiota and irritable bowel syndrome: friend or foe? Int J Inflam 2012;2012:151085. doi:10.1155/2012/151085.

[15] Lupp C, Robertson ML, Wickham ME, Sekirov I, Champion OL, Gaynor EC, et al. Host-Mediated Inflammation Disrupts the Intestinal Microbiota and Promotes the Overgrowth of Enterobacteriaceae. Cell Host Microbe 2007;2:119–29. doi:10.1016/j.chom.2007.06.010.

[16] Erdogan A, Rao SSC, Gulley D, Jacobs C, Lee YY, Badger C. Small intestinal bacterial overgrowth: duodenal aspiration vs glucose breath test. Neurogastroenterol Motil 2015;27:481–9. doi:10.1111/nmo.12516.

[17] Jalanka-Tuovinen J, Salojärvi J, Salonen A, Immonen O, Garsed K, Kelly FM, et al. Faecal microbiota composition and host-microbe cross-talk following gastroenteritis and in postinfectious irritable bowel syndrome. Gut 2013:1–9. doi:10.1136/gutjnl-2013-305994.

[18] Kiank C, Taché Y, Larauche M. Stress-related modulation of inflammation in experimental models of bowel disease and post-infectious irritable bowel syndrome: Role of corticotropin-releasing factor receptors. Brain Behav Immun 2010;24:41–8. doi:10.1016/j.bbi.2009.08.006.

[19] Villani AC, Lemire M, Thabane M, Belisle A, Geneau G, Garg AX, et al. Genetic Risk Factors for Post-Infectious Irritable Bowel Syndrome Following a Waterborne Outbreak of Gastroenteritis. Gastroenterology 2010;138:1502–13. doi:10.1053/j.gastro.2009.12.049.

[20] Zucchelli M, Camilleri M, Nixon Andreasson A, Bresso F, Dlugosz A, Halfvarson J, et al. Association of TNFSF15 polymorphism with irritable bowel syndrome. Gut 2011;60:1671–7. doi:10.1136/gut.2011.241877.

[21] Gwee KA, Graham JC, McKendrick MW, Collins SM, Marshall JS, Walters SJ, et al. Psychometric scores and persistence of irritable bowel after infectious diarrhoea. Lancet 1996;347:150–3. doi:10.1016/S0140-6736(96)90341-4.

[22] Lee YY, Waid A, Tan HJ, Chua ASB, Whitehead WE. Rome III survey of irritable bowel syndrome among ethnic Malays. World J Gastroenterol 2012;18:6475–80. doi:10.3748/wjg.v18.i44.6475.

[23] Gorbach SL, Mitra R, Jacobs B, Banwell JG, Chatterjee BD, Mazumder DN. Bacterial contamination of the upper small bowel in tropical sprue. Lancet 1969;1:74–7. doi:10.1016/S0140-6736(69)91091-5.

[24] Aziz I, Mumtaz S, Bholah H, Chowdhury FU, Sanders DS, Ford AC. High Prevalence of Idiopathic Bile Acid Diarrhea Among Patients With Diarrhea-predominant Irritable Bowel Syndrome Based on Rome III Criteria. Clin Gastroenterol Hepatol 2015:Epub ahead of print. doi:10.1016/j.cgh.2015.03.002.

[25] Ghoshal UC, Park H, Gwee K-A. Bugs and irritable bowel syndrome: The good, the bad and the ugly. J Gastroenterol Hepatol 2010;25:244–51. doi:10.1111/j.1440-1746.2009.06133.x.

[26] Dunlop SP, Jenkins D, Spiller RC. Distinctive clinical, psychological, and histological features of postinfective irritable bowel syndrome. Am J Gastroenterol 2003;98:1578–83. doi:10.1111/j.1572-0241.2003.07542.x.

[27] Darkoh C, Comer L, Zewdie G, Harold S, Snyder N, DuPont HL. Chemotactic chemokines are important in the pathogenesis of irritable bowel syndrome. PLoS One 2014;9. doi:10.1371/journal.pone.0093144.

[28] Marshall JK, Thabane M, Garg AX, Clark WF, Moayyedi P, Collins SM. Eight year prognosis of postinfectious irritable bowel syndrome following waterborne bacterial dysentery. Gut 2010;59:605–11. doi:10.1136/gut.2009.202234.

[29] Ford AC, Moayyedi P, Lacy BE, Lembo AJ, Saito Y a, Schiller LR, et al. American College of Gastroenterology Monograph on the Management of Irritable Bowel Syndrome and Chronic Idiopathic Constipation. Am J Gastroenterol 2014;109:S2–26. doi:10.1038/ajg.2014.187.

[30] Ford AC, Quigley EMM, Lacy BE, Lembo AJ, Saito Y a, Schiller LR, et al. Efficacy of Prebiotics, Probiotics, and Synbiotics in Irritable Bowel Syndrome and Chronic Idiopathic Constipation: Systematic Review and Meta-analysis. Am J Gastroenterol 2014:1–15. doi:10.1038/ajg.2014.202.

[31] Resta-Lenert S, Barrett KE. Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive Escherichia coli (EIEC). Gut 2003;52:988–97. doi:10.1136/gut.52.7.988.

[32] Jeffery IB, O'Toole PW, Öhman L, Claesson MJ, Deane J, Quigley EMM, et al. An irritable bowel syndrome subtype defined by species-specific alterations in faecal microbiota. Gut 2012;61:997–1006. doi:10.1136/gutjnl-2011-301501.

[33] Garsed K, Chernova J, Hastings M, Lam C, Marciani L, Singh G, et al. A randomised trial of ondansetron for the treatment of irritable bowel syndrome with diarrhoea. Gut 2013:1–9. doi:10.1136/gutjnl-2013-305989.

[34] Dove LS, Lembo A, Randall CW, Fogel R, Andrae D, Davenport JM, et al. Eluxadoline Benefits Patients with Irritable Bowel Syndrome with Diarrhea in a Phase 2 Study. Gastroenterology 2013;145. doi:10.1053/j.gastro.2013.04.006.

[35] Dunlop SP, Jenkins D, Neal KR, Naesdal J, Borgaonker M, Collins SM, et al. Randomized, double-blind, placebo-controlled trial of prednisolone in post-infectious irritable bowel syndrome. vol. 18. 2003. doi:10.1046/j.1365-2036.2003.01640.x.

[36] Lam C, Tan W, Leighton M, Hastings M, Lingaya M, Falcone Y, et al. A mechanistic multicentre, parallel group, randomised placebo-controlled trial of mesalazine for the treatment of IBS with diarrhoea (IBS-D). Gut 2015:Epub ahead of print. doi:10.1136/gutjnl-2015-309122.

Very Basic

GUT MICROBIOTA AND PERMEABILITY IN IRRITABLE BOWEL SYNDROME

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TAKE HOME MESSAGES

- IBS is an extremely common functional intestinal disorder and its pathophysiology is becoming better defined.
- IBS is a multifactorial disease characterized by changes in the brain-gut-axis and micro-organic abnormalities in the intestine, including modifications in gut microbiota and increased mucosal permeability.
- The gut microbiota is the ecosystem of microorganisms that normally inhabit the gastrointestinal tract, participating in metabolic, protective and trophic functions.
- Culture-independent, high-throughput molecular techniques have provided a better understanding of the phylogenetic framework of the intestinal microbiota in several diseases, including IBS.
- Several studies have demonstrated both quantitative and qualitative changes of mucosal and fecal gut microbiota in patients with IBS compared with healthy subjects.
- The intestinal barrier is a complex anatomical and functional structure that separates the internal milieu from the gut lumen.
- Alterations in the intestinal barrier and increased permeability have been found to play a pathogenic role in IBS as well as in many other intestinal and extra-intestinal diseases.
- Changes in intestinal microbiota may contribute to a loss of intestinal barrier function causing an increased flow of antigenic substances that activate mucosal immune responses. This low-grade inflammatory state activates nociceptive sensory pathways leading to symptoms that characterize IBS.

ABSTRACT

Abnormal interplay between the gut microbiota, the epithelial barrier and the mucosal immune system plays a key role in the pathophysiology of irritable bowel syndrome (IBS). The recent introduction of culture-independent techniques has allowed the detection of new gut microbial communities and a better understanding of the role of gut microbiota in IBS. At the same time, the understanding of the molecular organization and function of the gut barrier and the role of increased intestinal permeability in several diseases, including IBS, has dramatically increased. A leaky epithelial barrier increases the load of microbiota-related substances to the mucosal immune system. Low-grade immune activation in IBS primarily involves mast cells that, when activated, release several mediators. These mediators may evoke altered neuromuscular responses and increased visceral sensory perception. A better knowledge of pathophysiological mechanisms of IBS will potentially lead to the development of more effective and targeted drugs.

Keywords: irritable bowel syndrome, gut microbiota, epithelial barrier, immune activation.

INTRODUCTION

Irritable bowel syndrome (IBS) is a functional intestinal disorder characterized by abdominal pain and/or discomfort associated with changes in bowel habits[1]. IBS affects between 10% to 20% of the general population and represents one of most common reasons for seeking healthcare. Although IBS does not reduce life expectancy, it is associated with a considerable reduction in quality of life and a substantial economic burden. According to the predominant bowel habit, IBS is further classified into diarrhea predominant IBS (IBS-D), constipation predominant IBS (IBS-C), mixed bowel pattern IBS (IBS-M) or unsubtyped IBS, if there are no sufficient abnormalities of stool consistency meeting the criteria for IBS-C, D, or M[1]. The pathophysiology of IBS is complex and multifactorial. Traditionally, IBS has been considered as a disorder affecting the brain-gut axis; it is associated with psychosocial factors, abnormalities of gastrointestinal motility and visceral hypersensitivity. In recent years, molecular, biochemical and genetic abnormalities have been identified, including: genetic factors and polymorphisms, altered enteroendocrine metabolism (e.g., serotonin metabolism dysregulation), neuroplastic changes, mucosal and systemic immune activation, gastrointestinal infections, changes in gut microbiota, and increased mucosal permeability[2-4]. In particular, attention has been paid to alterations in gut microbiota and permeability.

GUT MICROBIOTA

The microbiota is defined as the complex community of microbes that normally inhabits the human body, including bacteria, viruses and fungi. It is estimated that the human microbiota consists of 10^{14} organisms, outnumbering the human cells by one order of magnitude[5]. The microbiota colonizes every surface of our body that is exposed to the external environment; therefore, it is located on the skin and in the genitourinary, respiratory and gastrointestinal tracts. Among these organs, the gastrointestinal tract is the most abundantly colonized, with an increasing microbe density from the stomach to the colon. The microbial composition differs along the axis of the digestive tract, with a progressive increase of the anaerobic species towards the colon[6].
In addition to the longitudinal heterogeneity, there is also a latitudinal variation in the microbiota composition. The microbial population present in the gut lumen differs significantly from that adherent and embedded in the mucus layer – with a ratio of anaerobes to aerobes lower at the mucosal surface than in the lumen[7]. Up until 15 years ago, there was limited knowledge about the gut microbiota because most of the microbial populations were uncultivable. However, during recent years the introduction of culture-independent techniques has allowed the identification and enumeration of new bacterial species, monitoring of changes in the gastrointestinal tract community, and better understanding of the role of gut microbiota in health and disease. Currently, more than 50 bacterial phyla have been described, of which 10 inhabit the colon and 3 predominate: *Firmicutes*, *Bacteroidetes* and the *Actinobacteria*[8]. Many intrinsic and extrinsic factors can modulate the distribution and composition of gut microbiota, including gastric acidity, gastrointestinal secretions and motility, anticommensal immunoglobulin (Ig)A and antimicrobial peptides, drugs blocking acid secretion and affecting gastrointestinal motility, antibiotics, and dietary modifications, including probiotic and fibre supplementation[8]. Furthermore, gut microbiota changes during the different stages of life: the fetal gut is virtually sterile, but it is rapidly colonized at birth by bacteria from mother's vagina or gut[9]. In childhood, the microbiota is very unstable and continues to evolve until adulthood with a gradual increase in *Bacteroides* spp., a decline in *Lactobacillus* spp. after the age of five and a decrease in *Bifidobacterium* spp. in the late teenage years[10]. Changes also occur in old age with a decline in *Bacteroides* spp. and an increase in *Enterococcus* spp. and *Escherichia coli*[11].

The intestinal microbiota is involved in important and specific tasks involving the host's homeostasis. In fact, gut bacteria are involved in many physiological, metabolic, nutritional and immunological processes. These include, among many others, the absorption of nutrients, the production of vitamins and hormones, host defense against pathogens and toxins, the control of epithelial cell proliferation and differentiation, and the development and regulation of the mucosal immune system[5].

Several lines of evidence suggest that intestinal microbiota may be involved in the pathophysiology of IBS. First, prospective studies have shown that 3% to 36% of gastrointestinal infections, inducing a marked disruption of the intestinal ecosystem, lead to a new diagnosis of the IBS, the so called post-infectious IBS[12]. Second, antibodies against flagellin, a component of indigenous bacteria inhabiting the human gut, and increased levels of human beta-defensin-2 (the first discovered inducible human antimicrobial protein) have been identified in at least a subgroup of patients with IBS, suggesting the existence of an abnormal host immune response towards components of the intestinal microbiota[13, 14]. Third, modulation of gut microbiota with probiotics and non-absorbable antibiotics has been shown to improve

symptoms in patients with IBS, providing a proof-of-concept for the implication of intestinal bacteria-host interactions in the pathophysiology and symptom generation of patients with IBS[15, 16]. Finally, a number of studies over time showed qualitative and quantitative changes in the composition and stability of intestinal microbiota in patients with IBS.

Earlier culture-based studies demonstrated a different composition of fecal and intestinal mucosal microbiota in patients with IBS compared with healthy individuals. In particular, these studies showed decreased numbers of *Lactobacilli, Bifidobacteria,* and anaerobic bacteria in patients with IBS in comparison with healthy individuals. However, only about 20% of the bacterial species and strains that inhabit the gut have been identified by conventional culture techniques. The advent of culture-independent, high-throughput molecular techniques has opened new avenues towards our understanding of the phylogenetic framework of the intestinal microbiota in several diseases.

Some studies showed a decreased proportion of the genera *Bifidobacterium* and *Lactobacillus,* and an increased ratio of *Firmicutes/Bacteroidetes* at phylum level in patients with IBS[17]. A recent study has investigated the correlation between microbiota profiles and psychological factors or bowel physiology. It showed that microbiota abnormalities were associated with peripheral changes, such as alterations in bowel transit times, while individuals with no modifications of the microbiota had more psychological disorders, including anxiety and depression[18]. A recent study has characterized the microbial composition of patients with post-infectious IBS (PI-IBS) and examined the associations between the fecal microbiota and the clinical features of these patients. The data suggested that the discriminant bacteria between the patients and healthy subjects consisted of 27 genus-like phylogenetic groups. Interestingly, these microbial profiles were associated with the mucosal expression of several host genes, including some involved in the inflammatory response and cell junction integrity, suggesting an impact of the altered microbiota on the immune system and impaired epithelial barrier function[19].

Attention is now focused on the potential functional consequences of altered microbiota taxa. Intestinal bacteria changes can contribute to abnormal bowel function and pain perception through the release of many metabolites, including short chain fatty acids, as a result of fermentation of unabsorbed polysaccharides in the small intestine. Interestingly, patients with IBS had increased fecal levels of acetic and propionic acids which correlated with the severity of abdominal pain and bloating[20].

Other effects of changes in microbiota on bowel physiology could be related to the activation of the innate immune system. The interaction between intestinal bacteria and the epithelium is mediated by microbiota-related substances (e.g., bacterial lipopolysaccharides) which are recognized by specific receptors (*toll-like receptors,* TLRs) resulting in mucosal immune activation in order to preserve homeostasis and epithelial barrier function. A recent study demonstrated that IBS patients have an increased colonic mucosal expression of TLR-4 compared with healthy controls. This finding suggests that components of the microbiota can cross the intestinal mucosa, inducing overstimulation of TLRs and activation of the mucosal immune response. This most likely occurs in the subgroup of patients showing altered intestinal barrier function and increased epithelial permeability, with subsequent immune system exposition to an abnormal microbial antigenic load[21].

Recent research supports the potential role of mucosal immune activation in the pathophysiology and symptom generation in IBS[3]. An increased number of activated immunocytes, mainly mast cells and T cells, has been detected in the mucosa of both the small bowel and colon in a subset of patients with IBS as compared with controls. In particular, mast cells, a key component of the innate immune system, are considered as sentinels strategically located at the interface between the host and the external environment. Upon activation, these cells release a number of biologically active substances contained in their granules, including histamine, serotonin and proteases. They can also release cytokines and membrane-derived arachidonic acid metabolites including prostaglandins and leukotrienes. The abnormal release of these bioactive mediators in the intestinal milieu may impact on gut nerve intrinsic and/or extrinsic activity, as demonstrated by their adoptive transfer to naïve animals or human tissues which increases intestinal intrinsic neuron excitability, mesenteric sensory nerve activity, and visceral sensitivity[3]. Furthermore, mast cells are found in close proximity to mucosal innervation and this spatial association is considered a key feature underlying the crosstalk between the immune and nervous system in the gut[22]. Interestingly, a positive correlation has been found between the number of colonic mast cells and both the intensity and the frequency of abdominal pain, supporting the potential relevance of these immune mechanisms in symptom generation in patients with IBS. The correlation of immune cell infiltration with bowel habits in IBS patients is controversial: some studies showed a similar degree of mast cell infiltration in patients with predominant diarrhea or constipation, while other research has reported that immune cell counts were higher in patients with diarrhea[3].

INTESTINAL EPITHELIAL BARRIER

As mentioned above, alterations in the intestinal barrier have been found to play a pathogenic role in IBS as well as in many other diseases, including inflammatory bowel diseases, celiac disease and also extra-intestinal disorders[23].

The intestinal barrier is a complex structure that separates the internal milieu from the gut lumen; it includes the vascular endothelium, the epithelial cells, the mucus layer, and also the intestinal microbiota. Apart from this physical barrier, there is a chemical barrier consisting of digestive secretions, antimicrobial peptides, and other cell products (e.g., cytokines and inflammatory mediators). In more detail, the main physical barrier is represented by enterocytes arranged in a single layer. The paracellular space is sealed by tight junctions (TJs), adherence junctions and desmosomes; all these components regulate the flow of water, electrolytes and small molecules and contribute to epithelial stability. TJ complexes are organized in the transmembrane proteins, occludin and claudins, interacting with zonula occludens (ZO), proteins that bind to the actin cytoskeleton. The contractions of actin result in the opening of TJs with an increase of permeability.

Any alteration of this network that constitutes the intestinal barrier increases the mucosal permeability, causing the passage of endoluminal antigens in the deeper layers. This results in the activation of the adaptive immune response leading to the low-grade inflammatory state demonstrated at least in a subgroup of patients with IBS[23].

The integrity of the intestinal barrier has been evaluated both *in vivo* and *in vitro*[23-26]. *In vivo*, intestinal permeability is assessed by the use of oral indigestible probes that are generally too large to cross TJs. Increased mucosal permeability determines a more permissive passage of these molecules and their urinary excretion can be quantified. Typical probes include poly-ethylene glycol (PEG) 400, ^{51}Cr-EDTA, or sugars, such as sucrose as a marker of gastric permeability, mannitol as a marker of small bowel permeability, lactulose as a marker of damaged small bowel permeability, and sucralose as a marker of colonic permeability. Recent studies assessing lactulose: mannitol ratios as a marker of intestinal permeability found that the optimal time for urine collection is 0–2 hours for small bowel and 8–24 hours for colonic permeability.
Intestinal permeability can also be evaluated *in vitro* using mucosal biopsies, by means of quantifying the passage of large molecules from the luminal to the basolateral side of the epithelium. Mucosal biopsies can be used to obtain other important information including TJ ultrastructure and the protein and gene expression of key TJ molecules.

Recently, some biomarkers of epithelial cell integrity have been proposed, including citrulline (i.e., an amino acid produced by small intestinal enterocytes that is assessed in plasma) and fatty acid binding proteins (i.e., small cytosolic proteins present in mature enterocytes of the small and large intestine) that are measured in both plasma and urine. The measurement of urinary or plasma levels of Glutathione S-transferases might be useful for assessment of intestinal damage, and urinary levels of claudins can be used as non-invasive markers for intestinal TJ loss[23].

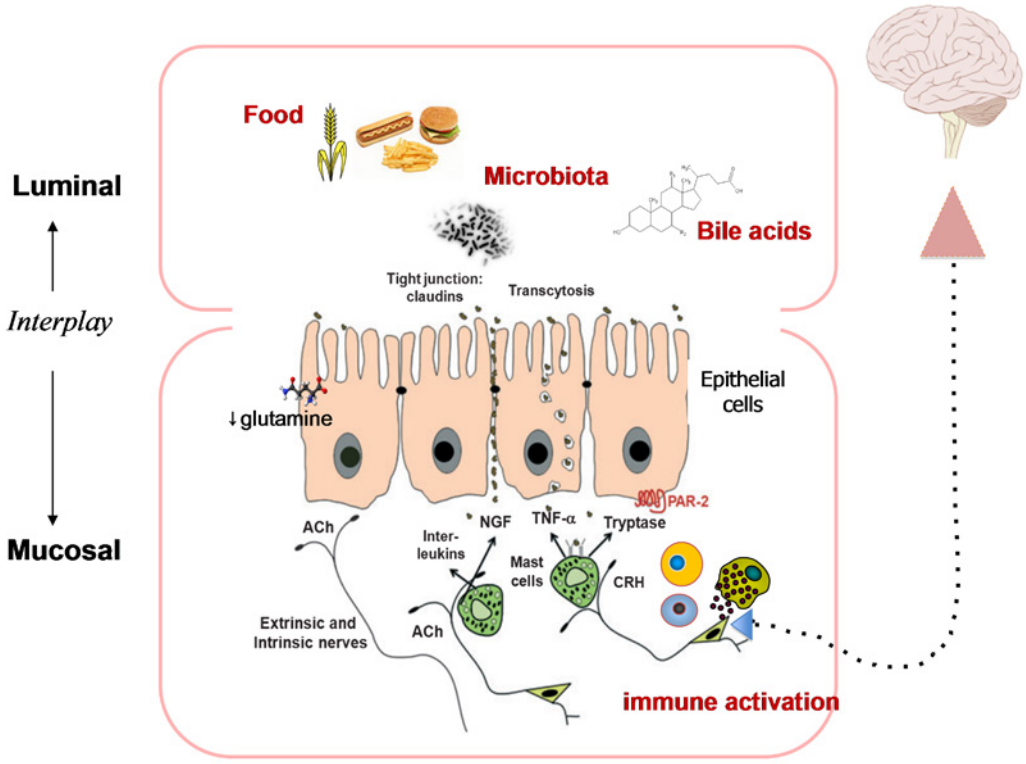
Studies primarily focused on PI-IBS, IBS-D and in all the subgroups of patients with IBS showed an increased intestinal permeability associated with changes in TJ expression. *In vivo* studies reported that increased intestinal permeability could be detected both in the small and large intestine of patients with IBS. The impaired permeability was confirmed by *in vitro* studies on mucosal biopsies obtained from the colon of both IBS-D and IBS-C patients[26, 27]. In particular, decreased mRNA expression of ZO-1 was found in colonic samples of patients with IBS in comparison with those obtained from healthy subjects[26]. ZO-1 expression was decreased at both gene and protein levels in the jejunum of patients with IBS-D, confirming that altered TJ expression may be a common mechanism involving both the small intestine and colon in IBS[28]. Altered TJ expression was also associated with the redistribution of ZO-1 from the apical membrane of the enterocytes to the cytoplasm, suggesting that endocytosis contributes to a ZO-1 down-regulation in IBS[28]. The factors involved in TJ alterations in IBS remain poorly defined, but they are likely to be multifactorial, involving stress, unrecognized food allergies, bile acid malabsorption,

Figure 1: Schematic representation of putative microenvironmental factors involved in the pathophysiology of IBS, with particular attention on microbiota and permeability. The figure highlights the interplay between luminal and mucosal factors. A leaky barrier may allow amplification of signaling from the lumen to the neural and immune elements. Adapted from Keita and Söderhol[31].

changes in gut microbiota and mucosal immune activation. Attention has been directed toward factors released in the luminal or mucosal milieu. In fact, the incubation of Caco-2 cell monolayer with supernatants obtained from colonic biopsies of patients with IBS induced an increased paracellular permeability associated with a decreased ZO-1 mRNA expression compared with supernatants obtained from healthy individuals[24]. Although the origin of the mediators affecting TJ function is yet to be defined, products released by bacteria adherent to the epithelium or molecules released by epithelial or immune cells, such as proteases, histamine and prostanoids, are likely participants in the induction of increased mucosal permeability[29]. Interestingly, a significant correlation has been repeatedly found between increased mucosal permeability and abdominal pain in patients with IBS. In particular, abdominal pain severity was significantly correlated with the increase of paracellular permeability and the down-regulation of ZO-1 mRNA expression in Caco-2 monolayers evoked by IBS colonic mucosal supernatants[26, 30].

CONCLUSIONS

In recent years, there has been a consistent increase in our knowledge of the pathophysiology of IBS. Several lines of evidence recognize luminal factors, such as intestinal bacteria, increased intestinal permeability and low-grade immune activation as important players. A variety of different triggers, including changes in gut microbiota, in genetically predisposed individuals may contribute to the loss of intestinal barrier function allowing the passage of antigens through the mucosal layer (Figure 1). This may elicit mucosal immune activation with release of numerous mediators, which induce neuroplastic changes and evoke the sensitization of afferent neuronal fibres, leading to abdominal pain perception and changes in bowel habit, the key symptoms of patients with IBS.



References

(1) Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC. Functional Bowel Disorders. Gastroenterology. 2006;130:1480–91.

(2) Barbara G, Stanghellini V. Biomarkers in IBS: when will they replace symptoms for diagnosis and management? Gut. 2009;58:1571–5.

(3) Barbara G, Cremon C, Carini G, Bellacosa L, Zecchi L, De Giorgio R, et al. The immune system in irritable bowel syndrome. J Neurogastroenterol Motil. 2011;17:349–59.

(4) Camilleri M. Peripheral mechanisms in irritable bowel syndrome. N Engl J Med. 2012;367:1626–35.

(5) Sekirov I, Russell SL, Antunes LC, Finlay BB. Gut microbiota in health and disease. Physiol Rev. 2010;90:859–904.

(6) O'Hara AM, Shanahan F. The gut flora as a forgotten organ. EMBO Rep. 2006;7:688–93.

(7) Swidsinski A, Loening-Baucke V, Lochs H, Hale LP. Spatial organization of bacterial flora in normal and inflamed intestine: a fluorescence in situ hybridization study in mice. World J Gastroenterol. 2005;11:1131–40.

(8) Simrén M, Barbara G, Flint HJ, Spiegel BM, Spiller RC, Vanner S, et al. Intestinal microbiota in functional bowel disorders: a Rome foundation report. Gut. 2013;62:159–76.

(9) Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci USA. 2010;107:11971–5.

(10) Balamurugan R, Janardhan HP, George S, Chittaranjan SP, Ramakrishna BS. Bacterial succession in the colon during childhood and adolescence: molecular studies in a southern Indian village. The Am J Clin Nutr 2008;88:1643–7.

(11) Enck P, Zimmermann K, Rusch K, Schwiertz A, Klosterhalfen S, Frick JS.. The effects of ageing on the colonic bacterial microflora in adults. Z Gastroenterol. 2009;47:653–8.

(12) Cremon C, Stanghellini V, Pallotti F, Fogacci E, Bellacosa L, Morselli-Labate AM, et al. Salmonella gastroenteritis during childhood is a risk factor for irritable bowel syndrome in adulthood. Gastroenterology. 2014;147:69–77.

(13) Schoepfer AM, Schaffer T, Seibold-Schmid B, Müller S, Seibold F. Antibodies to flagellin indicate reactivity to bacterial antigens in IBS patients. Neurogastroenterol Motil. 2008;20:1110–8.

(14) Langhorst J, Junge A, Rueffer A, Wehkamp J, Foell D, Michalsen A, et al. Elevated human betadefensin-2 levels indicate an activation of the innate immune system in patients with irritable bowel syndrome. Am J Gastroenterol. 2009;104:404–10.

(15) Moayyedi P, Ford AC, Talley NJ, Cremonini F, Foxx-Orenstein AE, Brandt LJ, et al. The efficacy of probiotics in the treatment of irritable bowel syndrome: a systematic review. Gut. 2010;59:325–32.

(16) Pimentel M, Lembo A, Chey WD, Zakko S, Ringel Y, Yu J, et al. Rifaximin therapy for patients with irritable bowel syndrome without constipation. N Engl J Med. 2011;364:22–32.

(17) Rajilić-Stojanović M, Biagi E, Heilig HG, Kajander K, Kekkonen RA, Tims S, et al. Global and deep molecular analysis of microbiota signatures in fecal samples from patients with irritable bowel syndrome. Gastroenterology. 2011;141:1792–801.

(18) Jeffery IB, O'Toole PW, Öhman L, Claesson MJ, Deane J, Quigley EM, et al. An irritable bowel syndrome subtype defined by species-specific alterations in faecal microbiota. Gut. 2012;61:997–1006.

(19) Jalanka-Tuovinen J, Salojärvi J, Salonen A, Immonen O, Garsed K, Kelly FM, et al. Faecal microbiota composition and host-microbe cross-talk following gastroenteritis and in postinfectious irritable bowel syndrome. Gut. 2014;63:1737–45.

(20) Barbara G, Cremon C, Stanghellini V. Inflammatory bowel disease and irritable bowel syndrome: similarities and differences. Curr Opin Gastroenterol. 2014;30:352–8.

(21) Brint EK, MacSharry J, Fanning A, Shanahan F, Quigley EM. Differential expression of toll-like receptors in patients with irritable bowel syndrome. Am J Gastroenterol. 2011;106:329–36.

(22) Barbara G, Stanghellini V, De Giorgio R, Cremon C, Cottrell GS, Santini D, et al. Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. Gastroenterol. 2004;126:693–702.

(23) Bischoff SC, Barbara G, Buurman W, Ockhuizen T, Schulzke JD, Serino M, et al. Intestinal permeability – a new target for disease prevention and therapy. BMC Gastroenterology 2014;14:189.

(24) Barbara G. Mucosal barrier defects in irritable bowel syndrome. Who left the door open? Am J Gastroenterol. 2006;101:1295–8.

(25) Rao AS, Camilleri M, Eckert DJ, Busciglio I, Burton DD, Ryks M, et al. Urine sugars for in vivo gut permeability: validation and comparisons in irritable bowel syndrome-diarrhea and controls. Am J Physiol Gastrointest Liver Physiol. 2011;301:G919–28.

(26) Piche T, Barbara G, Aubert P, Bruley des Varannes S, Dainese R, Nano JL, et al. Impaired intestinal barrier integrity in the colon of patients with irritable bowel syndrome: involvement of soluble mediators. Gut. 2009;58:196–201.

(27) Barbara G, Zecchi L, Barbaro R, Cremon C, Bellacosa L, Marcellini M, et al. Mucosal permeability and immune activation as potential therapeutic targets of probiotics in irritable bowel syndrome. J Clin Gastroenterol. 2012;46:S52–5.

(28) Martínez C, Vicario M, Ramos L, Lobo B, Mosquera JL, Alonso C, et al. The jejunum of diarrhea-predominant irritable bowel syndrome shows molecular alterations in the tight junction signaling pathway that are associated with mucosal pathobiology and clinical manifestations. Am J Gastroenterol. 2012;107:736–46.

(29) Coëffier M, Gloro R, Boukhettala N, Aziz M, Lecleire S, Vandaele N, et al. Increased proteasome-mediated degradation of occludin in irritable bowel syndrome. Am J Gastroenterol. 2010;105:1181–8.

(30) Zhou Q, Zhang B, Verne GN. Intestinal membrane permeability and hypersensitivity in the irritable bowel syndrome. Pain. 2009;146:41–6.

(31) Keita AV, Söderholm JD. The intestinal barrier and its regulation by neuroimmune factors. Neurogastroenterol Motil. 2010;22:718–33.

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THE ORAL AND GUT MICROBIOMES ARE PERTURBED IN RHEUMATOID ARTHRITIS AND PARTLY NORMALIZED AFTER TREATMENT

Zhang X, Zhang D, Jia H, Feng Q, Wang D, Liang D, et al. Nat Med. 2015;21(8):895–905. doi: 10.1038/nm.3914.

Rheumatoid arthritis (RA) is an autoimmune disorder with obscure etiology. Recent discoveries in genealogy indicated some genes are responsible in the etiology of RA such as HLA-DRB1, TNFAIP3, PTPN22 and PADI4[1]. However, environmental factors have also been shown to contribute to the disease pathogenesis[2]. One of the most popular research areas in epigenetic factors on chronic diseases is the gut microbiota. RA is not an exception, and many studies have shown that RA is associated with dysbiosis. Joint inflammation is the hallmark of RA but other body sites may also show preceding signs of inflammation. Gut microbiota is mostly stable in a given person, and in patients with RA, there are unique features of microbial composition. The oral microbiome is another body site with its own ecosystem and, in many studies in different disease conditions (cirrhosis, colon cancer, etc), oral microbiome is strongly correlated with gut microbiota. It is not a surprise that oral and gut microbiota are interrelated, since it is a continuum of longitudinal microbial axis along digestive tract.

Zhang *et al* (a large team of researchers from China) investigated oral (salivary and dental) and fecal microbiota in RA patients. They also followed these patients for therapy-related changes in the microbiota. To investigate the gut microbiome in RA patients, they carried out metagenomic shotgun sequencing of 212 fecal samples (77 treatment-naive individuals with RA and 80 unrelated healthy controls; 17 treatment-naive individuals with RA paired with 17 healthy relatives; and 21 samples from disease-modifying anti-rheumatic drug [DMARD]-treated individuals with RA). Gut microbial diversity and richness were similar between the 77 treatment-naive individuals with RA and 80 unrelated healthy controls. To delineate features of the RA-associated gut microbiome, they identified 117,219 gene markers that were differentially enriched in RA patients versus controls and clustered the genes into metagenomic linkage groups (MLGs) on the basis of their correlated abundance variation among samples. The 88 MLGs that contained at least 100 genes separated RA-enriched and control-enriched MLGs along the vector for RA status in canonical correspondence analysis, confirming that they were associated mainly with RA status, rather than with other complicating factors. A cluster containing Veillonella and Haemophilus strains, along with other MLGs including *Klebsiella pneumoniae, Bifidobacterium bifidum, Sutterella wadsworthensis* and *Megamonas hypermegale*, were enriched in the healthy controls compared with the RA subjects. In contrast, the RA-enriched MLGs formed a large cluster including *Clostridium asparagiforme, Gordonibacter pamelaeae, Eggerthella lenta* and *Lachnospiraceae bacterium*, as well as small clusters or single MLGs containing strains such as *B. dentium, Lactobacillus sp.* and *Ruminococcus lactaris*. The RA gut was enriched in Gram-positive bacteria and depleted of Gram-negative bacteria, including some Proteobacteria and Gram-negative Firmicutes of the Veillonellaceae family.

RA status had the strongest effect on the dental and salivary microbiomes among all available phenotypes. There was a strong correlation between oral and gut microbiomes. The researchers also performed a novel analysis, which the performance of gut microbiota in distinguishing RA patients (as a diagnostic marker) was analyzed for the first time in the literature. To illustrate the diagnostic value of the RA-associated microbiome, they first constructed random forest disease classifiers based on the gut MLGs. Tenfold cross-validation was done five times on the cohort (N = 157), and the final model contained 8 of the 88 gut MLGs, leading to an area under the receiver operating curve of 0.940 (specificity, 0.922; sensitivity, 0.838). Oral microbiome had also some diagnostic capability.

The authors also reported a partial recovery (improvement) of gut microbiota after methotrexate or DMARD treatment. Similar findings were also reported in Crohn's disease after anti-tumor necrosis factor therapy in a recent paper[3].

Finally, there is a tremendous progress in microbiota research and it is getting viral in various areas of medicine. RA is a solid evidence for microbiota-autoimmunity concept and probably future studies will delineate novel approaches for diagnosis, phenotyping and treatment of this epidemic disease.

References

(1) McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med. 2011;365(23);2205–19.

(2) Raychaudhuri S, Sandor C, Stahl EA, Freudenberg J, Lee HS, Jia X, et al. Five amino acids in three HLA proteins explain most of the association between MHC and seropositive rheumatoid arthritis. Nat Genet. 2015;44(3):291–6.

(3) David B, Teresa MD, Mireia LS, Margarita MM, Anna B, Miriam S, et al. Anti-TNF treatment with adalimumab induces changes in the microbiota of Crohn's disease. J Crohns Colitis. 2015 Jul 4. pii: jjv119 [Epub ahead of print].

EFFECT OF PROBIOTICS ON GLYCEMIC CONTROL: A SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED, CONTROLLED TRIALS

Ruan Y, Sun J, He J, Chen F, Chen R, Chen H. PLoS One. 2015;10(7):e0132121. doi: 10.1371/journal.pone.0132121.

Probiotics are defined as live microorganisms with potential health benefits for the host if consumed in adequate amounts[1]. Probiotics have beneficial effects on the immune system, gastrointestinal disorders, autoimmune and allergic diseases and other conditions associated with dysbiosis. Ruan *et al* analyzed the effect of probiotics on blood sugar levels. Animal models suggest a beneficial effect on blood glucose levels and insulin resistance. The authors searched 1,207 records after eliminating duplicates and assessed 72 eligible full-text articles. Finally, 16 articles were included in the meta-analysis. Seventeen clinical trials involving 1,105 participants (551 probiotics, 554 control) were included. Sixteen trials were double-blinded and one study was single-blinded.

This is the first study to systematically analyze the effect of probiotics on glycemic control. The rationale behind probiotic consumption in diabetic patients comes from gut microbiota research in the last decade. Metagenomic data have revealed that patients with type 2 diabetes exhibit a moderate degree of gut microbial dysbiosis compared with patients with inflammatory bowel disease[2]. The proportions of the phylum Firmicutes and the class *Clostridia* are significantly reduced, whereas the class of the gram negative Betaproteobacteria is highly enriched in the feces of patients with type 2 diabetes[3].

Overall, probiotics significantly reduced fasting blood glucose by 0.31 mmol/L, insulin by 1.17 μU/mL and improved homeostasis model assessment of insulin resistance by 0.48. The study showed that the glucose-lowering effect of probiotics is more pronounced in diabetic patients than in healthy controls. Another proposed mechanism of glucose lowering mechanism is the increased glucagon-like peptide-1 secretion. Some authors also suggested anti-oxidant mechanisms, anti-inflammatory effect (diabetic patients have low-grade inflammation), suppressing NF-κB pathway.

There are some limitations to this study. Meta-analyses are a difficult type of analysis, especially in the field of probiotics. Each strain has unique characteristics, and each probiotic has different effect on the immune/ecosystem of the gut. Combination of bacterial strains versus single bacterial products has totally different mechanism of action. So, this dilemma brings major question marks for gathering all of the probiotics into one basket. In the future, single probiotic preparations might be assessed for meta-analytic research.

This meta-analysis also identified some of the characteristics of probiotics which had a greater influence on glycemic control. Combinations of probiotic species were better than single bacterial products. Higher doses, $>10^{11}$ colony-forming units and a longer duration of therapy (>8 weeks) were also associated with a superior positive result. As a result, the authors clearly defined the role of probiotics in diabetic patients. Probiotics have a modest blood glucose and insulin resistance lowering effect in diabetic population. Further research is needed in order to clarify the type, dosage and duration of specific bacterial combination in this condition.

References

(1) Reid G, Charbonneau D, Erb J, Kochanowski B, Beuerman D, Poehner R, et al. Oral use of Lactobacillus rhamnosus GR-1 and L. fermentum RC-14 significantly alters vaginal flora: randomized, placebo controlled trial in 64 healthy women. FEMS Immunol Med Microbiol. 2003;35(2):131–4.

(2) Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature. 2012;490(7418):55–60.

(3) Larsen N, Vogensen FK, van den Berg FW, Nielsen DS, Andreasen AS, Pedersen BK, et al. Gut microbiota in human adults with type 2 diabetes differs from nondiabetic adults. PLoS One 2010;5(2):e9085. doi: 10.1371/journal.pone.0009085.

HAVING OLDER SIBLINGS IS ASSOCIATED WITH GUT MICROBIOTA DEVELOPMENT DURING EARLY CHILDHOOD

Laursen MF, Zachariassen G, Bahl MI, Bergström A, Høst A, Michaelsen KF, Licht TR. BMC Microbiology 2015;15:154. doi: 10.1186/s12866-015-0477-6.

Laursen *et al* recently published a very interesting study in *BMC Microbiology* about the effects of environmental influences on gut microbiota. The rationale for this hypothesis was the accumulating evidence that infections early in life and the presence of older siblings and furred pets in the household strongly influences the risk of allergic diseases in children. Laursen and colleagues proposed that this effect was due to early life development of gut microbiota. According to this hypothesis, they planned a study to investigate whether the presence of older siblings and furred pets and early life infections affected gut microbial communities at 9 and 18 months of age and whether these differences were associated with the cumulative prevalence of atopic symptoms of eczema and asthmatic bronchitis at 3 years of age. The pediatric cohort used in this study was the SKOT 1 cohort, which includes 311 Danish children followed until 3 years old for the analysis of relationship between early diet, growth and development and disease risks[1]. The researchers have already studied gut microbiota in another study; however, this study had a more extensive microbial analysis[2].

The authors standardized the patients by characteristics such as the prevalence of allergic heredity and C-section, average gestational age at birth, actual age at 9 and 18 month visits, infant age at start of daycare or nursery, breastfeeding duration and macronutrient intake at 9 and 18 months visits were similar between infants with and without older siblings, furred pets or early life infections. They found that infants with furred pets had a lower Firmicutes diversity at 9 months of age and higher abundance of Cronobacter at 18 months of age. The number of older siblings correlated positively with bacterial diversity ($p = 0.030$), diversity of the phyla Firmicutes ($p = 0.013$) and Bacteroidetes ($p = 0.004$) and bacterial richness ($p = 0.006$) at 18 months. Furthermore, having older siblings was associated with increased relative abundance of several bacterial taxa at both 9 and 18 months of age. However, gut microbiota characteristics were not significantly correlated with eczema and asthmatic bronchitis during the first 3 years of life.

What this study brings into the literature is that we have diseases in adulthood which are strongly affected by our early life developmental changes in the microbiome. These are inter-related with our surroundings, especially our household and family members. We are not sterile living beings in a lab environment and we should consider health and disease in a continuum of birth, childhood and then adulthood in its ecosystem.

References

(1) Madsen AL, Schack-Nielsen L, Larnkjaer A, Mølgaard C, Michaelsen KF. Determinants of blood glucose and insulin in healthy 9-month-old term Danish infants; the SKOT cohort. Diabet Med. 2010;27(12):1350–7.

(2) Bergström A, Skov TH, Bahl MI, Roager HM, Christensen LB, Ejlerskov KT, et al. Establishment of intestinal microbiota during early life: a longitudinal, explorative study of a large cohort of Danish infants. Appl Environ Microbiol. 2014;80(9):2889–900.

GUT-MICROBIOTA-METABOLITE AXIS IN EARLY RENAL FUNCTION DECLINE

Barrios C, Beaumont M, Pallister T, Villar J, Goodrich JK, Clark A, et al. PLoS One. 2015;10(8):e0134311. doi: 10.1371/journal.pone.0134311.

Metabolites derived from bacteria provide a readout of the metabolic state of an individual and are the product of genetic and exogenous (diet, lifestyle, gut microbial activity) factors under a particular set of conditions. Chronic kidney disease (CKD) is a chronic uremic condition with increased gut permeability and associated with dysbiosis[1, 2]. This study aimed to clarify the gut-microbiome-metabolite axis to improve strategies that manipulate the gut microbiota in the onset of kidney dysfunction.

Barrios *et al* investigated circulating plasma metabolites of gut bacteria in 4,439 individuals from the TwinsUK cohort. They adjusted the confounding variables which might affect gut microbial metabolites such as age, sex, body mass index (BMI), diabetes mellitus, family relatedness, etc. They also investigated the effect of diet and found no independent effect on gut bacterial metabolites.

Out of 4,439 individuals only 7.4% had estimated glomerular filtration rate (eGFR) <60 mL/min/1.73m^2. Indoxyl-sulfate (Beta [SE] –2.74 [0.24]; p = 8.8 x 10^{-29}), p-cresyl-sulfate (–1.99 [0.24]; p = 4.6 x 10^{-16}), and phenylacetylglutamine (–2.73 [0.25]; p = 1.2 x 10^{-25}) were significantly and negatively associated with eGFR after adjusting for confounding variables. Lachnospiraceae, Christensenellaceae and Ruminococcaceae bacterial families are well-known for their butyrate production and increased well-being. These bacteria are also found to be protective for renal function in this study. Serum levels of harmful bacterial end-products (indoxyl-sulphate, p-cresyl-sulphate and phenylacetylglutamine) were inversely correlated with these aforementioned bacteria. The researchers found that dysbiosis starts even at the early stages of renal decline.

As a result, this is first study combining metabolome and microbiome data in early renal function decline. Circulating levels of uremic toxins are correlated with dysbiosis and renal function, even at the beginning of renal disease. Strategies for manipulating gut microbiota to gain a better metabolomic state might be an adjunct for CKD treatment in the future.

References

(1) Viaene L, Thijs L, Jin Y, Liu Y, Gu Y, Meijers B, et al. Heritability and clinical determinants of serum indoxyl sulfate and p-cresyl sulfate, candidate biomarkers of the human microbiome enterotype. PloS One. 2014;9(5):e79682. doi: 10.1371/journal.pone.0079682.

(2) Shin SY, Fauman EB, Petersen AK, Krumsiek J, Santos R, Huang J, et al. An atlas of genetic influences on human blood metabolites. Nat Gen. 2014;46(6):543–50. doi: 10.1038/ng.2982.

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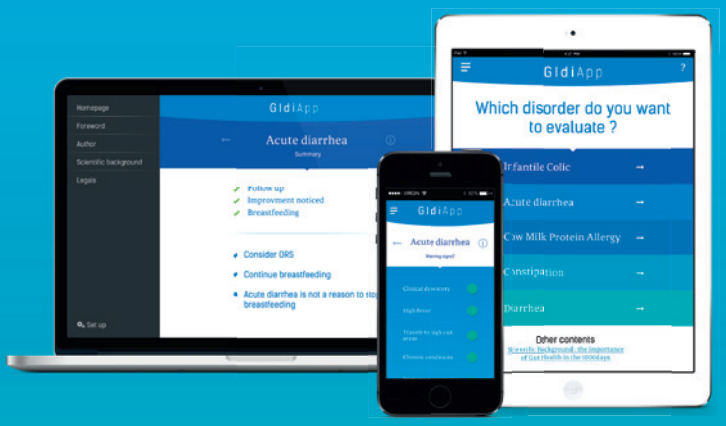

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THE ANNUAL DIGESTIVE DISEASE WEEK 2015

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INTRODUCTION

The annual Digestive Disease Week (DDW) was held in Washington from May 16 through May 19, 2015[1]. This meeting provided approximately 15,000 researchers, clinicians and policy makers from across the globe with a good opportunity to learn about the latest developments and research questions in the field of Gastroenterology. In 2015, DDW included presentations on microbiota and obesity, novel diagnostic tools, and the role of the gut microbiota from metabolites to neurotransmitters.

MICROBIOTA AND OBESITY

Where we stand

Obesity is an increasing problem, which is becoming an epidemic due to changes in the modern environment. In addition, obesity is associated with many diseases, and has a devastating effect on quality of life. In this year's DDW, special attention was given to obesity and its connection to the gut microbiome (GM).

Obesity – a state, not a process

In his lecture on obesity, Prof. Kaplan from the Massachusetts General Hospital at Harvard Medical School explained that the historical view of obesity is based on the assumption that it is a process and that lifestyle choices and characterological flaws (i.e. willpower, psychology) are responsible. Very recently science began to understand that the state of obesity is much more diverse and involves the dysfunction of a complex physiological regulatory system. The body tends to maintain a stable adipose tissue mass (much like a stable number of blood cells), even if it's abnormal. Obesity results from the failure of energy regulatory mechanisms irrespective of food intake or energy expenditure[2].

Variety of regulatory mechanisms (Kaplan)

Nutrients or the chemical compounds of nutrients present in the gastrointestinal (GI) tract can influence approximately 200 types of receptors present in this organ. The neuronal, hormonal and immune responses are the central mechanisms that can act on the brain, affect appetite and therefore regulate energy expansion. Conversely, the liver and the pancreas are involved in the regulation of metabolic processes. The brain, liver and pancreas make a triad of organs responsible for energy balance regulation and metabolic function[2].

Gut microbiota regulates metabolic activity (Kaplan)

The GM may send inflammatory signals, which result in a modified sensitivity to insulin. The GM also produces short-chain fatty acids which are known to increase adiposity. To show the interaction between GM and obesity, Prof. Kaplan and colleagues have carried out studies with germ-free mice colonized with GM. The results of this study demonstrate that microbiota colonization rapidly increases adiposity in mice without increasing food intake, suggesting that microbiota allow for greater energy harvest thereby helping to gain body mass. In another study, the microbiota was evaluated in mice with genetic obesity and diet-induced obesity (high fat/high sugar). This study showed that both genetic- and diet-induced obesity is linked to an increase of firmicutes and a decrease of *Bacteroidetes*. This means that it is not only the GM that influences obesity, but that obesity has an impact on the microbiota as well.

Another preclinical study designed to evaluate how the GM reacts to gastric bypass surgery showed that gastric bypass drastically changes the GM in mice. Among animals who underwent gastric bypass surgery, increased energy expenditure and weight loss was observed, despite receiving the same amount of food compared to the control group. This study shows that gastric bypass surgery modifies the GM by producing more proprionate, which is known to stimulate energy expansion[2].

Energy balance and homeostasis regulation – a new approach

Energy homeostasis in humans is fairly simple: when the human body consumes more energy than it uses, the excess energy is stored in adipose tissue causing obesity. The main questions that Dr. Diehl's team from Duke University in North Carolina posed were: what controls the energy homeostasis in humans?, and why human tissues are damaged when energy balance is challenged? To answer these questions, they presented recent findings from animal model studies[3].

Hedgehog pathway

The key pathway controlling energy balance was identified in a study conducted with flies by a group of scientists head by H. Esterhauser from the Medical University of Vienna, Austria[4]. The Hedgehog pathway (HP) is a morphogenic-signaling pathway which regulates tissue construction, controlling stem cell viability and modulating progenitor cell fate decisions (proliferation, migration and differentiation), involved in epithelial-mesenchymal cross-talk. The HP and its ligands are lipid modified and associate with lipoproteins, which are the most critical regulators of net energy balance (adiposity) during embryogenesis in flies. The studies in flies were later repeated in mammals (e.g. mice). These results demonstrated that over activating Hedgehog signaling in fat tissues of developing mammals produces visceral adiposity and the metabolic syndrome. The authors concluded that the HP can regulate fat mass. Moreover, active hedgehog stimulates glycolysis and inhibits lipid storage, in other words, turning hedgehog off inhibits these actions and promotes lipid storage.

The circadian clock

Another mechanism of regulating energy consumption and replenishment is the circadian clock. It is because of the circadian clock that we are active and consuming energy at certain times of the day and we are replenishing it at other times. It is proven that hedgehog regulates the circadian clock and vice versa, but the mechanisms responsible for this cross regulation are unclear [3]. Emerging research by Dr. Eugene Chang at Chicago University, revealed that the human microbiome is the energy sensor that sets the circadian clock and not light as previously thought[5]. It is the short-chain fatty acids produced by microbiota that "set the clock". The importance of GM in energy consumption was previously confirmed in studies performed with mice. Germ-free mice stay leaner than the conventional mice, irrespective of their dietary intake. Therefore, no GM means unrestricted energy "consumption" and restored gut microbiome means restricted energy consumption. Furthermore, GM transplanted from fat mice into lean mice results in weight gain in the latter. Therefore, an "obese" microbiome promotes energy replenishment and a "lean" microbiome promotes energy consumption (much like the hedgehog pathway)[3].

Prevention of obesity-related tissue damage

Obesity usually causes tissue damage. The HP is known for being reactivated during adult tissue injury. However, less active hedgehog can inhibit regeneration and hyperactive hedgehog can cause defective repair, resulting in scarring and cancer lesions. The circadian clock is regulated by the HP, and consequently the GM regulates the circadian clock; one way to activate HP and prevent tissue injury linked to obesity is to modulate the microbiome. In summary, deregulation of pathways that control energy utilization and storage as HP, results in disordered energy utilization and storage which can cause abnormal tissue growth and repair[3].

Obesity in Humans – every case is different

Another lecture by Prof. Kaplan from Harvard Medical School presented the factors that influence weight loss besides energy balance[6].

The human body tries to maintain stable adipose tissue mass by altering its energy balance regulation and that is why low-calorie diets do not always result in long-term weight loss. It has been shown that patients who lose weight on a low-calorie diet and then move on to a weight-maintenance diet gain weight while eating less. This is because certain hormone changes appear to promote fat storage instead of fat loss.

Regardless of which means of losing weight is chosen, there is always variability in the outcomes, because each case of obesity is different in terms of onset, fat localization and distribution, and metabolic consequences. Weight loss is even influenced by the genome. Studies using identical twins and their spouses, revealed that on the same diet twins demonstrated equivalent weight loss, while their spouse's exhibited a different pattern of weight loss[7].

The Western Diet modifies fecal metabolites and reduces the number of nitrergic myenteric neurons

In humans, the Western diet is generally associated with a reduction in stool frequency. Studies have shown that high-fat fed mice exhibit delayed GI motility, which is associated with a reduced number of nitrergic myenteric neurons. However, to date data on the mechanisms of these alterations remain unclear. Dr. Reichardt , and colleagues, from Munich's Technical University, evaluated the influence of a Western diet on modification of fecal metabolites associated with the development of enteric neurodegeneration together with the role of GM in this event. They hypothesized that a Western diet would induce GM dysbiosis.

Mice fed a Western diet for 12 weeks exhibited a reduced stool frequency in comparison to mice in the control group fed a regular diet, this was associated with a delayed intestinal

transit time. Moreover, mice fed a Western diet had a longer colonic transit time which was associated with a reduced number of nitrergic myenteric neurons in the proximal colon. Overall, 3,730 metabolites were quantified in the feces, however, 185 were significantly higher in mice on a Western diet when compared to the control group.
In order to understand the possible role of the GM in these changes, fecal citrulline in germ-free mice was measured for 6 weeks and similar concentrations were observed.

Long-term consumption of a Western diet is also linked to nitrergic myenteric neuronal loss and this is thought to be a contributing factor to delayed GI transit. These alterations were preceded by gut dysbiosis and elevated fecal citrulline. Investigators found that fecal citrulline was not affected by intake of a Western diet under germ-free conditions, suggesting that the GM is required for such effects[8].

A link between the use of antibiotics, disturbed gut microbiota and diabetes

Antibiotic therapy, which is increasingly used in Western countries, can cause bacterial dysbiosis. Recent animal data have confirmed that antibiotic exposure can disrupt the GM by altering metabolic genes and adiposity induction. These data show a profound influence of GM on metabolic pathways, which is linked to the pathogenesis of obesity, insulin resistance and diabetes. A case-control study, using a large population-based database from the United Kingdom, was carried out by B. Boursi and colleagues from Tel Aviv University, to evaluate the correlation between past antibiotic exposure and increased diabetes risk.

The study population included 208,002 diabetic cases and 815,576 matched controls. Exposure to a single antibiotic prescription was not associated with a higher adjusted risk of diabetes in all antibiotic groups. However, treatment with 2 to 5 antibiotic courses was associated with an increase in diabetic risk for penicillin, cephalosporins, macrolides and quinolones. The risk increased with the number of antibiotic courses, and reached 1.37 (95% CI 1.19–1.58) for more than 5 courses of quinolones[9].

The role of gut microbiota in absorption of lipids and the intestinal immune response

Obesity and diabetes are often associated with low-grade inflammation. On the other hand, the GM is believed to be implicated in endotoxemia, induced by a high-fat diet, and in increased intestinal permeability. Whether the GM is responsible for the lipid-induced activation of intestinal mucosal mast cells is unknown. The team of H. Sato from the National Defense Medical College in Saitama, Japan conducted an animal study with the objective of investigating the effect of antibiotics on mucosal mast cell activation, intestinal permeability, and the overall efficiency of fat absorption in lymph fistula rats. Investigators found that lymphatic diamine oxidase levels and rat mucosal mast cell protease II levels were significantly lower among the antibiotic-treated animals, suggesting a role for gut bacteria in the lipid-induced activation of mucosal mast cells. In addition, lymph fluorescein isothiocyanate was significantly lower in the antibiotic-treated group compared with the controls, suggesting that the presence of gut bacteria in the control group produced a leakier gut in response to lipid absorption. The results of this study suggest that the gut microbiota regulates the intestinal immune response to dietary lipids via the activation of intestinal mucosal mast cells. In addition, this study supports the effect of gut bacteria on the absorption of lipids. However, the mechanism of this interaction is unknown[10].

MICROBIOTA NOVEL DIAGNOSTIC TOOLS

Prof. Hashsham from Michigan State University presented new microbiota analysis chips which can evaluate bacterial samples cheaply and rapidly on site. This new technology is designed to analyze stool samples, in order to identify the cause of infectious or bacterial diarrhea. The system is designed as a chip with an assay where the samples are loaded, a device which conducts the sample analysis, and an application which contains the database and can be downloaded to a smartphone-, Android-/iPod-based operation or tablet. In addition, results can be obtained 10 to 60 minutes after sample collection. This new technology could become a fast and cheap method of analysis, and may be employed in developing countries where conventional analysis methods are less widely available[11].

FROM METABOLITES TO NEUROTRANSMITTERS: ROLE OF GUT MICROBIOTA

In his lecture, Prof. Savidge from the Texas Children's Hospital discussed the ways that gut microbiota interacts with the organism, with special emphasis on its role as a neurotransmitter producer.

Fast transitional alterations in the GM occur in the presence of changes in diet, pathogen exposure, or drug intake, as well as the use of pre- or probiotics. These changes interfere not only with the gut physiology, but can also influence the central nervous system function[12].

Microbiota produces neurotransmitters

There is strong evidence that *in vitro* microbiota actually produces neurotransmitters, for example it has been proven that spore-forming clostridia synthesizes the potent neurotransmitter serotonin. Gases, such as NO and H2S, are another important neuromodulator and the microbiota produces large quantities[12].

Microbiota and Clostridium difficile infection

One disease that is known to cause dysbiosis is *C. difficile* infection. The success of fecal transplantation in treating this infection proves that the microbiota has a protective effect which is yet to be understood.

Prof. Savidge presented his research on the role of microbiota in *C. difficile* infection. His team carried out a large analysis of microbiota from patients who have *C. difficile* infection. They noticed that all of the patients had specific changes in terms of microbiota composition – the number of *Bacteroidetes* decreases and there is an increase in *C. difficile* and *Streptococcus*.

In healthy patients, clostridia produce serotonin, but in the case of *C. difficile* infection these "good" clostridia disappear and are replaced by *C. difficile* which is a γ-aminobutyric acid (GABA) producer. To confirm the clinical relevance of this data, Prof. Savidge carried out a clinical trial, in which his team looked for a connection between the use of GABA receptor A agonists and cases of *C. difficile* infection. They found that patients taking zolpidem are five times as likely to be infected with this bacteria. This shows that *C. difficile* infection is at least partially caused by GABA imbalance in the body[12].

References

(1) Access online https://watch.ondemand.org/ddw (Accessed May 25 2015)

(2) Lee M. Kaplan, Massachusetts General Hospital, Harvard Medical School, "Microbiome and obesity", presented during DDW, May 16 2015

(3) Anna Mae Diehl, Duke University Durham, North Carolina. "Small Animal Models. Pathway Discovery to Advance Obesity Diagnosis and Management", presented during DDW, May 18 2015

(4) Teperino, Raffaele, et al. «Canonical and Non-Canonical Hedgehog Signaling and the Control of Metabolism.» Seminars in cell & developmental biology. Vol. 33. Academic Press, 2014.

(5) Leone, Vanessa, et al. «Effects of Diurnal Variation of Gut Microbes and High-Fat Feeding on Host Circadian Clock Function and Metabolism.» Cell host & microbe 17.5 (2015): 681-689.

(6) Lee M. Kaplan, Massachusetts General Hospital, Harvard medical School, "Scientific Exploration Using the Human "Model" of Obesity", presented during DDW, May 16 2015

(7) Hatoum, Ida J., et al. «Heritability of the weight loss response to gastric bypass surgery.» The Journal of Clinical Endocrinology & Metabolism 96.10 (2011): E1630-E1633.

(8) Francois Reichardt, Department of Human Biology, Technical University Munich, Freising-Weihenstephan, Germany, "Fecal Metabolites Associated With Nitrergic Myenteric Neuronal Impairment and Gastrointestinal Motility Disorders in Western Diet-Fed Mice", presented during DDW, May 16 2015

(9) Ben Boursi, Telaviv University, "The Effect of Past Antibiotic Exposure on Diabetes Risk", presented during DDW, 17 May 2015

(10) Hirokazu Sato, National Defense Medical College in Saitama, Japan," Targeting the Gut Microbiome With Antibiotics Reduces Mucosal Mast Cell Activation and Lipid Absorption in Lymph Fistula Rats", presented at DDW's Poster Session

(11) Syed Hashsham, Michigan State University, "Gut Microbiome, Human Health and Rapid Diagnosis Chips", presented during DDW, 16 May 2015

(12) Tor Savidge, Texas Children's Hospital, "From Metabolites to Neurotransmitters – What do Your Gut Bacteria Really Do for Motility", presented during DDW, 16 May 2015

ESPGHAN CONGRESS NEWS 2015

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The Annual meeting of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) attracts thousands of physicians, researchers and scientists from nearly 100 countries around the world.
The meetings are a platform for scientific exchange and offer a high standard of educational program, making them one of the best places to learn about the latest advances in the fields of gastroenterology, hepatology and nutrition.
The majority of attendees are university-based physicians, clinicians in private practice, and basic or clinical scientists.
The 48th Annual Meeting of ESPGHAN took place on May 6–9, 2015 in Amsterdam, The Netherlands.

SYMPOSIUM

Probiotics were a specific topic of the meeting, discussed at a symposium on the afternoon of Thursday, May 7. During this parallel symposium, Prof. Yvan Vandenplas from Belgium provided an interesting update on "Fecal Transplant in Children: Fact or Fancy." He underlined the risks and advantages of administering fecal material from healthy people to patients, which is a heavily debated topic. In adults, recurrent *Clostridium difficile* has become an accepted indication for fecal transplant. Besides all the possible indications, many other questions need to be answered before pediatric indications and recommendations can be established. Optimal donor selection, fresh *versus* frozen stools *versus* capsules containing only microbiota, volume, and route of administration are just a few examples of the areas with missing data. These data are necessary to formulate recommendations for fecal microbiota or fecal material administration in children. A careful but not-too-complex regulation is the first priority in order to minimize the risk of administering fecal slurry.

In her presentation "Probiotics in Health Care: a Food, a Food Supplement, or a Drug?", Prof. Hania Szajewska from Poland stressed the importance of clarifying the definition of the term "probiotic" in order to guide clinicians and consumers in differentiating the diverse products on the market. Probiotics are available under the status of either food supplement (FS) or drug. However, a wide gap exists between registration processes for FS and drugs and there are many differences between countries. Official authorities in Europe such as the European Food Safety Authority (EFSA) and European Medicine Agency (EMA) are ensuring that any claim of a FS sold in Europe is clear and justified by scientific evidence. Currently no probiotics have yet received a health claim from EFSA. *Saccharomyces boulardii* and some others are considered a drug in many European countries.

With his presentation entitled "Intestinal Microbiome in Pediatric Gastrointestinal Disease: Useful Diagnostic Biomarker", Prof. Tim De Meij from The Netherlands described "what to measure", "how to measure" and "when to measure" with regard to the intestinal microbiome to improve its role as a diagnostic biomarker. He pointed out that there are several possible samples (e.g., fecal sample, rectal swab and mucosal biopsy) with many differences between them. More than 90% of the intestinal microbiome isn't detectable with a culture but only with a DNA analysis. While it has been well established that a balanced microbiome is related to health, there is now increasing evidence that an imbalanced microbiome or dysbiosis is related to many health problems both within the gastrointestinal tract, such as diarrhea and inflammatory bowel disease, and outside the gastrointestinal tract, such as obesity and allergy. However the practical use of the intestinal microbiome as a diagnostic biomarker in pediatric gastrointestinal disease is not yet well defined.

ORAL PRESENTATIONS

During the symposium, there were oral presentations of two studies that have been conducted in fields where the microbiome is gaining growing importance: necrotizing enterocolitis (NEC) pathogenesis, diagnosis and prevention, and the microbiome of breast milk.

Prof. Tim de Meij from the Netherlands presented the results of a study entitled "Early Detection of Necrotizing Enterocolitis by Fecal Gas Analysis and its Association with Intestinal Microbiota" NEC is the most common severe gastrointestinal disease in very-low-birthweight (VLBW) infants, with incidence rates of 3–15%. Currently available biomarkers lack the accuracy to detect NEC at a preclinical

stage and cannot discriminate NEC from sepsis. Alterations in microbiota are considered an essential factor in the pathogenesis of NEC. The authors analyzed fecal volatile organic compounds (VOC) by means of an electronic nose (eNose) to describe microbial composition, consequently allowing for the early discrimination of children with NEC, sepsis and controls. Fecal VOC profiles of infants with NEC differed significantly from matched controls 2 to 3 days prior to clinical onset of NEC. NEC could also be differentiated from sepsis at the same time. In conclusion VOC profiling has potential as a noninvasive method to detect NEC in early stages.

Prof. Linxi Qian from China presented the results of a study entitled "Determination of Bifidobacterium and Lactobacillus in Breast Milk of Healthy Women by Digital PCR". This study showed that, in addition to its role as a provider of nutrients and bioactive/immunological compounds, breast milk also provides commensal bacteria. They used a quantitative real-time polymerase chain reaction (qRT-PCR) that is currently used for quantitative analysis of the probiotic 16S ribosomal RNA (16S rRNA) gene in human breast milk. In this study, they confirmed that breast milk contains *Bifidobacterium* and *Lactobacillus* species that may promote healthy microbiota development, and droplet digital PCR might be a better tool than conventional qRT-PCR to precisely quantitate the bacterial DNA in breast milk.

OTHER ORAL PRESENTATIONS

A retrospective study from Madrid University supported the routine use of a probiotic mixture (*B. bifidum* or other *Lactobacilli* or *Bifidobacteria* species) as safe for newborns hospitalized in neonatal intensive care units. No isolation of probiotics from clinical cultures occurred and no clinical episode of sepsis attributable to probiotics was recorded. In addition, there was no effect of probiotic administration on the incidence of NEC or sepsis.

A randomized open trial was conducted to assess the impact of adding *S. boulardii* CNCM I-745 to triple therapy for *Helicobacter pylori* eradication in children. A total of 194 *H. pylori*- positive children received a 14-day triple therapy regimen (omeprazole + amoxicillin + clarithromycin, or omeprazole + metronidazole + clarithromycin for participants with penicillin allergy) or a 14-day triple therapy plus *S. boulardii*. Children in the *S. boulardii* group had less frequent diarrhea that started later and was of shorter duration than in the control group.

Compliance was also significantly better in the *S. boulardii* group. Although there was a 10% better eradication rate in the group receiving *S. boulardii*, this was not statistically significant.

A randomized trial was conducted in VLBW neonates to evaluate the effects of a probiotic combination (*L. acidophylus, Enterococcus faecium* and *B. infantum* with the ratio of 1.5:1:1.5 at a dosage of 0.6 x 10^7 CFU twice a day from their first milk feeding until discharge) on late-onset sepsis incidence and growth. The authors of this study demonstrated that children receiving prophylactic probiotics had fewer late onset sepsis episodes. There was no effect on growth, but VLBW neonates were discharged at a smaller gestational age.

POSTERS

Newborns

In early life, the intestinal microbiota is dynamic with increased susceptibility to host and environmental factors. Under normal circumstances, the intestinal mucosal barrier prevents potentially pathogenic bacteria and toxins from entering the systemic circulation. In preterm babies, the mucosal barrier is immature and postnatal events (e.g., hospitalization, antibiotic treatment and formula feeding) may further reduce barrier function. This may result in translocation which is regularly cited in the pathogenesis of NEC. Probiotics may enhance intestinal barrier function via a number of different mechanisms thereby reducing translocation. Meta-analyses of probiotic studies in preterm babies have reported reduced rates of NEC in babies receiving this intervention but the data are controversial.

A randomized, placebo-controlled study was conducted in a cohort of premature infants (gestational age <31 weeks) to determine whether administration of *B. breve* BBG-001 could prevent NEC, sepsis or death, relative to placebo. Intestinal permeability was assessed 14 days after birth by the sugar absorption test (SAT) using lactulose and mannitol and intestinal protein loss by stool alpha-1-antitrypsin (A1AT). The administration of *B. breve* BBG-001 was not associated with a reduction in bacterial and endotoxin translocation, or in a statistically significant reduction in intestinal permeability or intestinal protein loss. Studies such as this may help to inform optimal strain selection for future probiotic trials in preterm babies.

A polyphasic study combining phylogenetic analysis (16S rRNA gene sequencing and multilocus sequence analysis) and phenotypic characterization with mass spectrometry to characterize *C. neonatale* clinical isolates from preterm neonates. This study demonstrated that *C. neonatale* is a new species within the *Clostridium* genus sensu stricto for which the authors proposed the name *C. neonatale* sp. nov.
The use of matrix-assisted laser desorption/ionization time-of flight mass spectrometry (MALDI-TOF MS) has proved useful in differentiating between *C. neonatale* and *C. butyricum*. The use of MALDI-TOF MS will allow the characterization of *C. neonatale* at the clinical level, particularly when considering NEC studies.

In a study with the aim of defining establishment and functionality of the intestinal microbiota of preterm infants born at varying gestational ages, the authors observed a temporal pattern in microbiota development in all enrolled preterm infants. They noted that, while the meconium of newborn infants has a highly diverse microbiota composition, this develops towards a Bifidobacteria-dominated microbiota at postnatal weeks 3–6. At this time, *Bifidobacterium* spp. are significantly more abundant in very preterm than in extreme preterm infants, indicating delayed colonization with *Bifidobacterium* spp. in the extreme preterm infants.

A study assessed the impact of mode of delivery and bacterial composition of human milk on the fecal microbiota of exclusively breast-fed infants. The bacterial diversity and richness in both human milk and infant feces were quite similar between the groups. The profiles of human milk microbiota were generally dominated by *Firmicutes* (44.9±18.8%), *Proteobacteria* (43.7±21.0%) and *Actinobacteria* (9.2±5.5%) at phylum levels, while no differences were found in the bacterial composition of human milk at both phylum and family levels. The gut microbiota of infants were dominated by *Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria*. Compared with infants born by vaginal delivery, those born by cesarean delivery had decreased richness of *Bacteroidetes*. These findings provided new evidence that delivery mode has an impact on the microbial community in early life.

Human milk and probiotic microbes could decrease the risk of NEC in premature infants; however, there is currently limited understanding of the mechanisms of protection and few comparisons of different species of probiotic organisms. Blooms of Enterobacteriaceae have been described just prior to the onset of NEC. A study analyzed the changes in the fecal microbiota of premature infants who were treated during neonatal intensive care unit hospitalization with *B. breve* at a dose of 3x 10^9 CFU/day. Administration of *B. breve* was associated with increased colonization with commensal *Bifidobacteria* and with increased levels of fecal Enterobacteriaceae. An increase in fecal Enterobacteriaceae in premature infants has previously been demonstrated with administration of *B. lactis*, whereas administration of *B. infantis* was associated with a decrease in fecal Enterobacteriaceae. The authors hypothesized that *B. lactis* and *B. breve* share common mechanisms of colonization that differ from those of *B. infantis*.

In a prospective, randomized, case–controlled trial conducted in infants with a gestational age of 30 to 37 weeks and a birth weight between 1500 to 2500 g, *S. boulardii* 50 mg/kg twice daily had a number of advantages compared with no intervention. *S. boulardii* supplementation appeared to bring the preterm infants' weight gain closer to that of intra-uterine growth rate, was associated with reduced feeding intolerance, and had no adverse effects.

GI-Infections

A recent meta-analysis confirmed the need for further studies to confirm the efficacy and safety of the treatment of acute diarrhea with *L. rhamnosus* GG (LGG).

A randomized multicenter double-blind placebo-controlled study conducted in infants and toddlers with acute gastroenteritis confirmed that diarrhea duration and severity under LGG plus oral rehydration solution (ORS) is shorter than during treatment with placebo plus ORS.

In a randomized clinical trial authors demonstrated that *B. lactis* plus inulin and *B. lactis* alone reduce the duration of diarrhea in the same way within ~ 30 hours. There was no effect of inulin alone on the duration of diarrhea.

A cost-effectiveness analysis of add-on *S. boulardii* CNCM I 745 in children with acute infectious diarrhea in Turkey showed that treatment reduced the number of children who required admission to hospital or emergency room visits. The total cost related to hospitalization and emergency care unit stay was reduced by 25% or $US 51 per patient. Over one year, if *S. boulardii* were administered to all children with rotavirus under 5 years of age, the total cost would be reduced by 23% or $US11.3 per patient.

Allergy, obesity and autoimmune diseases

A Chinese study characterized fecal microbial compositions of 153 Hong Kong infants with or without eczema. Among the top 5 genera, *Bifidobacterium* was more commonly found in controls than cases (those with eczema). The relative abundance of *Roseburia* was also higher in controls than cases (eczema). The Shannon diversity index was similar between cases and controls. Comparing microbial compositions in these newborns and the Swedish, *Escherichia coli* was found in the top 5 genera among only the Chinese in both cases and controls whereas *Enterobacter* was seen only in Swedish newborns. *Clostridium*, *Parabacteroides* and *Lactobacillus* were found only in Chinese newborns with eczema and in healthy Swedish newborns. *Bifidobacterium, Bacteroides* and *Streptococcus* were found among top 5 genera among cases and controls in both populations. In conclusion, *Bifidobacterium* and *Roseburia* appear to be less frequently detected in the stools of 4-week-old Chinese infants who subsequently develop eczema. Microbial diversity is not associated with eczema susceptibility. This study confirms ethnic-specific early-life fecal microbial compositions.

It is increasingly apparent that intestinal microbiota and gut-liver axis malfunction modulate body fat excess and its comorbidities. A study has confirmed several murine/human preliminary literature findings that suggest a peculiar intestinal microbiota dysbiosis and low diversity in obesity and non-alcoholic fatty liver disease. In these conditions, the coexistence of H_2-producing bacteria and H_2-utilizing methanogenic bacteria suggest that increased energy harvesting is occurring via H_2 transfer between bacterial and Archaeal species. These species might serve as a target in studies with tailored probiotics.

Inflammatory Bowel Disease

In a prospective study conducted in a large cohort of children with newly diagnosed inflammatory bowel disease the intestinal microbiota composition was described. There were clear differences in microbiota composition between pediatric inflammatory bowel disease patients and controls, affecting all major phyla. Changes were disease-specific and reversion towards normal control microbiota was only seen for Crohn's disease patients.

Nutrition and Basic Science

Fermented cow's milk with *L. paracasei* CBA L74 (FM-CBAL74) exerts a preventive effect against childhood infectious diseases. In a study, the authors related this effect to a direct interaction with human enterocytes. Through direct interaction with the enterocytes, FM-CBAL74 regulated cell growth and differentiation, innate immunity, and the expression of inflammatory mediators. These actions could be responsible, at least in part, for the positive effect of FM-CBAL74 observed in children.

In a metagenomic study, authors monitored the microbial colonization of the gastrointestinal tract in two different infant groups that were fed infant formula either with probiotic *B. longum* subsp. *infantis* CECT7210 (treated group) or without the probiotic bacteria (control group). Microbial biodiversity was higher in the group fed infant formula containing CECT7210 in comparison with the control group. The ratio of phylum Bacteroidetes/Firmicutes at the end of the nutritional intervention with probiotic CECT7210 was lower in the treated group than in the control group. Presence of the *Bifidobacterium* species and *B. longum* species were significantly increased in the treated group at the end of the study compared with the control group. Overall, fewer pathogens (*Escherichia, Clostridium, Salmonella* and *Yersinia*) were present in the treated group than in the control group but this difference was not statistically significant. In spite of global pathogen reduction not being significant, presence of *E. coli* in the treated group was statistically lower than in the control group.

References

(1) Scientific Abstracts from the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Annual Meeting, 6–9 May, 2015. Amsterdam, The Netherlands. J Pediatr Gastroenterol Nutr 2015: 60.

BIOTASCOPE

Translational Science in Microbiota

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