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Mini review

A dynamic partnership: Celebrating our gut flora

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Abstract

Emerging data indicate that humans enjoy health through a productive collaboration with their colonizing flora, the majority of whom reside in the colon. This minireview provides a perspective on recent data and the exciting scientific challenges ahead.

Keywords: Intestinal flora; Gut flora; Colonic flora; Bacteroides; Intestinal immunity; *Bacteroides fragilis*; *Bacteroides thetaiotaomicron*; Mucosal immunity

1. Introduction

Among body sites normally sporting a community of microbes, the human gut, predominately the colon, harbors the greatest number and diversity of organisms, primarily bacteria. Pasteur with prescient insight postulated that our health is intertwined with our resident flora [1]. Dr. Joshua Lederberg, a Nobel Laureate (1958) at the age of 33, later coined the term 'microbiome' or the collective genome of our indigenous microbes and further proposed that a comprehensive view of human genetics and physiology is a composite of human and microbial genetics [2]. Later, the human genome project revealed 233 proteins with homologues only in bacteria, suggesting that we have acquired these genes from our resident flora [3]. This has led to a fundamental question; namely, to what extent is human life dependent on its microflora? [4] Investigations addressing this question have spawned two new scientific disciplines. The first titled 'Eco-Devo' or ecological developmental biology pursues the hypothesis that human development is both hardwired in our genes and derived from our interactions with microbes [5]. The second field, cellular microbiology, is built on the principle that studies of normal flora as well as microbial pathogens provide new insights into host cell biology, biochemistry and development [6]. The goal of this paper is to provide a perspective on recent data supporting the hypothesis that the relationship between the host and the gut flora is not simply commensal (i.e. living together without injury to either partner) but rather symbiotic or mutualistic; namely, an interdependent relationship essential to our well-being (Table 1) [2,7].

2. Basic facts about the human gut flora

Comprised of 500 to 1000 bacterial species with two to four million genes, the microbiome contains about 100-fold more genes than the human genome and the estimated 10^{13} bacterial cells in the gut exceeds by 10-fold the total ensemble of human cells [2]. At least half of these organisms cannot be cultured but no one discounts the importance of these elusive microbes. In this vast community of gut bacteria, anaerobes outnumber aerobes by estimates of 100–1000 anaerobes to one aerobe. The mechanisms accounting for composition of the gut flora and how it is assembled are incompletely understood. However, it is clear that, at birth, humans become colonized with facultative aero-

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bes including streptococci and *Escherichia coli* but, at the critical juncture of weaning, there is a dramatic shift in the flora with obligate anaerobes, particularly *Bacteroides* species, becoming preeminent (Fig. 1) [5]. Intestinal *Bacteroides* consist of at least four key species, *B. thetaiotaomicron, B. vulgatus*, *B. distasonis* and *B. fragilis*, and comprise about 30% of the total gut flora suggesting that these organisms are likely the key anaerobes in health and disease [8].

Of these species, two dominate the medical literature and investigations, *B. fragilis* and *B. thetaiotaomicron. B. fragilis* are not only constituents of the normal gut flora of most humans but are the leading human anaerobic pathogens [8,9]. *B. fragilis* show an impressive capacity to regulate their surface structures by DNA inversions permitting display of up to nine distinct polysaccharide capsular variants as well as L-fucose-containing glycoproteins reminiscent of host proteins [10–13]. Further, decoration of *B. fragilis* with fucosylated molecules confers a competitive survival advantage in vivo [12]. The high level of antigenic variability and surface display of antigens similar to host intestinal epithelial cells may permit a certain 'tolerance' for *B. fragilis* allowing them to associate intimately with the mucosal surface [14]. In contrast, *B. thetaiotaomicron* lack adhesive molecules and are located in the gut lumen where they appear to assist in polysaccharide digestion (see below) [7].

Table 1
How the gut flora promote human health

Polysaccharide utilization and nutrient release
Enhanced fat storage
Induction of mucosal glucose transporters
Induction of villous capillary formation
Induction of select proteins of innate immunity
Contribute to mucosal homeostasis and repair capacity
Stimulate secretory IgA production
Induce development of gut-associated lymphoid tissue
Promote diversification of lymphoid populations and immunoglobulin genes

3. Nutritional benefits of the gut flora

In 1983, Wostmann and colleagues observed that germ-free rodents require 30% more calories to maintain their body mass than conventional rodents (possessing their 'normal' gut flora) [15]. The potential mechanisms accounting for this observation remained obtuse until recently when seminal studies by Drs. Lora Hooper, Jeffrey Gordon and others using germ-free mice colonized with conventional gut flora or *B. thetaiotaomicron* suggested that the gut flora contribute to carbohydrate and lipid absorption [7,16–19]. Sequencing of the *B. thetaiotaomicron* genome revealed, remarkably, that a majority of this genome is devoted to polysaccharide utilization and, importantly, contains enzymatic capacities lacking in the human genome permitting, for example, the digestion of nutrients otherwise inaccessible to the host [16]. The genome of these bacterial glycophiles, termed a 'glycobiome', predicts that they display receptors for complex polysaccharides as well as secrete a vast array of carbohydrate-degrading enzymes into the bacterial periplasm or extracellular fluid [7]. Consistent with the hypothesis that the metabolic capabilities of *B. thetaiotaomicron* are critical to host nutrition, these organisms are observed to associate with food particles and mucus and to modify their glycan foraging behavior (via differential gene expression) depending on the available nutrient sources [19].

The gut flora also likely regulates fat storage [17]. Eight-week-old germ-free mice have puny epididymal fat pads compared to the adipocyte hypertrophy evident in either conventional mice or mice conventionalized with gut flora at the time of weaning (10 to 14 days of age). In fact, colonization of germ-free mice with normal gut flora produces a 60% increase in body fat and the emergence of insulin resistance within 14 days despite a 30% reduction in food intake. Leptin, an adipocyte-derived hormone whose expression correlates with adipocyte lipid content and also suppresses appetite, glucose and insulin levels, increases significantly by 14 days after colonization of germ-free mice. The potential

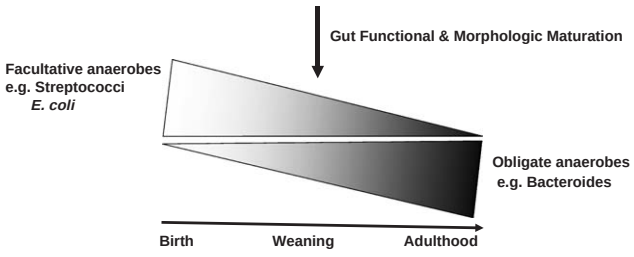

Fig. 1. Reproduced with permission from Trends in Microbiology 12:129, 2004.

mechanism may be that the normal gut flora inhibit fasting-induced adipocyte factor (Fiat, also known as angiopoietin-like protein 4) yielding enhanced lipoprotein lipase activity and triglyceride storage in adipocytes. Modulation of the gut flora or pharmacologic upregulation of Fiat have been proposed as potential targets helpful in addressing the public health epidemic of obesity in the United States [7,17].

4. Gut flora induce intestinal development further enhancing nutrition

Using colonic bacteria or *B. thetaiotaomicron* colonization of germ-free mice as an experimental model, several lines of evidence support the hypothesis that our gut flora promote intestinal development that may serve to further enhance the host's nutrition. First, *B. thetaiotaomicron* colonization induces expression of sodium/glucose transporters (Sglt1) in the intestinal epithelium providing assistance in uptake of the glucose liberated by the metabolic activities of the gut flora [18]. Second, colonic bacteria or *B. thetaiotaomicron* alone induce our intestinal epithelial cells to express fucose-containing glycoconjugates [5]. Intriguingly, if the *B. thetaiotaomicron* fucose-utilization pathway is mutated, these bacteria no longer can induce the host fucose-containing glycoconjugates indicating that this developmental program is interdependent. These results suggest that *B. thetaiotaomicron* use the terminal fucose of these glycoconjugates as an energy source but it is not understood what these particular glycoconjugates contribute to the host. Third, weaning of mice (a time point of marked changes in the gut flora, Fig. 1) is associated with development of an extensive villous capillary network but capillary development in the gut is quite limited in germ-free mice [1]. Notably, colonization of germ-free mice with conventional gut flora or *B. thetaiotaomicron* alone for 10 days stimulates robust villous capillary development. Although mechanistic details are sparse, capillary development stimulated by *B. thetaiotaomicron* is dependent on the presence of Paneth cells in the gut. Together, these developmental changes in the gut would be predicted to promote, at a minimum, host nutrition by enabling absorption of simple sugars and their delivery to the host via a web of intestinal capillaries.

5. Gut flora induce gut innate and adaptive immunity

Germ-free mice have an underdeveloped mucosal immune system with small lymphoid follicles, few IgA-secreting plasma cells and reduced submucosal T cell populations including CD4+ and intraepithelial CD8+ lymphocytes [20,21]. Stimulation of the development of the intestinal immune system by the gut flora occurs over a few months [5,22]. Conventional gut flora, but not *B. thetaiotaomicron* alone, have been shown to induce expression and secretion by Paneth cells of angiogenin-4, a bactericidal protein that specifically kills gram-positive organisms [5]. In contrast, other proteins, such as α-defensins, secreted by the innate immune system are not induced by conventional gut flora. It has been suggested that secretion of angiogenin-4 may help to shape the composition of the gut flora at the time of weaning when gram-positive bacteria decrease in the gut and a preponderance of gram-negative anaerobic organisms become established [22].

Recent data also suggest that commensal bacteria are essential to intestinal mucosal homeostasis and that Toll-like receptors (TLRs) are critical contributors to intestinal homeostasis [21,23]. TLRs, a key component of the innate immune system, are a family of proteins termed 'pattern-recognition receptors' that recognize conserved molecules (such as lipopolysaccharide or LPS) released by bacteria. These sensors of infection trigger inflammatory and immune host responses. However, it has been unclear how the gut, as an organ immersed in LPS, avoids constant inflammation induced by its bacterial population. One hypothesis to explain LPS tolerance in the gut is compartmentalization of TLRs in the submucosa where physical separation from the luminal bacteria would occur [21]. If this hypothesis is correct, then mice lacking intact intestinal innate immunity would not develop illness or an inflammatory response if the intestinal epithelial barrier were breached. However, contrary to the hypothesis, Rakoff-Nahoum and colleagues [23] showed that a subclinical breach in the barrier function of the gut was lethal to mice lacking intestinal innate immune responses (Myd88 knockout mice). In contrast, mice possessing their normal flora were unaffected by a subclinical breach in the gut barrier. Consistent with this result, mice whose gut contents were sterilized with an armamentarium of antibiotics also died when a subclinical breach in the gut barrier was induced. These intriguing results suggest that the normal commensal flora are essential to homeostasis in the gut and the ability to respond to, at least modest, gut injury. Further, TLRs, rather than routinely being triggers for inflammation in the gut, appear to critically contribute to intestinal mucosal health [21,24]. The circumstances and mechanisms by which TLRs shift from an essential physiologic role to a pathologic role in intestinal disease requires additional investigation. Additional mechanisms by which the gut flora may successfully colonize without inducing inflammation have been reported. Kelly and colleagues reported that *B. thetaiotaomicron* antagonizes, in vitro and in vivo, activation of the transcription factor, Nuclear Factor-κB (NF-κB), a 'first responder' in inducing innate inflammatory responses in the gut, by

enhancing nuclear export of its RelA subunit [25]. Non-pathogenic *Salmonella* also inhibit NF-κB activation but, in this case, by blocking degradation of its partner protein, IκBα [26].

Besides these effects of the gut flora on the innate immune system, development of the adaptive intestinal immune system appears equally dependent on the gut commensal flora [21,22]. Sampling of luminal commensal bacteria with subsequent presentation of antigens to submucosal macrophages and lymphocytes by intestinal dendritic cells contributes to regulation of the host:bacteria intestinal boundary [27,28]. Intestinal submucosal lymphoid tissue prevents the systemic escape of the engulfed commensal bacteria and these submucosal commensal bacteria further drive production of IgA in the gut including antibodies that specifically bind to commensal antigens [21,27]. Secretion of IgA (at the rate of at least 3–5 g/day) serves as another important host defense helping to maintain the host's mutualistic relationship with its flora [29]. Consistent with this thesis, there is a dramatic expansion of predominately non-cultivable anaerobes in the small bowel of IgA deficient mice [30]. Colonization of germ-free mice and studies in rabbits have also indicated that commensal organisms assist in inducing development of lymphoid follicles (such as Peyer's patches), the intestinal intraepithelial lymphocyte population and antibody and lymphocyte diversity in the intestine [22,31,32].

6. Summary and conclusions

A renaissance is underway regarding our understanding of the contributions of our flora to human health and development. The sequencing of the *B. thetaiotaomicron* [16] and *B. fragilis* genomes [10,11], microarray analyses and new animal models with their genetic variants have begun to provide a sophisticated understanding of the everyday interactions of the gut flora and the intestinal epithelium. Together these data indicate that 'commensals', suggesting simple coexistence, is a poor term for our flora and that the dynamic term, symbionts, indicating interdependence of host and flora in a mutualistic relationship is more accurate [2,7]. Nonetheless, our knowledge remains rudimentary with the most detailed information available for only one Bacteroides spp., *B. thetaiotaomicron*, with little known about the symbiontic role of *B. fragilis*, that preferentially adheres to the colonic mucosa where it is poised to invade the host [14]. *B. fragilis* illustrates well the thin line that separates gut symbiont from pathogen (Fig. 2) [33]. Future studies face the challenge of building from our understanding of individual anaerobes to developing insights into the complex interactions between the human host and members of the three major bacterial genera in the gut, *Bacteroides*, *Clostridium* and *Eubacterium* [7]. It seems likely that the journey to unravel the mechanisms of symbiosis at the gut mucosal surface and their 'breakdown' permitting an adversarial role between host and flora (such as illustrated by inflammatory bowel disease [21]) will be most exciting.

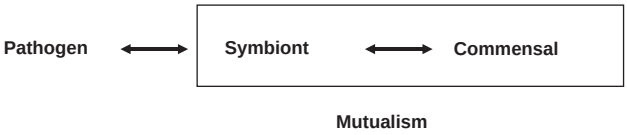

Fig. 2. Modified with permission from Science 292:1115, 2001 & Science 299:1999, 2003.

References

[1] Stappenbeck TS, Hooper LV, Gordon JI. Developmental regulation of intestinal angiogenesis by indigenous microbes via Paneth cells. Proc Natl Acad Sci USA 2002;99:15451–5.
[2] Hooper LV, Gordon JI. Commensal host-bacterial relationships in the gut. Science 2001;292:1115–8.
[3] Relman DA, Falkow S. The meaning and impact of the human genome sequence for microbiology. Trends Microbiol 2001;9:206–8.
[4] Ruby E, Henderson B, McFall-Ngai M. Microbiology. We get by with a little help from our (little) friends. Science 2004;303:1305–7.
[5] Hooper LV. Bacterial contributions to mammalian gut development. Trends Microbiol 2004;12:129–34.
[6] Cossart P, Boquet P, Normark S, Rappuoli R. Cellular microbiology emerging. Science 1996;271:315–6.
[7] Backhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. Science 2005;307:1915–20.
[8] Salyers AA. Bacteroides of the human lower intestinal tract. Annu Rev Microbiol 1984;38:293–313.
[9] Polk BF, Kasper DL. *Bacteroides fragilis* subspecies in clinical isolates. Ann Intern Med 1977;86:569–71.
[10] Cerdeno-Tarraga AM, Patrick S, Crossman LC, Blakely G, Abratt V, Lennard N, Poxton I, Duerden B, Harris B, Quail MA, et al. Extensive DNA inversions in the B. fragilis genome control variable gene expression. Science 2005;307:1463–5.
[11] Kuwahara T, Yamashita A, Hirakawa H, Nakayama H, Toh H, Okada N, Kuhara S, Hattori M, Hayashi T, Ohnishi Y. Genomic analysis of Bacteroides fragilis reveals extensive DNA inversions regulating cell surface adaptation. Proc Natl Acad Sci USA 2004;101:14919–24.
[12] Coyne MJ, Reinap B, Lee MM, Comstock LE. Human symbionts use a host-like pathway for surface fucosylation. Science 2005;307:1778–81.
[13] Krinos CM, Coyne MJ, Weinacht KG, Tzianabos AO, Kasper DL, Comstock LE. Extensive surface diversity of a commensal microorganism by multiple DNA inversions. Nature 2001;414:555–8.
[14] Namavar F, Theunissen EB, Verweij-Van Vught AM, Peerbooms PG, Bal M, Hoitsma HF, MacLaren DM. Epidemiology of the Bacteroides fragilis group in the colonic flora in 10 patients with colonic cancer. J Med Microbiol 1989;29:171–6.
[15] Wostmann BS, Larkin C, Moriarty A, Bruckner-Kardoss E. Dietary intake, energy metabolism, and excretory losses of adult male germfree Wistar rats. Lab Anim Sci 1983;33:46–50.
[16] Xu J, Bjursell MK, Himrod J, Deng S, Carmichael LK, Chiang HC, Hooper LV, Gordon JI. A genomic view of the human-

Bacteroides thetaiotaomicron symbiosis. Science 2003;299: 2074–6.
[17] Backhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semenkovich CF, Gordon JI. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci USA 2004;101:15718–23.
[18] Hooper LV, Wong MH, Thelin A, Hansson L, Falk PG, Gordon JI. Molecular analysis of commensal host-microbial relationships in the intestine. Science 2001;291:881–4.
[19] Sonnenburg JL, Xu J, Leip DD, Chen CH, Westover BP, Weatherford J, Buhler JD, Gordon JI. Glycan foraging in vivo by an intestine-adapted bacterial symbiont. Science 2005;307:1955–9.
[20] Macpherson AJ, Uhr T. Compartmentalization of the mucosal immune responses to commensal intestinal bacteria. Ann NY Acad Sci 2004;1029:36–43.
[21] MacDonald TT, Monteleone G. Immunity, inflammation, and allergy in the gut. Science 2005;307:1920–5.
[22] Cash HL, Hooper LV. Commensal bacteria shape intestinal immune system development. ASM news 2005;71:77–83.
[23] Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell 2004;118:229–41.
[24] Madara J. Building an intestine—architectural contributions of commensal bacteria. N Engl J Med 2004;351: 1685–6.
[25] Kelly D, Campbell JI, King TP, Grant G, Jansson EA, Coutts AG, Pettersson S, Conway S. Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. Nat Immunol 2004;5:104–12.
[26] Neish AS, Gewirtz AT, Zeng H, Young AN, Hobert ME, Karmali V, Rao AS, Madara JL. Prokaryotic regulation of epithelial responses by inhibition of IkappaB- alpha ubiquitination. Science 2000;289:1560–3.
[27] Macpherson AJ, Uhr T. Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. Science 2004;303:1662–5.
[28] Rescigno M, Urbano M, Valzasina B, Francolini M, Rotta G, Bonasio R, Granucci F, Kraehenbuhl JP, Ricciardi-Castagnoli P. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. Nat Immunol 2001;2:361–7.
[29] Kraehenbuhl JP, Corbett M. Immunology. Keeping the gut microflora at bay. Science 2004;303:1624–5.
[30] Suzuki K, Meek B, Doi Y, Muramatsu M, Chiba T, Honjo T, Fagarasan S. Aberrant expansion of segmented filamentous bacteria in IgA-deficient gut. Proc Natl Acad Sci USA 2004;101:1981–6.
[31] Rhee KJ, Sethupathi P, Driks A, Lanning DK, Knight KL. Role of commensal bacteria in development of gut-associated lymphoid tissues and preimmune antibody repertoire. J Immunol 2004;172:1118–24.
[32] Rhee KJ, Jasper PJ, Sethupathi P, Shanmugam M, Lanning D, Knight KL. Positive selection of the peripheral B cell repertoire in gut-associated lymphoid tissues. J Exp Med 2005;201:55–62.
[33] Gilmore MS, Ferretti JJ. Microbiology. The thin line between gut commensal and pathogen. Science 2003;299:1999–2002.