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From probiotics to therapeutics: another step forward?

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Commentary

Preclinical studies with probiotics continue to unravel mechanisms of cytoprotection and suggest that approaches utilizing microbial products as therapeutics in acute and chronic gastrointestinal disorders could be effective. However, clinical trials using these bacteria have thus far been inconsistent. In this issue of the *JCI*, Yan et al. describe a novel mechanism of cytoprotection by p40, a soluble product of *Lactobacillus rhamnosus* GG, mediated via EGFR. The efficacy of p40 in three models of chemically induced colitis indicates tremendous therapeutic potential, though this finding will need to be verified in human patients.

cortical function restoration after therapy (7) brings great promise. Depending on the affected gene and severity of the phenotype, the extension of the landmark achievements in LCA2 to other types of LCA may prove more challenging depending upon the age of intervention.

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From probiotics to therapeutics: another step forward?

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Preclinical studies with probiotics continue to unravel mechanisms of cytoprotection and suggest that approaches utilizing microbial products as therapeutics in acute and chronic gastrointestinal disorders could be effective. However, clinical trials using these bacteria have thus far been inconsistent. In this issue of the *JCI*, Yan et al. describe a novel mechanism of cytoprotection by p40, a soluble product of *Lactobacillus rhamnosus* GG, mediated via EGFR. The efficacy of p40 in three models of chemically induced colitis indicates tremendous therapeutic potential, though this finding will need to be verified in human patients.

The human body as a superorganism

A new view of human body has emerged as a result of the work of the NIH Human Microbiome Project (HMP), the European Metagenomics of the Human Intestinal Tract (MetaHIT) project, the International Human Microbiome Consortium (IHMC), and individual research laboratories. It is a picture of a superorganism in which a large number of different organisms coexist as one. In this superorganism, only 10% of cells represent *Homo sapiens* (1), with millions of microbial genes outnumbering the "mere" 20–25 thousand genes that constitute the human genome (2). The vast majority of the microorganisms coinhabiting humans are located within the confines of the gastrointestinal tract. Termed the gut microbiota, they have a collective mass of approximately 1.5 kg, and together with the gastrointestinal tract itself they create the most metabolically active system within human body. The gut microbiota is a very diverse, complex, and dynamic system forming varying niches along the GI tract segments, and its makeup and function are affected by host genetics, environment, diet, and disease states. Humans have coevolved with their microbial component over two

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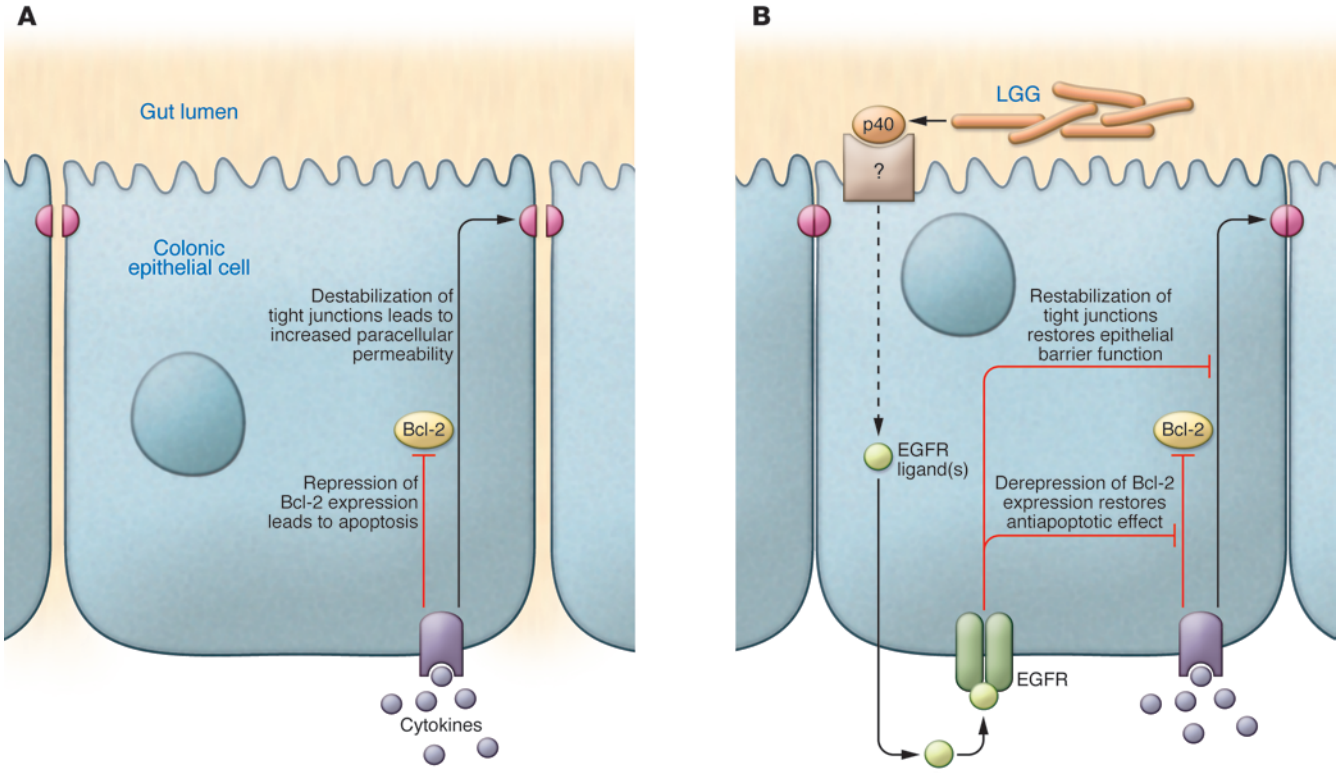

Figure 1
Schematic representation of the effects of p40 in colonic epithelial cells. (**A**) In the absence of p40, cytokine signaling inhibits the proapoptotic protein Bcl2, and contributes to the destabilization of tight junctions. (**B**) It remains unknown how p40 signals from the lumen, and which EGFR ligands are released from the colonic epithelial cell — and by what means — in response to p40. Although Akt activation was used as a reporter of EGFR activation, the role for this kinase in the effects of p40 remains to be elucidated; therefore, it is not depicted here.

million years, leading to a homeostatic and symbiotic system designed for optimized production and absorption of essential nutrients, tightly regulated epithelial cell differentiation and renewal, and balance between immune recognition of pathogens and tolerance of commensal microorganisms. However, the cultural, social, dietary, medical, and technological advances of the last two centuries continue to challenge this balance. It is now increasingly appreciated that changes in the microbial composition and metabolic activity of the gut microbiome may significantly contribute to several metabolic and autoimmune diseases in humans. As a consequence, there has been a growing interest in approaches to manipulate the gut microbiota as a means for disease prevention and treatment (3).

Functional foods

The concept of functional foods dates perhaps as far back as Hippocrates and his motto "Let food be your medicine." Although the origin of milk fermentation practices can be traced back long before the Phoenician era and placed in the Middle East as early as 7000 B.C., the use of probiotics (a term derived from Greek προ and βιοτοσ – "pro-life") was first advocated by the Russian biologist and Nobel Prize laureate Élie Metchnikoff (1845–1916). Metchnikoff defined them as "live microorganisms, which exhibit health-promoting effects," and his fascination with lactic acid–producing *Lactobacillus delbrueckii* ssp. *bulgaricus* laid the groundwork for the concept of probiotics, their protective effects against enteric infections and their possible role as a means to attain a physiological old age and disease-free death (4). Probiotic research and industry have continued to grow from these early observations, and the global market for probiotic ingredients, supplements, and foods is expected to reach $19.6 billion in 2013, with more than 500 probiotic products introduced in the past decade alone (5). However, the growth of the probiotic industry has not been paralleled by advances in basic probiotic research and clinical trials determining their efficacy. We have only begun to understand the mechanisms and limitations of probiotics, which vary greatly among the strains and among the treated individuals (6, 7). Moreover, many clinical studies yielded negative results, leading to very polarized views among scientists and clinicians on the usefulness of probiotics in general populations and in specific disorders.

The general concepts behind the reported beneficial effects of commensal bacteria, probiotic strains, and their combinations in microbial restoration have been recently reviewed by Reid et al. (3). These include: coaggregation; competitive exclusion; production of biosurfactant, bacteriocin, and H_2O_2; immunomodulation and modulation of cellular signaling pathways; and the stabilization of epithelial tight junctions. One can categorize these mechanisms as actions that occur within the intestinal lumen, at the mucosal surface, or within and beyond the intestinal mucosa. An example of luminal effects is the finding that *Lactobacillus salivarius* UCC118 protects against *Listeria monocytogenes* infection by the production of antimicrobial bacte-

riocin (8). Actions at the mucosal surface include induction of mucin and defensin expression, enhanced barrier function by modulation of tight junctions, and competitive exclusion via competition for the pattern recognition receptors expressed by the cells of the innate immune system (9). Some probiotics protect against pathogen-induced tissue injury by stimulation of innate and acquired immunity, notably by induction of regulatory T cells. Such action, representing the probiotic effects beyond the intestinal mucosa, has been documented for *Bifidobacterium infantis*, which downregulated the inflammatory response to *Salmonella* infection or LPS injection in mice (10).

Based on these and many other studies, probiotics have been successfully applied to treat several gastrointestinal disorders, with beneficial effects observed in necrotizing enterocolitis, irritable bowel syndrome, acute diarrheal illnesses, and antibiotic-associated diarrhea, and as protection against opportunistic infections by *Clostridium difficile* (7, 11, 12). However, the role of probiotics in the treatment or relapse prevention in patients with inflammatory bowel diseases (IBD) is more complex and still remains controversial. *E. coli* Nissle 1917 seems to have efficacy comparable to that of the antiinflammatory mesalamine for maintenance of remission in ulcerative colitis patients (13). In patients with pouchitis, a cocktail of 8 different bacteria (VSL#3) showed both therapeutic and prophylactic efficacy (14). However, the experience in ulcerative colitis clinical practice seems to be inconsistent, and controlled trials in Crohn disease have not yet provided encouraging data. The reasons for the discrepancy between the promising in vitro and preclinical data and the actual clinical trials are not clear. Choice of strain or strain combination, consistency and viability of preparation, colonization, and persistence of the probiotic strain in the gut, as well as potential limitations of using live bacteria (e.g., bacteremias in neonatal or immunocompromised patients), represent some of the challenges in successful translation of probiotics to the clinic.

From bugs to medicine: distilling the probiotics

Identification of soluble factors mediating the beneficial effects of probiotics may present an opportunity not only to understand their mechanism of action, but also to develop effective pharmacological strategies that would circumvent many of the problems posed by therapy using live bacteria. Such an approach was taken with *Lactobacillus rhamnosus* GG (LGG), a bacterium isolated from the healthy human intestine. LGG was shown to prevent cytokine-induced apoptosis in intestinal epithelial cells (15). Follow-up studies by the same group resulted in identification of two novel proteins secreted by LGG, termed p40 and p75, that reproduced the effects of live bacteria on cytokine-induced epithelial cell apoptosis and protected the epithelial barrier from peroxide-induced damage (16, 17). Activation of the PI3K/Akt signaling pathway and PKC- and MAPK-dependent signaling were implicated in these two respective mechanisms of protection. In a study published in this issue of the *JCI* (18), Yan et al. tested recombinant p40 protein in vivo in three models of pharmacologically induced colitis. p40 was administered in pectin/zein-based hydrogel beads, and colonic delivery was confirmed by p40-associated fluorescence at the colonic epithelial surface. The protein was effective in preventing or reducing symptoms of established disease in all three models, reducing the number of apoptotic colonocytes and inducing activation EGFR and Akt. Through a series of elegant in vivo and in vitro studies, Yan et al. demonstrated that the protective effects of p40 action are dependent on EGFR signaling, as no benefit from p40 administration was seen in mice carrying a kinase-dead EGFR allele or in epithelial-specific EGFR knockout mice. EGFR activity was also required for p40-mediated restoration of expression of the antiapoptotic protein Bcl-2, prevention of tight junction endocytosis, and reduction of paracellular permeability. p40-induced Akt activation was blocked in cultured colonocytes treated with tyrphostin (AG1478), an EGFR tyrosine kinase inhibitor, and absent in EGFR-deficient mouse colonic epithelial (MCE) cells. Interestingly, the kinetics of the Akt activation by p40 showed a considerable delay compared with EGF stimulation, suggesting that p40 may affect this pathway indirectly. The authors speculated that p40 acts to induce Src kinase– and metalloproteinase-dependent release of yet-unidentified EGFR ligand(s), which would then act via an autocrine or paracrine manner to activate EGFR.

Implications

While undoubtedly much remains to be done to fully understand the mechanism of action of p40 (Figure 1) or other related molecules of probiotic origin, the study by Yan et al. is an important step forward, providing an encouraging base from which to seek other bacterial products that could substitute for live bacteria and be administered to patients in better-controlled experimental and clinical settings. Since many of the clinical trials with probiotics yielded disappointing or inconclusive results, verification of the preclinical observations with p40 in clinical trials will be indispensable in the future. Much attention will have to be given not only to drug efficacy tests, but also to potential side effects that might not be identified in a phase I trial with healthy subjects. Both Crohn disease and ulcerative colitis patients are at increased risk of colorectal cancer, a condition that could be exacerbated by EGFR activation. Although animal models of inflammation-associated colon cancer may not fully represent the microenvironment in human colon, preclinical studies looking at epithelial hyperproliferation will be important to advance this research.

While they are generally safe, and marketed as "natural" cures, there is still insufficient data that would allow us to definitively say that probiotics treat autoimmune disease or improve human health in general. Many of the probiotic products have been scientifically verified, while others have not, and some outright lack the necessary quality control and purity. Although soluble probiotic-derived factors may not fully mimic the complex effects of living microorganisms, studies such as that reported by Yan et al. (18) pave the way to identifying reproducible and specific means of improving the health of IBD patients.

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Neuroanatomy of body weight control: lessons learned from leptin

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Rather than arising from the passive accumulation of excess calories, obesity is a state in which the biologically defended level of body fat stores increases due to defects in the homeostatic process that matches food intake and energy expenditure over time. By deleting leptin receptors from distinct brain regions and neuronal subsets, researchers are beginning to identify the neuroanatomical substrates responsible for this regulation. In this issue of the *JCI*, Scott et al. demonstrate that loss of leptin receptors in a subset of hindbrain neurons increases food intake in mice, but, unlike what is observed when leptin receptors are deleted from hypothalamic neurons, these mice compensate by increasing energy expenditure and hence do not become obese. Although many brain areas can regulate energy intake and/or energy expenditure, it is likely that only a small subset of neurons actively matches the two over time. It is vital to clarify how this works if we are to improve our understanding of obesity pathogenesis and options available for its treatment.

The alarmingly high prevalence of obesity and related metabolic disorders has emerged as one of the most costly public health problems facing developed countries. The lack of effective treatment options exacerbates this problem and reminds us that despite steady progress in our understanding of neural and endocrine pathways controlling energy balance, our insight into mechanisms that underlie common forms of obesity remains quite limited.

Leptin, a hormone secreted by adipocytes in proportion to fat mass, plays a critical role in energy homeostasis by acting through its neuronal receptors in multiple brain areas to decrease food intake and increase energy expenditure (1). Because mutations that disrupt either leptin production or leptin receptors cause extreme hyperphagia and obesity in rodents and humans, there is little question of its physiological importance. However, the question of how leptin's many effects are mediated remains unresolved. One approach to addressing this question involves deletion of the gene encoding the leptin receptor in specific cell types using mouse molecular genetics. In this issue, Scott and colleagues describe mice in which leptin receptor expression was deleted exclusively in hindbrain neurons that express the transcription factor paired-like homeobox 2b (Phox2b) (2). As predicted, these mice are hyperphagic, but, unlike the obese phenotype of mouse models in which leptin receptors were deleted from hypothalamic neuronal populations (3–5), body weight is not substantially altered because the increased caloric intake is offset by increased energy expenditure. This outcome points to meaningful differences in the roles of hypothalamus and hindbrain as targets of leptin action.

Brain mechanisms that control feeding behavior

Neural control of food intake involves the integration of diverse signals: long-term signals related to stored fuel, including leptin; short-term signals that arise from the gastrointestinal tract in response to ingested nutrients; and reward-related factors, such as the hedonic and incentive value of the food (6). The brain uses these signals to make decisions about eating on a meal-to-meal basis in ways that serve the longer-term goal of maintaining body weight within a stable range. Since the mid-20th century, the hypothalamus has

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