ly upon similar voice cues to encode individual identity within their vocalizations. Whereas the recipient must learn to recognize the voice of each individual, the speaker does not need to learn to produce voice cues. Speaker-specific cues stem from natural differences in the air-filled vocal tracts of each person. Diving mammals are unable to rely upon such vocal cues for individual recognition because, as they dive, the volume of gases in the vocal tract is halved with each additional atmosphere of pressure—this change in the vocal tract renders voice cues unreliable.

Diving mammals that rely upon individual-specific social relationships must learn to produce individually distinctive vocal signature signals. The best-known example is the signature whistle of the bottlenose dolphin (see the figure). Whether in captivity or in the wild, these animals produce signature whistles with an individually distinctive frequency pattern. Bottlenose dolphin calves develop a stereotyped signature whistle during their first year of life. Development of a signature whistle is strongly influenced by learning—most dolphins develop signature whistles that are different from those of their parents, but similar to other sounds present in their environment at birth (*2*).

An isolated captive or wild dolphin is most likely to produce its own signature whistle, but occasionally may also imitate the signature whistle of an associate (*4*). In his study, Janik (*3*) analyzed whistles from wild dolphins and considered them matching if the same whistle was emitted by two separate dolphins within 3 seconds of each other. In this circumstance, it is likely that one of the dolphins was producing its signature whistle and the other was imitating it. Janik proposes that one dolphin may be imitating the signature whistle of another in order to address that individual. Captive dolphins have been shown to associate a newly learned whistle with an arbitrary human object (termed vocal labeling) (*5*). Janik now provides important evidence that vocal labeling is used by wild dolphins for social communication.

Anthropologists who analyze the increase in the ratio of brain to body mass in our hominid ancestors often call their field "the study of the evolution of intelligence." Research that relates cognition to neural circuitry in marine mammals is still in its infancy, but some species are known to invest heavily in brain tissue. Bottlenose dolphins, for example, have a brain to body mass ratio that is higher than that of most mammals and is close to that of humans. Theories of the evolution of intelligence that emphasize the suite of adaptations for tool use (including bipedal gait, opposable thumbs, and increased ability to manipulate objects) would not have predicted the large brain to body mass ratio in dolphins. Few mammals are less adapted for tool use than dolphins, porpoises, and whales (collectively called cetaceans)—selection for a hydrodynamic shape has reduced their appendages to fins, and they are poorly adapted for manipulating objects, compared with, say, a raccoon. Other theories explaining the evolution of large brains in primates emphasize the social aspects of intelligence (*6*). Dolphins provide a good fit for these models—both dolphins and higher primates learn the signals to establish both cooperative and competitive relationships within their social groups.

There is a healthy pressure in the biological sciences to study simple systems. Yet these do not capture all of the important features of life. The genetics of viruses do not tell us all we need to know about multicellular organisms. Similarly, studying social insects is unlikely to unfold to us the whole story about the evolution of social behavior in dolphins and humans. There is a growing appreciation among those who study communication and complex social behavior of the fascinating similarities in the ways that birds and mammals use vocal imitation to interact with specific individuals (*1, 7*). As Janik points out, these similarities may provide an important comparative perspective on how capabilities for imitation evolved in our hominid ancestors.

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PERSPECTIVES: IMMUNOLOGY

Therapeutic Manipulation of Gut Flora

Fergus Shanahan

Inflammatory bowel disease—a collective term embracing both ulcerative colitis and Crohn's disease—is a significant health-care problem affecting between 0.1% and 0.2% of the population in developed countries. These important and disabling conditions are characterized by diarrhea, pain, and other intestinal symptoms, and by lifelong relapses. Ulcerative colitis is confined to the mucosal layer of the large bowel, whereas Crohn's disease can affect any portion of the intestinal tract. The pathogenesis of inflammatory bowel disease is complex but appears to involve interactions among three essential ingredients: host genetic susceptibility, intestinal bacteria, and the gut mucosal immune response.

Despite impressive advances in drug therapy, most treatment strategies have two major limitations: first, they suppress or otherwise alter the host immune response, thereby neglecting the contribution of enteric bacterial microflora to disease pathogenesis; and second, current immunomodulatory drugs lack organ specificity, affecting both mucosal and systemic host responses and resulting in unpleasant side effects. On page 1352 of this issue, Steidler and colleagues (*1*) address both of these concerns in their report of a therapeutic approach for local drug delivery in two mouse models of colitis. They show that dietary administration of the murine enteric bacterium *Lactococcus lactis*—genetically engineered to produce the anti-inflammatory cytokine interleukin-10 (IL-10) within the gut—is therapeutically effective in the mouse models. Their work demonstrates that convergence of the traditional research avenues of immunology and microbiology into a hybrid discipline yields new therapeutic strategies for combating complex diseases.

The immune response in the intestinal mucosa is conditioned by the indigenous bacterial microflora with which it exchanges regulatory signals (*2*). In susceptible individuals, inflammatory bowel disease arises when the immune system misperceives danger within the normal gut microflora and interprets the harmless enteric bacteria as pathogenic in-

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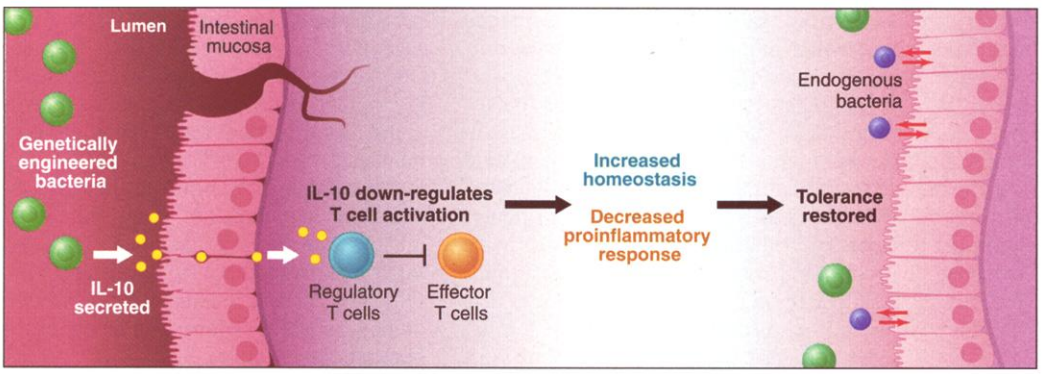
vaders; this leads to a breakdown in the normal regulatory constraints on mucosal immune responses to enteric bacteria (*3*). The profile of cytokines generated within the gut mucosa, which is genetically controlled and may differ from person to person, determines the features of the inflammatory process. Crohn's disease is associated with a predominance of type 1 helper T cell (T_H1) cytokines such as tumor necrosis factor–α (TNF-α), interferon-γ, and IL-12, whereas type 2 helper T cell (T_H2) cytokines such as IL-4 and particularly IL-5 are usually found in ulcerative colitis (*2*). Despite redundancy among mediators of inflammation, a hierarchy of importance has emerged with TNF-α as a key effector and regulatory molecule in T_H1 responses. This explains the rationale and efficacy of therapies that are designed to manipulate the intestinal cytokine milieu, for example, the treatment of Crohn's disease patients with antibodies to TNF-α (*4*).

The selection of IL-10 by Steidler *et al.* for therapeutic delivery to the gut is also based on a sound rationale. Mice with targeted disruption of the IL-10 gene develop enterocolitis, a condition similar to Crohn's disease in humans. Administration of IL-10 has provided therapeutic benefit not only to IL-10–deficient mice but also to other murine models of inflammatory bowel disease and to Crohn's disease patients (*5*). Evidence from murine studies shows that IL-10 is an essential modulator of the regulatory T cells that control inflammatory responses to intestinal antigens (*6*); this cytokine can restore tolerance of T cells to resident intestinal bacteria (*7*) (see the figure). Currently, the clinical usefulness of IL-10 is limited because it must be administered by frequent parenteral injections or by rectal enemas to ensure organ-specific delivery. In the Steidler *et al.* study, genetically engineered bacteria synthesized IL-10 within the intestinal lumen, thus avoiding systemic exposure; this approach provided therapeutic benefit at lower doses than would be required if the cytokine were to be administered systemically.



Small talk in the gut. The cytokine IL-10 (yellow) is secreted in the intestinal lumen of mice by nonpathogenic genetically engineered bacteria (green) administered as a food supplement. IL-10 traverses the gut epithelium, most probably by a paracellular route as epithelial permeability is increased during inflammation. It suppresses the inflammatory immune response in the gut mucosa by promoting the activity of regulatory T cells (blue) that hold effector T_H1 cells (orange) in check. In addition, indigenous enteric bacteria that are not genetically modified (lilac) may also condition the mucosal immune system and influence the cytokine milieu by interacting with the gut epithelium (red arrows).

Although the strategy adopted by Steidler and colleagues is new, the concept of therapeutically manipulating the enteric microflora by feeding nonpathogenic bacteria (probiotics) to patients is not. Probiotics are live microorganisms that confer a health benefit by altering the indigenous microflora. Lactobacilli, bifidobacteria, and other members of the resident microflora with no apparent capacity to induce mucosal inflammation are commonly selected as probiotics. Probiotic therapy has been effective for treating mice deficient in IL-10 and other animal models of inflammatory bowel disease (*2*, *8*); preliminary trials of probiotics in human colitis patients are encouraging (*2*, *9*). Probiotics might alter the gut microflora by competitive interactions with indigenous bacteria, production of antimicrobial metabolites, or modulation of the local immune response to enteric bacteria (*2*). Anticancer properties have also been attributed to probiotics (*10*) but the evidence is still inconclusive. Nevertheless, probiotics diminish the rate of progression from inflammation through dysplasia to colon cancer in IL-10–deficient mice (*8*). Oral delivery of genetically engineered bacteria may now redefine the scope of probiotic action.

Several questions and caveats remain to be addressed. Chief among these is a safety concern if bacteria of human intestinal origin are engineered to secrete biologically active agents such as IL-10. What might be the outcome of person-to-person transmission of such organisms? It is also noteworthy that both Crohn's disease and ulcerative colitis are heterogeneous disorders, and that cytokine patterns may vary within an individual at different phases of the disease. It is probably too simplistic to assume that a given probiotic will be suitable for all patients. Thus, the pathophysiological status of the host may need to be matched to the appropriate composition of enteric microflora and to the probiotic prescription or choice of cytokine to be manipulated. If safety, efficacy, and localized delivery of bioactive drugs by genetically modified, food-grade bacteria can be assured in humans, convenience of administration will definitely appeal to patients. As Steidler and colleagues point out, given the prohibitive cost of biological therapeutics for many patients, cost-effectiveness would be another advantage. Finally, there are wider applications for genetically modifed enteric bacteria, including delivery of vaccines and other biologically important molecules.

For now, Steidler *et al.* have established proof of principle in animal models with an exciting new approach to the treatment of inflammatory bowel disease. By using genetically modified enteric bacteria to manipulate the intestinal immune response, they provide new insight into the interactions among genes, bacteria, and inflammation that underlie the pathogenesis of this disorder. There remain significant gaps in our understanding of the normal interactions between the host and its intestinal microflora, but the new work gives us a glimpse into the untapped potential for therapeutically manipulating the content of enteric bacteria.

CREDIT: K. SUTLIFF

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