Will We Ever Have “God’s Probiotic”?

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“A galaxy is composed of gas and dust and stars—billions upon billions of stars,” astronomer Carl Sagan famously said, and “billions upon billions” almost instantly became part of our everyday language.

But inside us hums and thrums another incalculable universe—trillions, not just billions. A hundred trillion microbes thrive and reproduce inside us. Nine out of every ten cells in our body are microbial. We are, as Nobel laureate Joshua Lederberg said in the year 2000, “a chimera of sorts”—a superorganism with a huge genetic range, forged out of bacteria and our own cells. The majority of those microbes make their home in the gut, and are crucial to human life.

This issue of In Focus looks at new findings on the very specific and astounding ways gut bacteria orchestrate our health—based on the science of genome sequencing.

Sure, we’ve known since the time of Metchnikoff that the “good” bacteria—probiotics—help digestion and generally improve health, and that “bad” guys like C. difficile can make us sick or even kill us. But knowing that is like knowing the stars from afar—rather than holding them in your hands and learning what they’re truly made of.

Invisible organisms we’ve never been able to cultivate are being exposed as we sequence the genome of the entire gut microbiota. By mapping the DNA of microbes in our gut, we have been able to isolate known bacteria as well as species new to science. In 2008 the National Institutes of Health launched the $150 million dollar Human Microbiome Project, to parlay stunning genome sequencing technologies to peer into the phenomenal complexity of the human microbial community living on and within us. They are sequencing some 900 species in our overall microbiome, and have already discovered over 29,000 genes that are unlike any known genes, and must be from microbes.\(^\text{1, 2}\)

We can now cost-effectively sequence millions of fragments of DNA, not just hundreds. The resulting information explosion has opened up our understanding, so that we can examine distinct mucosal layers of the gut, understand how bacterial “misfits” derail our health, and learn what molecules “good” bacteria use to re-set the immune system.

Some highlights of the new findings:

- Our “metagenome”—the total genome of our gut microbes—offers genes that actually complement our own. These genes help break down fiber and medications, produce vitamins such as Vitamin K required for blood clotting and calcium metabolism, and extract energy from carbohydrates.
- Just like we have four blood types, we may have three or more “gut types”, known as enterotypes. In new findings just out from the European Molecular Biology Laboratory in Heidelberg, Germany, we have at least three distinct enterotypes, with different dominant bacteria with distinct specialties.\(^\text{3}\)
- The field of “metabolomics” looks at the metabolism of the gut bacteria, and how they can increase susceptibility to asthma, Type 1 diabetes, multiple sclerosis and other conditions. Scientists have isolated bacterial metabolites
that are systemic, and end up in our blood, tears and urine— influencing overall health and illness.\(^4\, 5\)

- Diseases like multiple sclerosis are strongly linked to gut bacteria. Scientists gave sterile, “germ free” mice a dose of one single species of bacteria that triggered an inflammatory molecule that entered the central nervous system and caused multiple-sclerosis like symptoms.\(^6\)

- Some of our gut bacteria have potent anti-inflammatory properties, and actually help regulate our own immune system. Flares in conditions as far-ranging as asthma, atopic eczema, rheumatoid arthritis and lupus may be quieted with the right bacteria.

- Broken cell walls from lysed probiotics, which are non-living, also provide crucial signals to our immune system. And they can be easier to utilize, and work more rapidly.

- Our flora can be “fat”. Gut bacteria that harvest carbohydrates more efficiently can lead to weight gain. Even more amazing, skinny mice who received transplants of flora from fat mice gained significantly more weight than those who receive the flora of skinny mice.\(^7\) Does obesity run in families in part because they share common flora—and are those flora shaped by shared genes? Scientists are now studying the gut biota and relatives who are lean or obese.\(^8\, 9\)

- It may be possible that a disordered gut flora can become like a superorganism that is infectious. Studies in mice show that mice bred to carry a mixed, pathogenic gut microbiota that lead to colitis, can transfer the entire flora and the disease to other “wild” seemingly genetically resistant mice that were formerly healthy.\(^10\)

- We don’t all share the same flora. Each person’s microbiome is unique and like the bacterial equivalent of a thumbprint. But the different bacteria we all harbor do tweak the same chemical pathways, and train the immune system toward both tolerance and efficacy.

- Even fatty liver may be influenced by the microbiome. A recent study found one bacterial species helped process choline and could help allay fatty liver, while another was detrimental.\(^11\)

This issue also offers clinical pearls from physicians who have learned to tweak their patients’ immunity with both live and broken cell wall, lysed probiotics. We also summarize a new study on the effect of lysed, broken cell wall bacteria on childhood eczema. Finally, we update the maturing picture around Vitamin D supplementation, and look at a new, more absorbable form of glutathione.

Where will all this research lead? Perhaps some day we’ll have what gastroenterologist Alexander Khoruts of the University of Minnesota jokingly calls “God’s probiotic”— a pill containing bacteria proven to fight serious infections. God’s probiotic. A worthy and lovely goal, and one science is now poised to achieve.\(^12\, 13\)

References available in online version at:

\textbf{http://www.nutricology.com/in-focus-sp-113.html}
In Focus: What’s so special about the gut?

Wendy Garrett: The distal gut is one of the most densely populated ecosystems on the planet. And it’s charged with the amazingly difficult task of creating an intelligent and flexible barrier—one that can protect us from invaders but not shut out important nutrients and cells. It tolerates trillions of microbes and countless food particles and yet is poised to act quickly if a pathogenic invader shows up. The gut ecosystem is truly nimble.

One reason the gut is so interesting is because of the bacteria that live in it. Right from the start the bacteria themselves give vital development clues to the lineages of cells that make up our immune system. When you breed “germ-free” mice without gut bacteria, their spleen, lymph nodes, and thymus all look abnormal. The gut itself looks abnormal. And many of our gut bacteria contribute to our immune resilience. They provide us with capacities we haven’t had to evolve on our own. They actually make us inhospitable shelters for disease-causing pathogens.

Finally, it’s captivating that gut microbial communities show such a remarkable degree of variation between individuals. Even so, microbial communities are more similar between family members, suggesting that shared genes and environment play a strong role in health—especially early on when the microbiota is being formed.

Garrett: The mice are known as TRUC (T-bet knock out/Rag2 knock-out ulcerative colitis). They are deficient in T-bet, a molecule that helps regulate immune function and Rag2, a gene necessary for T cells, B cell, and natural killer T cells. With these immune system defects, they have too much Klebsiella pneumoniae and Proteus mirabilis and too little bifidobacteria in their intestinal tract. This abnormal microbiota seems to contribute to ulcerative colitis. A majority of TRUC mice also spontaneously develop colonic dysplasia and rectal cancer. What is surprising is that the

Living in Harmony With Our Gut Microbiota:
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What is surprising is that a collection of abnormal gut microbes, once they have developed, can not only lead to colitis in the sick mice, but infect healthy mice, and create colitis.

A microbiome might be transmitted over many generations, and along with genes, shape the innate and adaptive immune system within families. That points to a path to study disease with new insight and tools.

In Focus: What a fascinating idea—that the microbiome can be transmitted. In fact, that’s what your research in mice has shown. You studied a special breed of genetically vulnerable mice that inevitably end up with pathogenic, inflammatory gut microbes that leads to ulcerative colitis. But what is interesting is this microbiota could be transferred to healthy, wild type mice that should be genetically resistant. And then they get colitis too. Though mice aren’t humans, this seems really powerful and relevant.

Garrett: The mice are known as TRUC (T-bet knock out/Rag2 knock-out ulcerative colitis). They are deficient in T-bet, a molecule that helps regulate immune function and Rag2, a gene necessary for T cells, B cell, and natural killer T cells. With these immune system defects, they have too much Klebsiella pneumoniae and Proteus mirabilis and too little bifidobacteria in their intestinal tract. This abnormal microbiota seems to contribute to ulcerative colitis. A majority of TRUC mice also spontaneously develop colonic dysplasia and rectal cancer. What is surprising is that the
abnormal microbiome itself, once it is developed, may actually be able to create colitis in healthy mice. It may do that through the actual bacteria in the stool, but exposure to maternal factors—such as milk—from a mom with intestinal inflammation may contribute to the process as well. TRUC mothers transmit colitis to their pups in the neonatal period. Yet if the mothers are treated with antibiotics to normalize their gut microbes, their pups develop normally. At the same time, the mothers can also transmit colitis to healthy, wild-type pups that have no genetic vulnerability—if they foster and nurse them. It turns out that the milk itself contains high levels of cytokines that are inflammatory. The connections between breast milk and the developing microbiome is a fascinating topic and a recent paper has begun to shed light on how milk and milk sugars influence the gut microbiota and disease susceptibility later in life. This area of study needs to be explored further.

What’s also surprising is that the colitis can be transferred horizontally to other adult, healthy, wild-type mice if they cohabit with TRUC mice. We need to understand why the healthy mouse with supposedly resistant genes and a functioning immune system gets sick, too. We do know that mice regularly eat each other’s poop, which is a good way to pick up the microbes—but why and how do they drive disease? We are investigating how the colitogenic microbes we have identified change the behavior of these wild-type resident microbial communities.

A classical and simple view of infectious diseases is that a single organism invades and leads to an infection. In inflammatory bowel disease, obesity, diabetes, and perhaps colon cancer; it looks like a collection of organisms may contribute to disease or disease susceptibility. In Focus: Can the TRUC mice help us learn why an entire gut microbial community can be curative or deadly? Gastroenterologist Alexander Khoruts of the University of Minnesota and his team have cured 13 of 15 refractory C. difficile colitis patients with fecal transplants. One woman, whose story was written up in the New York Times, was suffering terribly from C. difficile colitis—she was incapacitated from her intractable diarrhea and wheelchair-bound. Available antibiotics could not combat her infection but her husband’s fecal microbiota cured her. So sometimes the good microbes can take over and reverse a potentially deadly process.

In Focus: How has genomic sequencing changed your world since medical school? Could you even imagine back then the information at your fingertips today?

Garrett: Today we’re looking at a medical chart on a cancer patient, we can make decisions about drugs based on genetic tests—so we can select targeted therapies appropriate for individual patients. A decade ago many hospitals in the United States did not have electronic medical records let alone access to cancer genomics-based tests for their patients that were a mouse click away. When I was an undergraduate,
I ran my own sequencing gels and painstakingly read out a few hundred base pairs one nucleotide at a time.[editor note: a base pair consists of two nucleotides on opposite complementary DNA or RNA strands. The size of an individual gene is often measured in base pairs or kilo base pairs.] Now a single sequencing run can produce millions of sequenced nucleotides.

This technology has allowed us to identify single organisms in a fecal sample, and even identify bacteria we could never culture before. It allows us to look at the collective genome of our microbial communities, and at the immune networks that support the homeostasis of our “supraorganismal” self and begin to understand the dynamic interactions between a host and its microbiota in real time.

**In Focus:** Is our microbiome a bit like our fingerprint—recognizably human but individual? If so, how much do the differences matter? And how do we maintain our own homeostasis in the face of gut infections, food poisoning, antibiotics, and so on?

**Garrett:** We carry about 500-1000 different species of bacteria. We humans also have tremendous diversity at the strain level. Different microbes have evolved distinct strategies for co-existing with our mucosal immune system. For instance, the laboratories of Sarkis Mazmanian at Cal Tech and Dennis Kasper at Harvard have determined that *Bacteroides fragilis* uses a sugar molecule to modulate the immune system, and the Mazmanian laboratory has recently shown has this sugar drives the development of special regulatory T cells that help quell inflammation. The laboratory of Kenya Honda in Japan has discovered that a collection of 46 different Clostridia species can induce the development of similar regulatory T-cells, but in a totally different way—in fact, these bacteria don’t seem to use any currently known pathway of cross-talk between the immune system and bacteria. Research clearly shows that host genetics controls “who” shows up as far as microbes in gut microbial communities, but that different microbes can carry out similar tasks.

I like to use the image of a wobble board when discussing a healthy gut. A wobble board is a round disc with a ball in the middle, and you use it to practice maintaining balance. You feel sort of tippy on a wobble board, and you need to shift your weight, or stand, or crouch with your arms out, and generally find different ways to maintain a stable state. Your microbiome can do the same thing.

**In Focus:** Can you talk a bit about how an abnormal microbiome can lead to systemic illness?

**Garrett:** There’s strong metagenomic data emerging that gut microbiota may be associated with disease from asthma to type 1 diabetes and multiple sclerosis. As these fascinating associations between bacterial community membership and disease states crop up, the obvious and interesting questions of why and how emerge. Microbes are metabolic machines and their metabolism and metabolites participate in our everyday physiology. Bacterial metabolites make it into the systemic circulation and can be measured in blood, urine, and tears. We’ve begun to examine the benefits of so-called functional foods that contain beneficial microbes in TRUC mice and found that fermented milk products, like yogurts, can reduce intestinal inflammation. The yogurt influences the metabolism of resident gut bacteria and this resultant pattern of metabolites eliminates the home for the pro-inflammatory bacteria we’ve identified in the TRUC mice.

**In Focus:** Any final thoughts?

**Garrett:** The microbiota outweighs our own gene pool 100:1. Our gut immune system is being exposed to trillions of bacterial cells at any moment (with effective barriers in place). What is most amazing to me is that the vast majority of people are able to live in harmony with the gut microbiota. The co-adaptation and co-evolution that has occurred and is still occurring between our host tissues, immune system and the gut microbiota is dazzling.

References available in online version at: [http://www.nutricology.com/In-Focus-sp-114.html](http://www.nutricology.com/In-Focus-sp-114.html)
Thousands of years ago, legend has it, a peasant in Mesopotamia noticed that milk in his goatskin gourd had soured in the blazing heat into a delicious, fizzy beverage—yogurt. Flash forward to 1905, when a Bulgarian named Stamen Grigorov discovered one of the fermenting organisms—*Lactobacillus bulgaricus*—in yogurt, by then a staple that had long since been adopted widely across many countries for its health-promoting properties.

Yet neither that ancient peasant, nor that Bulgarian scientist, could have conceived a day when sequencing technologies would allow us to analyze the “metagenome” of the trillions of gut microbes we carry within us, analyzing the rich assortment of metabolites our friendly bacteria produce to guide our immune system.

In fact, one of the more surprising findings is that microbes don’t necessarily have to be alive to help us—many times the digested or “lysed”, broken molecules of cell walls give crucial information to our immune system. When they are already broken and “pre-digested”, they actually seem to work more rapidly, and can stay stable indefinitely without the requirements for food and controlled temperature that live bacteria have.

Here, we look at the newest research and clinical applications of gut microbes—both living and lysed.

**Messages From the Dead**

There is a stunning complexity to the impact our gut microbes have on our body—and sometimes a dead organism is quite potent. Alive or dead, the cell wall still contains molecules that cue our immune system: “These bits are from a friend, so turn down the inflammation and increase tolerance. But these other bits here suggest a serious invader has breached the ranks, and you’d better send cytokines, interleukins, tumor necrosis factor, superoxide, peroxide, and anything else you can think of, because this could be war!”

Different classes of bacteria have unique cell wall components to send those signals. Gram-negative bacteria (such as *Klebsiella pneumoniae*) create inflammation in part through the lipopolysaccharides (LPS) on their cell walls. In contrast, the thick outer wall of gram-positive bacteria (such as *Staphylococcus aureus* and flesh-eating *streptococci*) is made of peptidoglycan. According to biochemist Michael Pabst, formerly of the University of Tennessee: “Peptidoglycan forms a giant basket that holds the bacterium’s inner membrane and “guts” together against osmotic forces. Whenever a bacterium dies, the basket is broken down by bacterial and macrophage enzymes.”

That broken basket contains proteins called muramyl peptides, and abundant research has shown that muramyl peptides are superb triggers of fever, sleep and flu-like symptoms. In their presence, the immune system responds by becoming rapidly activated to resist infection. This resistance to infection is nonspecific. In mice, muramyl peptides cure lethal infections by bacteria, fungi and parasites. Pabst speculates that “muramyl peptides may be critical for the proper functioning of the immune system...[they] may act like vitamins for the immune system.”

In fact, the entire balanced microbiota might be like vitamins for the immune system, and the proteins, sugars and other key molecules on cell walls may provide crucial information. In one fascinating 2008 study, both fresh and heat-treated yogurt were found to alter the proliferation of gut flora. Sixty-three individuals consumed either fresh or heat treated yogurt...
for 15 days. The yogurt contained *Lactobacillus delbrueckii bulgaricus* and *Streptococcus thermophilus*. Three fecal samples were obtained: before the study, after eating fresh yogurt, and after eating heat-treated yogurt. These were compared to fecal samples from 60 individuals who did not consume any yogurt. A molecular analysis of gut microbes present in the fecal samples found that both fresh yogurt and heat-treated yogurt led to a significantly higher density of lactic acid bacteria and a significant decrease in the density of *Bacteroides*. The researchers concluded that the probiotics, whether alive or dead, had decreased the resident *Bacteroides*, and led to proliferation of lactic acid bacteria.\(^3\)

A recent study from Argentina also found that living or lysed *Lactobacillus casei*, a common probiotic in fermented dairy products, stimulated immune cells. “The whole bacterium or its fragments make contact with the gut-associated immune cells...reinforcing the non-specific barrier and modulating the innate immune response in the gut, maintaining the intestinal homeostasis.”\(^4\)

Studies from the early and mid-1990’s found that a lysate of *Lactobacillus bulgaricus* increased resistance to infections in mice. Fewer mice died of infections with *Klebsiella pneumoniae* or *Listeria monocytogenes* when given the lysate. Lysed *Escherichia coli* improves colitis in mice.\(^5\) Lysed, heat-killed *Propionibacterium acnes* contains a complex sugar that causes a “remarkable increase” in natural killer and other immune cells in mice.\(^6\) Purified cell walls from eight different gram-positive bacterial species spiked the production of TNF-alpha, suggesting that the content of the cell walls themselves “may contribute to the septic shock induced by gram-positive bacteria.”\(^7\)

Other studies show that both whole cell and cell wall fragments of *Lactobacillus* are capable of stopping pathogens from adhering to the urogenital tract in women.\(^8\) Heat-killed *Lactobacillus casei* protects mice from *Cytomegalovirus* infection, by increasing natural killer cell activity.\(^9\) A lysate of *Bifidobacterium longum* can even calm reactive skin, decreasing vasodilation, swelling, mast cell degranulation, and the inflammatory tumor necrosis factor alpha (TNF-a). In that particular skin study, 33 female volunteers with marked skin sensitivity were given a cream with an extract of the lysed probiotic, and 33 were given a placebo cream. For two months the cream was applied to the face, arms and legs twice a day. After a skin stinging test using lactic acid, the women receiving the bifido cream fared significantly better: “The number of strippings required to disrupt skin barrier function was significantly increased for volunteers treated with the active cream.”\(^10\)

After antibiotic treatment, several types of gut microbes did not recover to their pre-treatment levels, even as long as six months later.

The immune system. In the 1960’s, researchers showed that cell wall lysates of *L. rhamnosus* contain sugars and proteins that modulated the immune response.\(^11\) In the 1980’s, further research confirmed that both the whole cell and the cell wall of *Lactobacillus rhamnosus* strengthens immunity.\(^12\)

Is it really so surprising that “dead” bugs help shape our immune system? What, after all, is the basis of a vaccine? However, a vaccine works through the antibody response. Our gut microbes generally work in a different and more fundamental way. They speak to our immune system more generally—sending messages to help it decide just how to behave. That is why, for instance, a lysate of *Escherichia coli* taken to treat recurrent urinary tract infections also seems to help rheumatoid arthritis. The researchers speculate that “long-term activation of regulatory T-cells and oral tolerance, both key functions of the gut, may be boosted” by the molecules in the lysate.\(^13\) And in this insight lies one key to the way gut microbial cell walls and their molecular fragments dance with the immune system—leading to either systemic health or breakdown.

**Messages from the Living...**

As potent as heat-killed and lysed bacteria can be, living microbes offer the ultimate and complete communication system. In an ideal world, a baby born to a healthy mother with abundant *Lactobacilli* in her vaginal tract will be colonized by that flora, and then will be breastfed for months, leading to a healthy gut flora with many *Bacteroides* and...
Bifidobacterium species. Immune tolerance will be set early by this healthy microbiota, nourished by the balanced immune molecules in the mother’s milk.

But life is not always that simple. A mother’s milk, if the mother suffers from a disrupted flora, can lead to a disrupted flora in the baby (See Garrett interview, pp 4). A caesarean birth will colonize the newborn with skin microbes rather than gut. Formula feeding can never match breastmilk, and will alter the flora as well, but often it’s a necessary choice for working mothers.

If antibiotics are given too freely, the flora will be further disrupted, and may not completely recover. In one study using special sequencing of DNA in fecal samples, treatment with Ciprofloxacin significantly impacted over one-third of the microbes, although within four weeks most had completely recovered. However, several types of microbes did not recover to their pre-treatment levels, even as long as six months later. And in a 2011 study from the University of British Columbia, investigators used powerful mass spectrometry techniques to analyze the molecules shed by microbes in mouse feces before and after antibiotic treatment. The levels of 87 percent of the molecules detected had been shifted up or down by factors ranging from 2-fold to 10,000-fold. The most profoundly altered pathways involved steroid hormones, eicosanoid hormones (derived from fatty acids), sugar, bile acids and more. The hormones in particular, note the researchers, help control our immune system, reproductive functions, mineral balance, and sugar metabolism.

As biologist June Round of the California Institute of Technology notes, “Antibiotic treatment can... allow pathogenic bacteria an opportunity to colonize.”

A disrupted flora can also lead to allergic disease. According to researchers at the University of Michigan, the gut flora of allergic individuals differs from that of non-allergic ones. The researchers proved this in mice: after five days of drinking water that contains Cefoperazone (a third generation Cephalosporin), mice given a dose of Candida albicans have an increase in gut microbial fungus. If the mice are then exposed to an intranasal challenge of an allergen from Aspergillus fumigatus, they develop T-cell mediated airway autoimmune disease. Are skyrocketing asthma rates in children and adults due to a similar mechanism? What about rheumatoid arthritis—since patients with early rheumatoid arthritis turn out to have significantly less Bifidobacterium and Bacteroides fragilis—both of which are proven to help immune tolerance.

Probiotic bacteria have profound suppressive effects on pathogens. Small molecules secreted by probiotics reduce the level of expression of virulence genes in a strain of E. Coli that produces hemorrhages. A bacteriocin secreted by Lactobacillus salivarius prevents mice from succumbing to invasive infections caused by Listeria monocytogenes. In a review of the literature on probiotics and the microbiome, Baylor College of Medicine pathologist and immunologist Geoffrey Preidis notes that probiotics can lead to high levels of anti-inflammatory molecules like transforming growth factor B (TGF-B) and Interleukin-10. Probiotics can stimulate regulatory T-cells, says Preidis, and suppress and prevent synthesis of powerfully inflammatory cytokines. Different probiotics have different special abilities; some suppress cytokines stimulated by bacteria, and some suppress our own cytokines, says Preidis. Proteins secreted by L. rhamnosus GG help the gut barrier, by tightening the junctions and reducing permeability—and thus combating pathogens like virulent strains of E. coli.

Even the course of cancer might be altered by our flora: a new study on probiotic fermented milks in cancer found that yogurt consumption inhibited tumor growth, by increasing an anti-inflammatory molecule known as Interleukin-10. The researchers noted that the same effect occurred in a previous study on intestinal inflammation, by the same mechanism. “The consumption of fermented milks can modulate the immune system,” the researchers concluded. Another study found that probiotic milk stimulated mucosal immunity, reinforced the gut lining barrier, and modulated the innate immune response.

Our Fat Flora: The New Science of Bacteria and Obesity

Can your flora make you fat? Apparently so. The nutrient value of food depends in part on the ability of a variety of gut microbes to...
metabolize it. Mice bred to be obese have microbes with an increased ability to harvest indigestible dietary sugars, turning the sugars to fatty acids the mouse can absorb.\(^{(23)}\) When “wild-type” germ free mice (bred to have no gut microbes) are given the gut flora of genetically obese mice, they gain significantly more weight than if they receive the gut flora of genetically lean mice. In other words, the flora itself makes you “fat”. And studies in human obese and lean twins find the same distinction—the obese microbiome is enriched in the ability to process carbohydrates.\(^{(23)}\)

In fact, not only can your flora make you fat, it can lead to metabolic changes in the gut, liver, kidney and brain associated with obesity (such as high triglycerides). This specific effect has been demonstrated by biological chemist Jeremy Nicholson of Imperial College at London. Nicholson inoculated germ-free mice with an assortment of microbes modeled after a healthy human baby’s flora, and then examined the effect of two *Lactobacillus* species plus prebiotics. He found metabolic changes in the liver, kidneys, gut and parts of the brain. He also found effects specific to the organism. While *Lactobacillus paracasei* plus prebiotics led to lower levels of triglycerides in the liver, *Lactobacillus rhamnosus* plus prebiotics led to better intestinal absorption of dietary sugars. There is, it seems, a marvelous specificity to the action of each microbe on the body.\(^{(27)}\)

Even more remarkable, Nicholson found that the actual microbial content of the mouse gut hardly changed at all during the experiment. It seems the probiotics worked by molecular signals that stimulated the microbes already in

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**The Healing Species in Your Feces... A Quick Guide to Bacterial Terms**

**Bifidobacteria:** Bifidobacteria are found mostly in the large intestine. In breastfed infants bifidobacteria comprise more than 95% of intestinal bacteria. They are anaerobic, and unlike other probiotic bacteria, they can ferment carbohydrates to both acetic and formic acids. They also produce lactic acid, creating a healthy pH in the colon. They produce vitamins B1, B6, folic acid, and enzymes such as casein phosphatase and lysozyme.

**Lactobacillus GG:** The most studied probiotic, with hundreds of peer review studies, *Lactobacillus GG* was isolated from the gut of a healthy human by a team at Tufts University (see *In Focus* October 2009). Like other *Lactobacillus* species, it takes up residence primarily in the wall of the small intestine, where it helps normalize pH, promote digestive function, help suppress the growth of harmful bacteria, and stimulate a healthy immune response. It can help normalize fecal enzyme and short-chain fatty acid levels and in animal studies, reduced plasma endotoxin levels.

**Saccharomyces boulardii:** A non-colonizing yeast species closely related to Brewer’s yeast, *S. boulardii* “blooms” and quickly becomes established in the gut, where it can produce lactic acid and some B vitamins. Both extensive studies and clinical use suggest it can help displace unfriendly yeast species in the GI tract and increase levels of secretory IgA, which is crucial for gut immune function.

**Bacteroides fragilis:** *Bacteroides* are the most common anaerobic bacteria in the gut. They preferentially process complex polysaccharides from plants, although they can use simple sugars as well. Over 20 species in the fragilis group have been identified, and fascinating research has shown that one single sugar on the cell wall of the fragilis group—polysaccharide A (PSA)—is capable of reversing immune defects in germ-free mice bred to have no gut microbes. Scientists believe it’s serving a similar purpose in humans, since the sugar suppresses the production of the potent, pro-inflammatory cytokine IL-17.

**Lysate:** The cell wall fragments and molecules produced by the dissolution or destruction of cells, often by enzymes. For instance, our macrophages digest bacteria and spit out lysates that signal our immune system. An abundant scientific literature shows lysates of gut and other bacteria to be profound immune stimulators.

**Microbiota:** The microorganisms that typically inhabit a bodily organ or part; flora. Our gut contains a microbiota as diverse and abundant as the most lush rainforest.

**Microbiome:** The collective genomes of all the microbiota in our gut.

**Metagenome:** Most commonly refers to our own genome plus the genome of all our microbial flora.

**Metabolome:** The complete set of molecular metabolites (such as hormones and other signaling molecules) found—in this case—in the human gut. The metabolome is dynamic and ever-changing. In January 2007 scientists at the University of Alberta and the University of Calgary finished a draft of the human metabolome. They have catalogued and characterized 2,500 metabolites, 1,200 drugs and 3,500 food components that found in the human body. Metabolomics is already being used in pharmacology, pre-clinical drug trials, toxicology, and transplant monitoring.
residence, and perhaps the immune system itself.\(^{(27)}\)

This little gem of a finding once again points to the key on which the mystery of the microbiome turns. It may be the microbes’ potent messages and signals—the complex sugars, peptides, fatty acids and more—which impact health, often through a cascade of effects. The microbes’ metabolic activity—called the metabolome—holds many answers to the mysteries of health and illness. This microbiota is potent enough when transplanted to cause us to gain weight, succumb to colitis, or even to cure deadly colitis when given through a fecal transplant (see Garrett Q&A, p. 4).

### What Lies Ahead?

“The first decade of bacterial genomics has afforded unprecedented insights into the evolution of bacterial pathogens,” writes Marco Ventura, head of the Probiogenomics Laboratory at the University of Parma in Italy. “The next decade holds the promise of being even more rewarding, as the new discoveries about probiotic bacteria can be exploited.”\(^{(28)}\)

In coming years we are likely to discover, enhance or recombine new probiotics. Geoffrey Preidis notes that a nonpathogenic *E. coli* can neutralize cholera toxin, and that an early, Phase I trial of 10 Crohn’s disease patients taking a genetically modified strain of *Lactococcus lactis*, which over-produces anti-inflammatory IL-10, showed promise.\(^{(25)}\)

Sequencing and microbiology are so sophisticated now that we can watch exactly how a specific probiotic works. Marco Ventura reports that one species of Bifidus—*Bifidobacteria longum* subspecies longum, promoted Interferon-\(\gamma\) production from our own T-cells, and reduces our own production of specific antibacterial proteins. Another species of Bifidus downregulates inflammatory TNF-A and inflammatory cytokines produced by our own natural killer cells.\(^{(28)}\) *L. bulgaricus* is so widely used as a starter culture in yogurt that it has genetically adapted to milk and prefers to grow on lactose. *L. helveticus*, which is used as a cheese starter culture, has additional genes for fatty acid synthesis. Each species may have its own niche, its own particular response to what we eat, and its own conversation with our immune system. With this precise, zoom-in view of what probiotics are actually doing, we may, says Ventura, be able to select “the most appropriate probiotic strain for a particular health benefit…or for a particular human genotype.”

Diabetes might be altered by probiotics, according to researchers from Italy. Prebiotics and probiotics influence insulin sensitivity, glucose tolerance, and inflammation.\(^{(29)}\)

We also might learn how stress alters our gut microbiota and contributes to disease; research from Ohio State University shows that stress decreases beneficial *Bacteroides* and increases *Clostridium* species in the mouse. Cytokines then shift as well; the researchers conclude that, remarkably, the gut microbes themselves are necessary for stressor-induced increases in circulating cytokines.\(^{(30)}\)

One day we may even be able to alter the course of common conditions like fatty liver, which can lead to cirrhosis, by manipulating our gut microbes. Some cases of fatty liver seem due to a genetic weakness in the ability to process choline, but genes are not the sole cause. Bacteria matter as much, according to a new study of fecal bacteria, which found that individuals with gut microbes called *Gammaproteobacteria*, which can synthesize choline, had the lowest rate of fatty liver development. In contrast, those with lots of another group of bacteria, *Erysipelotrichi*, had significantly higher rates of fatty liver. Are the first set of bacteria contributing choline, while the second set are depleting it?\(^{(31)}\) Could we someday tweak our microbiota to avoid fatty liver?

The human body is a super-organism composed of ten times more microbial cells than our own. There are 3.3 million unique genes in the bacteria of the human gut, and as many as 1000 different bacterial species.\(^{(32)}\)

We are exquisitely sensitive to these commensals, fine-tuned to cohabit with us over millennia of evolution. And they are as sensitive to us. Which species are in your feces? It sounds humorous, but it may be the million dollar question for 21st century medicine.

References available in online version at: [http://www.nutricology.com/In-Focus-sp-115.html](http://www.nutricology.com/In-Focus-sp-115.html)
Frank Nochimson, M.D., is in private practice in Brooklyn, New York.

**Dr. Nochimson:**

“Whenever I put a patient on long-term antibiotics, as in the case of chronic Lyme disease, I recommend and prescribe probiotics. I often prescribe large doses of a probiotic mixture that contains five different live organisms. In many cases I also prescribe *Lactobacillus rhamnosus* lysate (non-living broken cell wall fragments). Together they often seem to have a complementary and synergistic effect.

This approach also seems to work in some cases of mixed autoimmune and infectious illness marked by gut inflammation. I recently treated a woman suffering from Crohn’s disease for twenty years. She was on a number of medications for her condition. I put her on live probiotics, *Saccharomyces boulardii,* and *Lactobacillus rhamnosus* lysate. With this combination, we were able to slowly bring down the dosages of some of her medications. Her next colonoscopy showed her to be in complete remission for the first time in two decades.

Another woman I’ve been treating is severely allergic to most foods. She can only eat five foods. We put her on an antifungal medication, and then added the combination of five live probiotics, *S. boulardii,* and *L. rhamnosus* lysate. It took three months to slowly get her up to par on these supplements, but when we did, she had a full blown skin reaction and full body rash. I’ve seen that before in other cases, and it appears to be the body secreting some of its stored toxins. She went back to a lower dose of the probiotics a month ago, and we will see if that is a good balance.

A male patient of mine had cervical stenosis, and when he first came to us his spine was quite inflamed. He was in terrible pain and scheduled for surgery. After testing, we put him on antimicrobials including artemisia and uña de gato, as well as live probiotics and the lysate. We also gave him body work twice a week, focusing on separating the ribs to take pressure off the spine. When ribs get tight from stress they can pull on the spine and vertebral column. Within three months he was out of pain and surgery was cancelled, but we are still treating him for the inflammatory process in his spine. You may wonder why we treated a spinal condition with antimicrobials and probiotics, but I find that often these autoimmune and inflammatory conditions, no matter where they manifest in the body, are due to an underlying infection. So we look at not only changing the diet, and treating the biochemistry with nutrients, but adding in probiotics and anti-infectious herbs.

The dosages of probiotics depend on the person, of course, but I do feel that sometimes high dosages are necessary. For someone taking antibiotics several times a day for chronic Lyme or a chronic autoimmune condition, I might suggest up to a dozen a day of each of these. These are not little doses, and this is not a fast process, but it does work, and when we see patients improve so remarkably it is very rewarding.”

Pieter deWet, M.D., is in private practice in Tyler, Texas and is the author of the book *Heal Thyself: Transform Your Life, Transform Your Health.*

**Dr. deWet:**

“I recommend probiotics to virtually every patient I see and feel it’s a critical part of healing—at least on the physical level. They say health starts in the intestinal tract, and today with a food supply containing so many genetically engineered and microwaved foods, as well as pesticides and herbicides, there is tremendous pressure on the gut flora. The organisms in the gut are a critical part of maintaining health. Dysbiosis is becoming almost universal in our society. You can look at almost any disease—from inflammatory bowel disease to dementia, fibromyalgia, chronic fatigue syndrome, Parkinson’s—and you’ll find a component of that disease is sourced in the gut. And when we talk about inheritance, that doesn’t apply just to our genes. We inherit the bacteria and microbes of our forefathers. What’s in your intestinal tract today is linked to what was in your mother’s and father’s intestinal tracts, and all the way back for generations. Isn’t that fascinating?

My general approach to treating patients with significant, chronic illness or a history of antibiotic use leading to fungal overgrowth, is to first give them *Saccharomyces boulardii,* the “good” yeast. After a course of the good yeast, I switch over to a combination formula containing *S. boulardii,* *Bifidus* and *acidophilus.* For healthy individuals,
I recommend a course of probiotics twice a year, as well as fermented foods like kefir and yogurt. In patients with severe immune dysfunction or cancer, I also use L. rhamnosus lysate as an immune booster and to get their immune systems reactivated. This is part of my overall approach to healing, which includes many other modalities including nutrient therapy, detoxification therapies, and emotional and spiritual work.

### Probiotics: An Effective New Approach to Childhood Eczema

One out of every ten American children suffers from eczema.(1) Sixty-five percent of those children are afflicted by 18 months of age, and less than half have complete resolution by 7 years of age, while only 60% have resolution by adulthood.(2) Not only is the presence of eczema a risk factor for asthma, the patches of itchy, dry, red skin can lead to unsightly, weeping and scaly sores. For parents and children alike, eczema is a frustrating problem.

Probiotics may provide one simple and healthy treatment solution. A study of 415 mothers, half of whom drank a daily glass of milk with a probiotic supplement, and half of whom drank plain milk, showed that the probiotic milk was able to cut the incidence of eczema in their children by forty percent. The mothers were followed from pregnancy until their children were two years old.(3)

In another study, researchers found that daily supplements of a multi-bacterial strain food (two strains of Bifidobacterium and one of Lactococcus) may reduce the risk of eczema by 58 percent during a child’s first two years of life. Probiotics become established during the first three months of life.(4)

A broken-cell wall probiotic may also help treat eczema, a new study shows. In fourteen children aged 8 to 64 months with treatment-resistant eczema, a lysate of Lactobacillus rhamnosus proved surprisingly effective, without side effects.(5) The researchers of the 2010 study, published in Inflammation and Allergy*, were intrigued by a review study of over 2,000 infants from eight

Hoang BX, Shaw G, Pham P, Levine SA. Lactobacillus rhamnosus cell lysate in the management of resistant childhood atopic eczema. Inflamm Allergy Drug Targets. 2010 Jul 1;9(3):192-6. PMID: 20687891

In an open, small trial, the researchers treated 14 children with a history of resistant eczema for at least six months. The children received 300-500 milligrams of standardized Lactobacillus rhamnosus lysate daily as an immunobiotic supplement. The results of this open label non-randomized clinical observation showed a substantial improvement in quality of life, skin symptoms and day- and nighttime irritation. There were no intolerance or adverse reactions observed. They conclude: “Lactobacillus rhamnosus cell lysate may thus be used as a safe and effective immunobiotic for the treatment and prevention of childhood eczema and possible other types of atopy.”(5)

*In Focus publisher Stephen A. Levine was one of four authors of this study.

References available in online version at:

http://www.nutricology.com/In-Focus-sp-117.html
A Spoonful of Sunshine: The Vitamin D Picture Matures
By Stephen A. Levine, Ph.D.

The much-discussed August 2010 issue of In Focus was devoted entirely to vitamin D and received tremendous positive feedback from physicians. We offered an in-depth distillation of the peer-review literature, explored the profound cross-talk that occurs between vitamins D and A, presented the views of experts in vitamin K, D and A, and supported a balanced approach to supplementation of this pro-hormone, making sure that other fat-soluble vitamins are not ignored. Two of the most important insights from that newsletter were that 1) too much vitamin D and too little vitamin A will create a functional deficiency of vitamin A with associated immune issues and 2) testing is mandatory for anybody concerned about vitamin D levels, as individual variation is so great.

Since that time, numerous exciting studies on vitamin D have been published, showing that a deficiency of vitamin D is connected to illnesses such as breast cancer, multiple sclerosis, and cardiovascular disease. As the picture matures, a balanced approach is the consensus.

In November, the big news came from the Institute of Medicine (IOM), which for the first time since 1997 issued new recommendations on safe levels of vitamin D supplementation. They called for at least 600 IU of vitamin D daily up to age 70, and 800 IU daily after age 71. They raised the safe upper limit of 2000 IU daily to 4,000 IU daily. A source no less prestigious than the Harvard School of Public Health Nutrition Source offered commentary co-authored by Heike A. Bischoff-Ferrari, MD, DrPH and Walter C. Willett, MD, DrPH, who noted that the IOM’s recommended safe threshold of 50 nmol/l (20 ng/ml) for 25(OH)D was not adequate for preventing fractures. In contrast to the IOM report, the International Osteoporosis Foundation (IOF) 2010 position paper had recommended a threshold of 75 nmol/l (30 ng/ml) for optimal fall and fracture reduction and recommended 800 to 1,000 IU of vitamin D per day for seniors age 60 years and older. (1) The Harvard newsletter approved of the upper limits: “In support of a greater safety margin in research and supplementation strategies, the IOM doubled the safe upper limit from 2,000 IU to 4,000 IU of vitamin D per day, which is appropriate.” (2)

In other news reflecting the complexity of the Vitamin D picture, a study presented at the American Association for Cancer Research (AACR) 102nd Annual Meeting in 2011 found that vitamin D significantly reduced development of estrogen receptor-positive breast cancer in both lean and obese mice, but had no beneficial effect on estrogen receptor-negative cancer. The researchers, from the Georgetown Lombardi Comprehensive Cancer Center found that Vitamin D reversed insulin resistance in obese mice, but did not reduce insulin sensitivity in lean mice. In earlier studies from the Lombardi Center, vitamin D reversed both early and advanced endometrial cancer in obese mice, but had no benefit on the same cancer in lean mice. The lead researcher, oncologist Leena Hilakivi-Clarke, PhD concluded: “For those who want to boost their use of vitamin D, it is important that they have their individual levels tested by a physician, and that they discuss their desire to use supplements.” (3) We might add that it would be useful to look at vitamin A in these conditions, since retinoic acid derivatives are being studied in cancer.

The cardiovascular connection to vitamin D was highlighted in a new study suggesting that a lack of the vitamin may be linked with stiffer arteries. The research comes from the Emory/Georgia Tech Predictive Health Institute. The findings were presented at the annual American College of Cardiology meeting in New Orleans. 554 participants in the study were Emory or Georgia Tech employees – average age 47 and generally healthy. The researchers monitored the ability of participants’ blood vessels to relax by inflating and then removing a blood pressure cuff on their arms. As blood flows back into the arm, blood vessels relax and enlarge – which can be measured by ultrasound. Resistance to blood flow imposed by the arteries can be measured in smaller blood vessels as well. Even after controlling for factors such as age, weight and cholesterol, people with vitamin D deficiency had vascular dysfunction comparable to those with diabetes or hypertension. (4)
A new study confirms that low sunlight exposure and low Vitamin D raises risk of multiple sclerosis, according to a new study published in Neurology in February, 2011. The multi-site study looked at 216 people age 18 to 59 who had a first event with symptoms of the type seen in multiple sclerosis (MS). Those people were matched with 395 people with no symptoms of possible MS who were of similar ages, of the same sex and from the same regions of Australia. The differences in sun exposure, vitamin D blood levels, and skin type accounted for a 32 percent increase in a first event. (5) And in a second study that refines the link between sunlight and MS, and looks deeper, it appears that sunlight exposure and a mononucleosis history together explain 72% of the variance in multiple sclerosis incidence within England. Is adequate sunlight--and the resulting healthy blood levels of vitamin D—helping keep this common virus in check, and in ways we do not yet understand, lowering the risk of multiple sclerosis? (6)

These are just a few of the new studies and recommendations on Vitamin D, which we feel reflects a maturing picture and an increasingly nuanced and balanced approach to this potent molecule.

References available in online version at:
http://www.nutricology.com/In-Focus-sp-I18.html

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The antioxidant glutathione, known as GSH, is arguably the most important antioxidant the body makes, and most certainly the most powerful intracellular antioxidant. In its reduced form it plays a pivotal role in DNA repair, immunity, flushing of toxins, removal of heavy metals, quenching of free radicals, and recycling of other antioxidants such as vitamins C and E. Glutathione supports detoxification in the lining fluid of the lung and intestines, enhances macrophage function, and slows virus production. Low levels of glutathione are associated with an astonishing range of diseases, from diabetes to Parkinson’s to asthma to kidney problems, and many other conditions. (See In Focus July 2008 p 11).

Unfortunately, oral supplementation of glutathione has proved tricky and sometimes ineffective, since the molecule when taken orally is not able to effectively reach and be absorbed into the intracellular space where it is needed. (1, 2) Optimal exposure to the potential benefits linked to GSH have been achieved with IV therapy but it is expensive and inconvenient, and has only short-term benefits, and so needs to be repeated frequently.

“The contribution of GSH deficiency in many pathologies has stimulated a number of researchers to find new potential approaches for maintaining or restoring GSH levels,” write Italian researchers in a review in the journal Molecules in 2010. (3) And as it turns out, those approaches have borne fruit. One novel formulation of the molecule, S-acetylglutathione (S-GSH), has been shown to be surprisingly well absorbed by cells and of great potential benefit. (3) It crosses the cell membrane more easily than GSH itself, and is easily de-acetylated in the cell, becoming active GSH. The fact that S-GSH can be effectively absorbed by cells after an oral dose argues for its great potential in comparison to IV therapy.

S-GSH proved a significant anti-viral agent both in vitro and in animal studies in a 2005 study from Johann Wolfgang Goethe University Hospital in Germany. Remarkably, it was stable in plasma and taken up directly by cells with subsequent conversion to GSH (the active, reduced form). In cell culture, S-GSH efficiently restored intracellular glutathione, and in mice, S-GSH but not plain glutathione, significantly decreased virally induced mortality. This novel form of glutathione was active and stable. (4)

S-GSH has also been shown to cause the death of certain cancer cells. In a study in the International Journal of Oncology, S-GSH induced significant cell death in three human lymphoma cell lines. It did not have the same effect on normal lymphocytes. The researchers concluded that “S-acetyl glutathione specifically activates programmed cell death in lymphoma cells.” In fact, their analysis showed that this form of glutathione depleted intracellular glutathione in the cancer cells, in a selective effect that was the opposite of its action in normal cells. (5)

Finally, in mice infected with a viral complex, S-GSH was able to reduce spleen viral content by 70% and lymph node viral content by 30%—and to do so at half the concentration of GSH. (6) As the Italian researchers note in Molecules, glutathione analogues such as S-GSH “may offer a promising therapeutic alternative for reducing the GSH functional loss related to many human diseases.” (3)

References available in online version at:
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