Bugs as Drugs: Bacteria as Therapeutics against Diseases

Bacterial therapeutics are being developed to treat human diseases, including gastrointestinal disorders, osteoporosis, and cancers

Shannon Weiman

While widely recognized for their pathogenic potential, bacteria are now at the forefront of biomedical research for their therapeutic capabilities. During the 2014 ASM General Meeting, held in Boston last May, several researchers who spoke in the plenary session, “Bugs as Drugs,” and in other sessions, described candidate bacterial therapeutics that are being developed to treat a wide variety of human diseases, including gastrointestinal disorders, osteoporosis, and cancers.

Some bacteria are being evaluated as disease treatments in near-native form, whereas others are genetically modified to make them safer and to enhance specific disease-fighting attributes. All take advantage of host-bacterial interactions, many of which include immune-manipulation, to achieve success in cases where traditional medical treatments do not always succeed.

Lactobacillus-Based Probiotics Ease Osteoporosis and Autoimmune Conditions

One approach to harnessing bacteria against disease is to use them as probiotics, which typically involves delivering specific microbial species to the gastrointestinal (GI) tract. Some experts have long held that bacterial probiotics benefit the digestive tract. However, recent reports indicate that the influence of such microbes on health and disease reaches far beyond the GI tract, benefiting patients with diseases ranging from type 2 diabetes, to cardiovascular disease, and cancer. Researchers report a particularly strong role for Lactobacillus spp. in preventing and treating various diseases and conditions, including osteoporosis and infections caused by a variety of pathogens. They also can be easily administered in foods.

Surprisingly, gut microbes influence the development of osteoporosis, according to Robert Britton of Michigan State University in East Lansing. “Recent studies suggest an important role for gut-bone signaling pathways and the microbiota in regulating bone health,” he says. “Bone is a dynamic organ that undergoes continuous turnover to adapt to the physiological needs of the organism.” However, hormone changes associated with menopause alter development of osteoblasts and osteoclasts, the specialized cells responsible for this dynamism, shifting the balance towards bone resorption, which underlies osteoporosis.

Administering the probiotic Lactobacillus reuteri to mice with osteoporosis can reverse this process of resorption, by suppressing osteoclast development, Britton says. For example, female mice given L. reuteri three times a week for four weeks had significantly improved bone density and strength compared to animals that were fed a diet lacking that probiotic. “Osteoclast bone resorption markers and activators (Trap5 and RANKL) as well as osteoclastogenesis are significantly decreased in L. reuteri-treated mice,” he says.

L. reuteri and menopause act through the

SUMMARY

➤ Some bacteria are being evaluated as disease treatments in near-native form, whereas others are genetically modified to make them safer and to enhance specific disease-fighting attributes.

➤ One approach to harnessing bacteria against disease is to use them as probiotics, including in cases of infectious diseases where they compete for resources or produce antimicrobial compounds.

➤ Probiotics are also being evaluated for their effectiveness in treating various autoimmune diseases, osteoporosis, and metabolic disorders such as diabetes.

➤ Listeria and Salmonella are among the bacteria being modified in various ways for potential treatment of a variety of tumors.
same mechanism to influence osteoclast formation, according to Britton. Both alter tumor necrosis factor (TNF-α) production, the master regulator of osteoclast development. Because estrogen blocks T-cells from producing TNF-α, decreases in estrogen during menopause result in increased TNF-α, formation of new osteoclasts and bone resorption. L. reuteri corrects this imbalance by creating immunosuppressive signals that decrease TNF-α production, he says. "L. reuteri is able to convert a dietary component, L-histidine, into an immunoregulatory signal, histamine, which suppresses pro-inflammatory TNF-α production." Thus, treating mice with this probiotic prevents and also reverses osteoporosis. "L. reuteri treatment may be a straightforward and cost-effective approach to reduce post-menopausal bone loss," he says.

L. reuteri also can mitigate diabetes-related bone loss in mice, which is caused by metabolic disease-associated inflammation, Britton continues. The anti-inflammatory, anti-TNF-α properties of L. reuteri also appear to ease the symptoms of Crohn’s disease, an autoimmune condition involving excessive inflammatory responses within the colon, in mouse models, according to Britton. “The identifcation of bacterial bioactive metabolites and their corresponding mechanisms of action with respect to immunomodulation may lead to improved anti-inflammatory strategies for chronic immune-mediated diseases,” he says.

**Probiotics Can Counteract and Compete against Microbial Pathogens**

Probiotics can also protect individuals against bacterial pathogens by competing for resources or producing antimicrobial compounds. Indeed, L. reuteri is named for its production of reuterin, which inhibits the growth of harmful gram-positive and gram-negative bacteria such as Listeria monocytogenes and Escherichia coli O157:H7, as well as yeasts, molds, and protozoa.

Various Lactobacillus strains, including L. reuteri (strain LR92), L. plantarum (strain LP-115), and L. salivarius (strain LS-33), inhibit the growth in vitro of pathogens that are found on teeth and in the oral cavity, according to Mette Kirstine Keller of the University of Copenhagen, Denmark, and her collaborators. She presented some of their recent findings during a poster session at the 2014 ASM General Meeting. These probiotics might be used to treat or prevent diseases caused by Streptococcus mutans, Porphyromonas gingivalis, and other bacteria that can cause dental caries and periodontal disease.

Meanwhile, Lactobacillus pentosus (strain LPS16) may protect against gastric ulcers by inhibiting the growth of Helicobacter pylori, according to Juinn-Jong Wu of National Cheng Kung University Medical College in Tainan, Taiwan. By producing lactic acid, this probiotic is active against at least 60 different clinically isolated, multidrug resistant strains of H. pylori, Wu says. In addition, L. pentosus adheres to the surfaces of gastric epithelial cells, protecting them against invasion by this pathogen. L. pentosus thus might provide a novel and antibiotic-free probiotic approach to treating and preventing gastric ulcers.

**Delivering Probiotics and Beneficial Metabolites in Fermented Foods**

Probiotics are easily administered orally, either in pill form, or alternatively, as components of fermented foods, including kambucha, a fermented tea; miso, a paste made from fermented beans; and yogurt and other forms of fermented milk or nondairy products. These probiotic foods are also reported to have a range of beneficial health effects.

For example, fermentation products in soy milk can reduce blood pressure, according to Tsung-Yu Tsai of Fu Jen University in New Taipei City, Taiwan, who presented recent findings during a poster session at the 2014 ASM General Meeting. Specifically, when soy milk was fermented by L. plantarum (strain TWK10) and administered daily to rats, both the systolic and diastolic blood pressures decreased. The mechanisms underlying these anti-hypertensive effects include suppression of angiotensin converting enzyme (ACE) activity in kidney and liver, as well as increased nitric oxide (NO) production due to upregulation of NO synthase, and increased superoxide dismutase activity. This probiotic food might provide an alternative to reliance on conventional drugs to control hypertension in humans, Tsai suggests.

Fermentation of goat milk by the same L. plantarum strain produces compounds that inhibit melanogenesis, part of the pathway leading to malignant melanoma or to other less deadly skin pigment disorders, Tsai continues. These compounds include radical-scavenging peptides and...
antioxidants that inhibit melanin production in a mouse melanoma cell line. This work could lead to a probiotic treatment for skin pigment disorders, or possibly melanoma.

**Bacteria-Based Vaccines: Recombinant Attenuated Salmonella Vaccines (RASVs)**

Bacteria can be used as vaccines, engineered to express specific antigens that induce protective immune responses in humans and animal hosts. “A significant benefit of using live bacterial vaccine vectors is their ability to invade and colonize deep-effector lymphoid tissues after mucosal delivery, which is critically important for inducing memory T cells,” says Roy Curtiss III of Arizona State University. “Salmonella enterica serotype Typhimurium is the best at this.”

Curtiss and his collaborators spent the past few decades improving the safety and efficacy of *Salmonella* candidate vaccines. A delayed-onset attenuation system introduces orally delivered *Salmonella* cells that can withstand harsh stomach conditions. However, shortly thereafter, the modified bacteria cease to produce key cell-wall constituents, and become increasingly susceptible to clearance by the immune system and lysis. This self-destruct system eliminates the agent from the host after inducing its therapeutic effects. Lysis further enhances efficacy by releasing a bolus of antigens that induce protective immune responses. This approach is also a form of biologic containment, preventing the bacteria from exiting or surviving outside the host, where they might interact with other hosts or environmental microbes.

Curtiss’s delayed-onset systems can drive expression of other factors that the bacteria require to survive in the GI tract or to elicit therapeutic effects, but might otherwise prove harmful to the...
host during prolonged systemic infection, such as lipopolysaccharide (LPS). “These technologies are now being used to develop vaccines to protect agriculturally important animals against pathogens, as well as S. typhi vaccines with genetic modification to protect against a number of important human pathogens,” says Curtiss. Most notably, an RASV against Streptococcus pneumoniae is currently in human clinical trials.

**Bacteria-Based Vaccines against Cancers: Listeria as a Vector**

While most vaccines are aimed at protecting individuals against infectious diseases, recent research is exploring whether vaccines can also be used therapeutically, both to protect against and treat various forms of cancer. Various types of bacteria, including Clostridia, Listeria, and Salmonella, are being studied as vectors for therapeutic cancer vaccines.

Intracellular bacteria could prove effective as cancer vaccine vectors in part for their ability to induce multifaceted immune responses, according to Daniel Portnoy of the University of California, Berkeley, who studies Listeria. “Despite the potential safety concerns for using wild-type Listeria as a vaccine vector, there are a number of desirable features of the natural biology of this bacterium that make it an attractive platform for continued development toward clinical evaluation. The central rationale is that the intracellular life cycle of Listeria enables effective stimulation of CD4 and CD8 T cell immunity.” In particular, Listeria targets antigen presenting cells, including dendritic and macrophage cells, both of which help to orchestrate cellular immune responses, generating lasting protective immunity.

Portnoy and his collaborators at Aduro BioTech in Berkeley developed Listeria-based cancer vaccines, several of which are planned for or are currently in clinical trials for treating pancreatic, ovarian, and prostate cancer, as well as mesothelioma and non-small cell lung carcinomas (NSCLC). One of them, designated CRS-207, shows considerable promise against pancreatic cancer thus far, according to Portnoy.

CRS-207 is engineered to express mesothelin, a signature protein of pancreatic cancers and malignant mesothelioma and also about half of ovarian cancers and NSCLCs. This cancer-specific antigen is recognized by cells of the immune system. “Mesothelin-specific T-cells kill human pancreatic tumor cells in vitro, and are correlated with long-term survival in patients,” Portnoy says.

The CRS-207 strain of Listeria was made safer for use in humans by deleting key virulence factors, reducing its toxicity more than 1,000-fold. “The immunopotency of Listeria was maintained and its toxicity was diminished in vivo, largely by blocking indirect ActA-mediated infection by cell-to-cell spread, and the direct internalin B-mediated infection of nonphagocytic cells, such as hepatocytes,” Portnoy says. “Infection of phagocytic cells was not affected, leaving intact the ability of Listeria to stimulate innate immunity and to induce antigen specific cellular responses.” During a phase 1 clinical trial, cancer patients who were treated with CRS-207 tolerated it well, even when it was given by intravenous infusion at doses of $1 \times 10^9$ bacteria.

Not only is the vaccine safe for recipients, it also appears to work well when tested in rodents and in humans. In animals, the vaccine induced antitumor immune responses that resulted in long-term survival. During a phase 2 clinical trial, CRS-207 more than doubled median survival time, from 4.6 to 9.7 months, among patients with metastatic pancreatic cancer, according to Portnoy. With agreement from officials at the Food and Drug Administration (FDA), the blinded part of the trial was halted to allow all patients to receive the treatment.

**Bacteria-Based Cancer Treatments: Direct Cytotoxic Effects of Tumor Infection**

Salmonella and Listeria can also directly infect tumors, exerting virulence mechanisms against tumor cells and eliciting immune attack of the infected tumor. Moreover, cytotoxic mechanisms can be engineered into such bacteria to enhance their efficacy against tumors, according to Neil Forbes of the University of Massachusetts, Amherst. “Recent advances in engineered therapeutic delivery include temporal control of cytokinin release, enzymatic activation of pro-drugs, and secretion of physiologically active biomolecules,” he says.

For example, Forbes and his collaborators engineered Salmonella to secrete Staphylococcus aureus hemolysin (SAH). When SAH-producing Salmonella cells are injected into mice with tumors, total tumor tissue is reduced by 90%, he says. “As a payload protein, SAH will enable effective bacterial therapy because of its ability to
Diffuse in tissue, kill cells, and expand tumor necrosis.” By contrast, conventional chemotherapy agents typically do not penetrate tumor tissues very well because they are limited to areas fed by blood vessels, which are lacking in the hypoxic tumor core.

Salmonella and Listeria are adept at propelling themselves into tumor tissue, according to Forbes. “Motile bacteria can overcome the penetration limitations of cancer chemotherapeutics because they can actively migrate into solid tumors,” he says. Mutants with impaired mobility no longer prove adept at colonizing or destroying tumors, he adds. Chemotactic mechanisms help to drive this capacity to infect tumors, with receptors for aspartate, serine, and ribose all helping to steer Salmonella toward tumors. “This new understanding of the mechanisms of Salmonella migration in tumors will allow for the development of bacterial therapies with improved targeting to therapeutically inaccessible regions of tumors,” he says.

Tumor cores, which are particularly inaccessible, tend to include cells that are resistant to chemotherapy and radiation and which seed metastases and relapses. As facultative anaerobes, Salmonella and Listeria can thrive in this hypoxic environment that is also protected from immune-surveillance. Their ability to target tumor cores is, potentially, a powerful advantage. In fact, when properly attenuated, these bacteria preferentially infect tumors where immune responses are suppressed, according to Forbes. “Approximately 10,000-fold more S. typhimurium accumulated in tumors than any other organ one week after the injection, thus demonstrating their specificity,” he says. This specificity is essential for safety, which requires minimizing the risk of systemic infection. In animal studies and during phase 1 human clinical trials, bacteria-based cancer treatments have induced very few side-effects and are well-tolerated, in contrast to conventional chemotherapy and radiation, whose toxic side effects sometimes kill patients before their cancers do.

**Targeted Cytotoxicity and Other Advantages over Traditional Cancer Treatments**

The cytotoxic effects of bacteria on tumor tissue can be further augmented by introducing toxic genes, under the control of an inducible promoter that limits their expression to within tumor tissue. Forbes uses the radiation-inducible PRecA promoter to control expression of the murine TNF-related apoptosis-inducing ligand (TRAIL) in Salmonella, subjecting tumors to two different kinds of treatment—radiation and the apoptosis-inducing toxin—simultaneously. “Systemic injection of Salmonella and induction of TRAIL expression using 2 Gy γ-irradiation caused a significant delay in mammary tumor growth and reduced the risk of death by 76% when compared with irradiated controls,” he says. “Repeated dosing with TRAIL-bearing Salmonella in conjunction with radiation improved the 30-day survival from 0 to 100%.” He and his collaborators also are experimenting with a quorum-sensing promoter, which would trigger toxic gene expression at high bacterial densities in tumor tissues and not require an external inducer. Such a system could be useful against metastases and other lesions that are too small to identify clinically.

Salmonella and Listeria treatments are proving effective against a broad spectrum of cancers when tested in animals, including primary and metastatic prostate, breast, pancreatic, colon, bone, and brain cancers. Their efficacy against metastases is particularly encouraging because metastatic cancers are the leading cause of death among cancer patients, and new treatments are often ineffective against this type of disease. Bacterial treatments, and the immune responses they induce, effectively target metastatic lesions when they are still microscopic and most vulnerable. By intervening at such an early stage, before lesions can be identified and conventional treatments begun, bacteria appear to offer new treatment strategies that could prove safer and more effective than any modern medicine has thus far.

Treating cancers with modified bacteria also seems less likely to select for treatment-resistant forms of this disease, according to Forbes and others in the field. Such treatments involve multipronged mechanisms that include direct cytoxic effects as well as host immune responses against tumors, which together are more difficult for cancer cells to evade. “Bacterial therapies have the potential to overcome resistances that cause chemotherapies to fail,” he says. With many advantages over traditional approaches, bacteria have the potential to transform the field of cancer treatment and, in animal studies and some human studies, prove effective against otherwise incurable cancers.