Scientists find that common dietary elements cure lethal infections, eliminating the need for antibiotics

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Salk scientists find that common dietary elements cure lethal infections, eliminating the need for antibiotics. From left: (front) Yujung Lee and Karina Sanchez; (back) Janelle Ayres, Samuel Redford, Grischa Chen and Alexandria Palaferri Schieber Credit: Salk Institute

Antibiotic use is driving an epidemic of antibiotic resistance, as more susceptible bacteria are killed but more resilient strains live on and multiply with abandon. But if antibiotics aren't the end-all solution for infectious disease, what is?

Salk Institute researchers report that giving mice dietary iron supplements enabled them to survive a normally lethal bacterial infection and resulted in later generations of those bacteria being less virulent. The approach, which appears in the journal *Cell* on August 9, 2018, demonstrates in preclinical studies that non-antibiotic-based strategies—such as nutritional interventions—can shift the relationship between the patient and pathogens away from antagonism and toward cooperation.

"Antibiotics and antimicrobials are one of the most important advances in medicine, and we definitely need to continue efforts focused on developing new classes of antimicrobials," says Associate Professor Janelle Ayres, who holds the Helen McLoraine Developmental Chair and is senior author of the new paper. "But we need to learn from history and think about other ways to treat infectious diseases. Our work suggests that instead of killing bacteria, if we promote the health of the host, we can tame the behavior of the bacteria so that they don't cause disease, and we can actually drive the evolution of less dangerous strains."

Ayres, a pioneer in researching the interactions between microbes and their hosts, is finding increasing evidence that in addition to our immune system, which kills pathogens, we have what she calls the cooperative defense system, which promotes health during host-microbe interactions. For example, in 2017 her team discovered that *Salmonella* bacteria can overcome a host's natural aversion to food when sick, which results in more nutrients for the bacteria and a gentler infection for the host. And in 2015 the Ayres lab found a strain of *E. coli* bacteria in mice that was capable of improving the animals' tolerance to infections of the lungs

and intestines by preventing wasting—a common and potentially deadly loss of muscle tissue that occurs in serious infections.

For the current work, Ayres' team studied a naturally occurring gastrointestinal infection in mice caused by *Citrobacter rodentium* (*CR*), which leads to diarrhea, weight loss and, in extreme cases, death. (*CR* is related to pathogenic *E. coli* that are associated with human food recalls.)

To elucidate novel mechanisms of the cooperative defense system, the Salk team used an innovative approach called lethal dose 50 (LD50), which is the dose of bacteria that kills 50 percent of the host population, while the other half of the population survives. Using what's known as a systems biology approach, they analyzed the gene activity that was induced in the infected healthy population compared to the infected sick population, as well as the uninfected healthy mice. From this analysis, they found that host iron metabolism was increased in the infected healthy population.

To test the importance of iron metabolism in promoting the cooperative defense system during infection, Ayres and her coauthors (including undergraduate Karina Sanchez, who conducted experiments with Ayres for two years until graduating and is now a technician in the lab) gave a population of mice an LD100 dose of *Citrobacter* (which should kill 100 percent of the host population) and fed half the population a normal diet and the other half a diet supplemented with iron for only 14 days, after which they were returned to a normal diet.

By day 20, all of the infected mice in the no-iron group had succumbed to the infection. However, in the supplemental-iron group, 100 percent of the infected mice were alive and healthy, even at day 30. The researchers found that even if they dosed animals with 1000 times the LD100 dose of the pathogen, a two-week course of iron kept the animals alive and healthy.

Tissue analysis over the course of the experiment showed that both groups of infected mice had comparable levels of bacteria, yet the iron group appeared healthy while the no-iron group got sicker. Ayres and her team used dietary iron as a tool to investigate the mechanism by which iron metabolism cured the infection.

They found that the short course of dietary iron caused an acute state of insulin resistance in the mice. This reduced the amount of glucose (sugar) absorbed from the intestine, increasing the amount of sugar in the intestine for the pathogen to metabolize. Increased glucose metabolism prevented the pathogen from turning on its genes that cause disease. Additionally, the team found they could bypass iron and use glucose supplementation instead and achieve all the same results.

Interestingly, Ayres and her team found that a year later animals that were infected with *Citrobacter* and had received a single two-week course of dietary iron were alive and healthy, and surprisingly still colonized by the pathogen in their gastrointestinal tract. "This was so exciting to us because it suggested that we basically drove the evolution of weakened strains of the pathogen," says Ayres.

To determine if this was the case, the team sequenced the genomes of *Citrobacter* that were isolated from these animals and found that in the genes necessary for causing disease, the bacteria had accumulated mutations, rendering those genes non-functional. This implied that, by increasing the amount of glucose available to the pathogen, the team was preventing the bacteria from turning on genes that cause more symptoms of sickness in its host. And, over time, by having its nutritional needs met, the pathogen was becoming less antagonistic and more cooperative.

While her team found dietary iron to be an effective treatment for infectious diarrhea in their preclinical studies, Ayres cautions that iron is not going to be the solution for all infectious diseases. "There are some

infections, such as malaria, in which giving iron would be a terrible idea, as the parasite thrives on iron," says Ayres. "However, I'm really encouraged by our findings because they suggest that manipulating the metabolic state of the host and the pathogen with common dietary elements can be extremely effective in curing infections. This means we can treat infections with strategies that are more globally accessible," says Ayres.

The group next plans to explore whether weakened bacterial strains might be used as a type of live vaccine or whether (and under what conditions) a weakened strain could revert to virulence or lethality.

Provided by Salk Institute