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Team shows how antibiotics enable pathogenic gut infections

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A new study by researchers at the Stanford University School of Medicine could help pinpoint ways to counter the effects of the antibiotics-driven depletion of friendly, gut-dwelling bacteria.

A number of intestinal pathogens can cause problems after antibiotic administration, said Justin Sonnenburg, PhD, assistant professor of microbiology and immunology and the senior author of the study, to be published online Sept. 1 in *Nature*. Graduate students Katharine Ng and Jessica Ferreyra shared lead authorship.

"Antibiotics open the door for these pathogens to take hold. But how, exactly, that occurs hasn't been well understood," Sonnenburg said.

In the first 24 hours after administration of oral antibiotics, a spike in carbohydrate availability takes place in the gut, the study says. This transient nutrient surplus, combined with the reduction of friendly gut-dwelling bacteria due to antibiotics, permits at least two potentially deadly pathogens to get a toehold in that otherwise more forbidding environment.

In the past decade or so, much has been learned about the complex microbial ecosystem that resides in every healthy mammal's large intestine, including ours. The thousands of distinct bacterial strains that normally inhabit this challenging but nutrient-rich niche have adapted to it so well that we have difficulty living without them. They manufacture vitamins, provide critical training to our immune systems and even guide

the development of our own tissues. Antibiotics decimate this gut-microbe ecosystem, which begins bouncing back within a few days but may take a month or more to regain its former numbers. And the ecosystem appears to suffer the permanent loss of some of its constituent bacterial strains.

It is thought that our commensal, or friendly, bacteria serve as a kind of lawn that, in commandeering the rich fertilizer that courses through our gut, outcompetes the less-well-behaved pathogenic "weeds." It has also been suggested that our commensal bugs secrete pathogen-killing factors. Another theory holds that the disruption of our inner microbial ecosystem somehow impairs our immune responsiveness.

"While these hypotheses are by no means mutually exclusive, our work specifically supports the suggestion that our resident microbes hold pathogens at bay by competing for nutrients," Sonnenburg said.

When that defense falters, as it does shortly after a course of antibiotics begins, marauding micro-organisms such as salmonella or *Clostridium difficile* can establish beachheads. Once they reach sufficient numbers, these two parasitic invaders can mount intentional campaigns to induce inflammation, a condition that impairs the restoration of our normal gut ecosystem but in which salmonella and *C. difficile* have learned to prosper.

The particular nutrients Sonnenburg's team looked at were sialic acid and fucose, a couple of members of the sugar family. While not household words like glucose, fructose or lactose, these two sugar varieties are produced in every cell in our bodies and are absolutely necessary for our healthy survival. They are also found in meat, eggs and dairy products.

Cells that line the gut extrude long chains consisting of exotic and

familiar sugars linked together and known by a catch-all term: mucus. This homely product serves two valuable functions. First, by coating the inside intestinal wall, mucus forms a reasonably impervious protective barrier to keep the resident microbes, which serve useful purposes inside the gastrointestinal tract, from getting out of the gut and into the bloodstream, where they could be lethal. But the mucus has a second function as well: It gives our resident microbes a guaranteed source of various sugars, like sialic acid and fucose, that they can snap off and use in a number of ways. They can, for example, break these sugar molecules down and derive energy from them.

"Our gut microbes have become very adept at eating mucus," Sonnenburg said.

For the *Nature* study, he and his associates experimented on mice that had been born and bred in a germ-free environment. These mice's guts were devoid of bacteria, unlike normal mice, which harbor hundreds of bacterial species in their bowels just as humans do. Into these germ-free mice the Stanford investigators introduced a single bacterial strain, *Bacteroides thetaiotaomicron*. *B. theta* is a member of a well-studied and important class of commensal microbes that populate the human gut. It has the enzymes necessary to pry sugar molecules loose from the mucus chains streaming from the gut lining. In the case of sialic acid, it lacks the enzymes that would allow it to break down either of these two molecules for its own snacking purposes.

Chipping off sugar molecules it can't chop up for food may seem a waste of *B. theta*'s time. But in a normal gut, there are plenty of other microbes that have tools for digesting sialic acid and fucose, and that can produce other materials *B. theta* needs. It's a barter system, which ecologists call symbiosis. (It also may just be that *B. theta* lops off these sugar residues to get to other, edible sugar residues underneath.)

In a series of separate experiments, the investigators introduced either *S. typhimurium* (a salmonella strain) or *C. difficile* in the *B. theta*-loaded experimental mice. Both types of bacteria can cause severe and potentially life-threatening disease associated with antibiotic use. They also share a common capacity for using sialic acid as an energy source, but not for slicing it off intestinal mucus. After discovering that *C. difficile* can neither liberate nor lunch on fucose, the Sonnenburg team focused their efforts on determining how the two pathogens make use of sialic acid.

Introducing one friendly and one pathogenic bacterial strain into the guts of the formerly germ-free mice, the scientists were able to show that, in this approximation of an antibiotic-decimated gut-microbe ecosystem, the levels of sialic acid soared to high levels in the absence of a complete set of intestinal microbes that ordinarily would keep those levels from climbing. In the presence of these sugars and absence of competition, both pathogens were able to replicate more rapidly. *B. theta* generated a sialic-acid surplus that, in the absence of the other hundreds of normal bacterial species, were bequeathed to the pathogenic strains.

When the researchers investigated the effects of antibiotics on mice with normal intestinal ecosystems, they saw the same sialic-acid spike—and pathogen population explosion—in the wake of the carnage. If the mice were not exposed to the pathogens, but only treated with antibiotics, the sialic acid concentrations returned to their original levels after about three days post-antibiotic treatment as commensals began to recover.

"The bad guys in the gut are scavenging nutrients that were liberated by the good guys, who are casualties of the collateral damage incurred by antibiotics," said Sonnenburg. "Antibiotics cause our friendly gut bacteria to unwittingly help these pathogens.

"We believe that bacterial pathogens in the gut cause disease in two

steps," he continued. "Others have shown that once these pathogens attain sufficient numbers, they use inflammation-triggering tricks to wipe out our resident friendly microbes—at no cost to the pathogens themselves, because they've evolved ways to deal with it. But first, they have to surmount a critical hurdle: In the absence of the inflammation they're trying to induce, they have to somehow reach that critical mass. Our work shows how they go about it after a dose of antibiotics. They take advantage of a temporary spike in available sugars liberated from intestinal mucus left behind by slain commensal microbes."

Sonnenburg said he thinks researchers may someday be able to find drugs that, co-administered with antibiotics, could inhibit the enzymes our friendly gut-bugs use to liberate sialic acid from intestinal mucus, so that a pathogen-nourishing spike doesn't occur. Alternatively, probiotics in the form of bacterial strains that are especially talented at digesting sialic acid could achieve a similar effect.

More information: Microbiota-liberated host sugars facilitate post-antibiotic expansion of enteric pathogens, *Nature*, DOI: 10.1038/nature12503

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