

Good Bacteria Trigger Proteins to Protect the Gut

New studies by Howard Hughes Medical Institute researchers indicate that the millions of beneficial bacteria living in the human gut may actually be helping to stave off injury to the lining of the intestines.

According to the researchers, their unexpected findings suggest that the practice of giving antibiotics to cancer patients to prevent infections might render the gut more vulnerable to damage - a danger that might be overcome by administering substances that mimic the protective presence of gut bacteria. The findings may revise thinking about the treatment of inflammatory bowel disease (IBD), in which the intestine is believed to mount an inflammatory response to benign, or commensal, bacteria.

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Ruslan Medzhitov

The researchers, led by Howard Hughes Medical Institute investigator Ruslan Medzhitov at Yale University School of Medicine, reported their studies in the July 23, 2004, issue of the journal *Cell*.

"Until now, almost everything we knew about the benefits of commensal bacteria had to do with their biological activity," said Medzhitov. "They metabolize nutrients to enable us to absorb them more readily. They aid in the early development of the gastrointestinal system. And they produce factors that prevent colonization by pathogenic bacteria. Our work, however, has revealed a role that is quite different."

Specifically, Medzhitov and his colleagues discovered that beneficial bacteria trigger proteins called Toll-like receptors (TLRs) to maintain the health of intestinal epithelial cells and to activate machinery that responds to injury. Previously, TLRs were thought to function strictly as assassins, recognizing molecules on the surface of pathogenic bacteria and triggering the innate immune system to attack. "It's always been a major paradox that our immune system evolved to recognize pathogenic microorganisms and to react to them with a powerful, destructive response; but at the same time, it tolerates a very close, continuous contact with myriad commensal bacterial species that share many molecular characteristics of pathogenic bacteria," said Medzhitov. The predominant view, said Medzhitov, was that TLRs were somehow prevented from recognizing commensal bacteria, yet unleashed specifically against pathogenic microbes.

To better understand the interaction between commensal bacteria and TLRs, the researchers used mice that lacked a key component of the TLR signaling machinery, called MyD88. Without this protein, mice are unable to activate TLR signaling. In initial experiments, they gave the mice a drug called DSS, which is toxic to epithelial cells in the colon.

Normal mice recovered easily from DSS exposure, but the drug severely damaged the intestines of the MyD88-deficient mice. The researchers found essentially the same results when they tested the effects of DSS on mice lacking the TLR proteins themselves.

"This was the exact opposite of what we expected, because everything we know about TLRs in mammalian systems has to do with immune response to infection," said Medzhitov. "We thought that since these mice couldn't mount a TLR-dependent inflammatory response to commensal bacteria, they would show reduced injury from DSS. We had no idea that these receptors might be involved in protecting these tissues from damage."

To explore whether the overgrowth of commensal bacteria contributed to the damage, the researchers depleted the bacteria from the mice's intestines using antibiotics. These antibiotic-treated MyD88-deficient animals showed the same DSS-induced damage as MyD88-deficient animals in which bacteria were allowed to grow normally. The researchers also found no evidence that the infiltration of white blood cells triggered the increased injury in MyD88-deficient mice.

The researchers observed a generalized disruption in proliferation and differentiation of intestinal epithelial cells in the MyD88-deficient mice, implicating TLRs in overall maintenance of the tissue. The animals' intestines were particularly sensitive to radiation, which could be attributed in part to the unusually rapid cell division occurring there.

Further, recovery from radiation-induced damage requires maintenance and repair processes that, the researchers found, depend on TLRs. They found that in response to DSS-induced damage, intestinal cells in the normal mice produced numerous proteins involved in tissue repair. In contrast, mice lacking a TLR response did not.

The researchers' experiments also demonstrated that commensal bacteria were necessary for TLR activity. When they used antibiotics to sterilize the colons of normal mice, they found that these mice were as susceptible to damage from DSS as MyD88-deficient mice.

Finally, when they treated bacteria-depleted normal mice with only the bacterial surface molecules by which TLRs recognize the pathogens, they found these molecules alone were enough to completely protect the mice from damage by DSS.

"These bacterial products completely rescued the commensal-depleted mice," said Medzhitov. "None of them even got sick, and when we analyzed their colons, they were healthy and normal. This was definitive proof that the beneficial effects of commensal bacteria were due to their recognition by TLRs and not their biological activity."

"Now we know that this recognition has a completely unexpected and novel function - triggering TLRs to control genes and processes that foster homeostasis of intestinal epithelium, which confer a protective effect on these cells and induce tissue repair." According to Medzhitov, TLRs may work both by a continual recognition of commensal

bacteria and by responding to damage to initiate a repair process.

Medzhitov said that his team's findings have two major clinical implications. First, he said, they will likely affect the clinical practice of treating cancer patients with antibiotics when they receive chemotherapy or radiation, which weaken the immune system.

"These antibiotics are intended quite correctly to protect patients against opportunistic infections. However, our work shows that such antibiotics can have the detrimental effect of preventing the natural and essential pathways that are important for protection and repair. Thus, it may be that such patients could be treated with a version of the bacterial cell surface products that would mimic the presence of commensal bacteria and maintain TLR activity," said Medzhitov.

Additionally, the findings may also influence the treatment of inflammatory bowel disease. "We know that bacteria play a detrimental role in these diseases, inducing a pathological inflammatory response. Often antibiotics are used in such cases as Crohn's disease, to eliminate gut bacteria. However, given our findings, the beneficial role of commensal bacteria might have to be taken into account in such treatments," said Medzhitov.

In further studies, Medzhitov and his colleagues are exploring whether the response of TLRs to bacteria plays a role in maintenance and repair of other epithelial tissues, including the skin, mouth, lungs and respiratory and urinary tracts. He said that similar TLR responses might play a role in the maintenance and repair of internal organs that are not exposed to bacteria, through a tissue response to endogenous triggering factors.