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Mouse study explains bacterium's unique role in periodontitis

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Scientists say they have solved in mice the mystery of how an unusual bacterium can trigger the common dental condition periodontitis while residing in low numbers in the space between tooth and gum.

The researchers report that the microbe Porphyromonas gingivalis hacks into the front-line immune cells that police the space between tooth and gum, known as the subgingival crevice, and reprograms them to create living conditions more to its microbial liking. As more immune cells are co-opted to follow the wrong program, the usually benign bacterial residents of the subgingival crevice — not P. gingivalis, as long suspected — opportunistically rise up in number, altering their community dynamics and prompting them to infect the tooth's supportive structures, or periodontium.

The study in the Nov. 17 issue of the journal *Cell Host and Microbe* was supported by the National Institute of Dental and Craniofacial Research (NIDCR), part of the National Institutes of Health.

"As the other bacteria attack, and immune cells counterattack, progressively damaging the integrity of the tooth in the process, P. gingivalis sits in the shadows and feasts on the inflammatory spoils," said George Hajishengallis, D.D.S., Ph.D., a researcher at the University of Louisville School of Dentistry and a co-lead author on the study. "This is one ingenious little bug."

According to Hajishengallis, their discovery marks the first documented

case in microbiology of a keystone species, or more specifically, a keystone pathogen. Coined by ecologists in the late 1960s, the keystone concept refers to a low-abundance species that exercises a disproportionate influence on its environment. In this case, low-abundance P. gingivalis shapes a microbial community and catalyzes the onset of an oral disease.

The next step is to test whether P. gingivalis follows a comparable evolutionary strategy in people. If so, they already have in their sights drugs that can be applied topically to the affected tooth to block this keystone pathogen from reprogramming the immune cells, providing a more targeted approach to prevent and/or treat periodontitis.

Periodontitis has afflicted humans from time immemorial, and today remains one of the most common causes of tooth loss worldwide. An estimated 53 million American adults have the condition, approximately 2 out of every 10 adults, at a total annual healthcare expenditure of more than $14 billion.

In the late 1970s, P. gingivalis became a subject of research interest when scientists noticed this rod-shaped anaerobe popping up consistently at low levels in studies that explored the microbiology of periodontitis. Further laboratory work showed that this late colonizer of the subgingival crevice arrives bearing an extensive arsenal of noxious chemicals. The assumption being, P. gingivalis employs them selectively to thwart the native, or innate, immune cells that patrol the gingival crevice and keep bacterial levels in balance.

But showing that P. gingivalis actually can cause periodontitis has remained scientifically tricky. The assumption has been that the bacterium could directly cause damage to the periodontium in animal models, independent of periodontitis and its natural disease process. Moreover, scientists have found themselves traversing new conceptual

territory to explain how such a low-abundance — some say stealth — pathogen could wreak such havoc in the mouth.

The research story has picked up in recent years as scientists have gained more technologically sophisticated tools to study P. gingivalis, and more biological information from which to form workable hypotheses.

A case in point is the progress in understanding how immune cells process incoming information to respond to the threat at hand. Studies show that some incoming defense signals converge within innate immune cells, a process called signaling crosstalk, which helps them to coordinate their early internal response to infection.

Last year, Hajishengallis and colleagues reported in mouse studies that P. gingivalis has learned through the millennia to exploit this crosstalk. The bacterium does so by jointly activating convergent defense signals involving the Toll-like receptor 2 (TLR-2), which helps to recognize pathogens, and the C5a receptor that is a part of the complement system that will respond to the threat.

"P. gingivalis exploits both receptors to induce a signal with an emergent property," explained Hajishengallis. "In other words, the property doesn't emerge from manipulating just one receptor. Both must be punched."

Hajishengallis said the emergent property impairs the affected immune cell from killing P. gingivalis. It also seems to initiate low-level inflammation that serves no immunological purpose other than possibly to generate nutrients for P. gingivalis.

This discovery pointed to an evasion mechanism that P. gingivalis employs to subvert innate immunity. Left unanswered was the larger question of whether this stealth mechanism also played a role in causing periodontitis.

To get their answer, the scientists revisited two unexplained laboratory observations. First, after mice are inoculated with P. gingivalis and develop advanced periodontitis, the levels of normally benign , or commensal, bacteria increase in the affected periodontium. Secondly, P. gingivalis is extremely difficult to detect at the site of the disease. These observations and last year's finding on signaling crosstalk suggested that P. gingivalis might compromise the immune system, but the commensals infect the periodontium.

In the current paper, that's exactly what they found. The scientists report that mice bred to produce immune cells without C5a receptors — meaning P. gingivalis can't co-opt their innate immunity — did not develop periodontitis after being inoculated with the bacterium. Commensal levels also remained within the range typically seen in the subgingival space.

In healthy mice kept under conventional laboratory conditions, the opposite was true when inoculated with P. gingivalis. The mice developed active periodontitis and its characteristic damage of the periodontium. They also had increased commensal levels and hardly detectable P. gingivalis.

"In contrast, mice bred to be germ-free were resistant to periodontitis after they were colonized by P. gingivalis," noted Michael Curtis, Ph.D., a co-lead author on the study and a scientist at Queen Mary University in London. "This was especially telling because these mice lacked the normal commensal bacteria in their mouths. In other words, in these sterile animals, P. gingivalis had no bacterial accomplices capable of causing periodontitis."

"There is one key word that explains everything: homeostasis," said Richard Darveau, M.S., Ph.D., a scientist at the University of Washington in Seattle and another co-lead author on the paper.

"Periodontal health is a matter of maintaining homeostasis, or equilibrium, among the bacterial inhabitants of the subgingival space and innate immunity cells. P. gingivalis is a master at disrupting homeostasis. Although this bacterium certainly is not the only cause of periodontitis, it stands out as a major culprit."

The scientists say their findings have encouraging therapeutic implications, in part because the microbial conditions in mice likely will be comparable in humans. They point to studies in monkeys that indicate the comparability.

They also note their findings provide a molecular target with tremendous specificity. "To counteract P. gingivalis and the negative outcomes that it orchestrates in patients, we will need most likely to target one of the signaling receptors," said John Lambris, Ph.D., an author on the paper and the Dr. Ralph and Sallie Weaver Professor of Research Medicine at the University of Pennsylvania. "P. gingivalis needs to manipulate both to work its tricks."

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