New Target for Periodontal Disease

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In a paper published on March 25 in *Nature Immunology* [doi:10.1038/ni.2260], a team of researchers reports on a potentially high-value new target in the fight against periodontal disease. The new lead is a glycoprotein called Del-1 that has the unique ability in animal studies to negatively regulate, or turn down, the inflammatory immune response, the body's first line of defense. In the current *Nature Immunology* paper, NIDCR grantees and colleagues show for the first time in the gingiva that a breakdown in Del-1's normal regulatory control of the immune-signaling protein IL-17 can drive the onset of periodontitis, particularly in aging. Based on this finding, the authors propose that periodontitis may be best characterized as a disruption of homeostasis, which then allows infectious and inflammatory conditions to proceed on their destructive paths. The Science Spotlight recently spoke about the paper and its implications with senior author George Hajishengallis, D.D.S., Ph.D., an immunologist at the University of Pennsylvania School of Dental Medicine. Here's what he had to say.

Let's start by defining a term. What is a neutrophil?

A neutrophil is a ubiquitous white blood cell, or leukocyte. In fact, the neutrophil is the most abundant white blood cell in the body and a major player in innate immunity. By innate, I'm referring to our inherited, or non-specific, immune system.

The body's first line of defense against infection.

Exactly. Neutrophils are present in the blood stream, patrolling for biochemical clues of injury or infection. When they detect trouble, neutrophils exit the blood stream and migrate to the problem site. Upon arrival, they display a docking protein, called an integrin, which briefly tethers them to the appropriate protein receptors displayed on the cell surface.

And the tether isn't permanent. It is as flexible as Velcro, correct?

That's right, the bonds are easily broken and reformed. This is important because neutrophils, like parachutists hitting the ground, tumble to a full stop and then transmigrate, or adhere to the cell surface, squeeze through the extracellular space, and attack the invading bacteria. This multi-step process is called the leukocyte adhesion cascade.

Your paper builds on a seminal discovery from 2008 involving the regulation of the leukocyte

adhesion cascade and a protein called Del-1. What was the previous discovery?

Let me turn the clock back a little further to set the stage. In 1998, researchers discovered the Del-1 gene while performing genetic studies in mice. The acronym is short for developmental endothelial locus-1. This three-part name implied that during development Del-1 promotes the adhesion of endothelial cells, which line the walls of our blood vessels. The suggestion was Del-1 has a regulatory role in endothelial differentiation and thus the formation of new blood vessels, a process called angiogenesis.

That's where the Del-1 literature focused for several years. But in 2008, Dr. Triantafyllos Chavakis, then at NIH's National Cancer Institute, and colleagues found Del-1 has a role in innate immunity. They showed that endothelial cells express Del-1 to inhibit the neutrophil's integrin proteins from transmigrating and mounting an inflammatory response.

And this was a first?

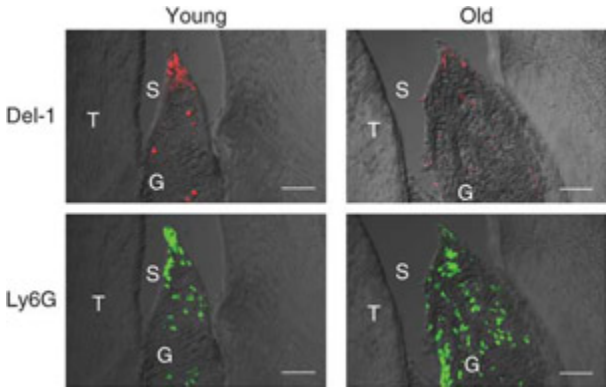
Yes. Del-1 was the first endogenous *negative* regulator of the leukocyte adhesion cascade. In other words, the finding showed endothelial cells have a regulatory say in stopping the immune response. This was completely unknown. Up to that point, it was assumed that leukocytes called the shots and the endothelial cells passively went along with the program.

How does your paper take the Del-1 story in a new direction?

Well, in collaboration with Dr. Chavakis, we show that changes in Del-1 expression, most notably as a part of the aging process, can cause periodontitis. This is the first report to link Del-1 to a chronic inflammatory disease. Although the work was performed in mice, our initial data suggest our findings certainly will apply to humans. If correct, I think this work has great preventive and therapeutic potential for chronic periodontitis, particularly for seniors. It's been so rewarding to watch the data fall into place. That's not often the case in science.

Well, how did you become interested in Del-1?

It was a combination of chance and luck. I had an NIH grant to study aging and periodontitis, and the lab was busy looking in a mouse model for genes whose expression changes with age. The funny thing was the old mice – our controls that hadn't been treated – naturally developed periodontal disease just like people (with bone loss). That got us thinking that these mice might serve as a good model to study the impact of aging on innate immunity in periodontal disease. We compared the young and the old mice to see which genes were up- and down-regulated. In truth, we were just fishing for a lead. But we got what amounted to an



Lower expression of Del-1 in old mice is correlated with periodontal bone loss.

interesting catch. In the old mice, we noticed the expression of Del-1 was greatly downregulated.

Was the Del-1 protein downregulated specifically in the gingiva?

Yes, both at the gene and protein levels. We also saw that there was an inverse correlation among the mice. By that, I mean the old mice had less Del-1 but more bone loss and inflammation than the younger ones. Well, I had never heard of Del-1 up to that point. I did a literature search and read about Del-1 and its reported role in angiogenesis. I thought we're not going to delve into angiogenesis. What possibly could be the relationship? This was early 2008.

And that's when luck entered the equation?

Precisely. In the summer of 2008, I went to Greece to attend an international meeting on innate immunity. And one of the speakers was the aforementioned Dr. Chavakis. He presented on Del-1 as an inhibitor of the leukocyte adhesion cascade. He showed that Del-1 specifically inhibits a protein called LFA-1 that neutrophils rely on to transmigrate and cause inflammation.

And you're thinking?

Oh my goodness, our gene of interest *does* play a role in innate immunity. So I talked to Dr. Chavakis afterwards and asked, "Has this work been published?" He said it was under review at the journal *Science*. I told him about my work, and we started to collaborate in late 2008. Four years later, we've cobbled together the complete story in periodontal disease. It's yet another example of how an oral disease can serve as an invaluable model to study the immune system.

The cobbling involved different types of knockout mice. What was your strategy?

Well, we first wanted to confirm that Del-1 was relevant to the periodontal system. Dr. Chavakis sent us Del-1 knockout mice, meaning they lacked both copies of the Del-1 gene, and we took a detailed look. Sure enough, we saw that even the young knockout mice had spontaneous periodontitis. We didn't need to induce anything experimentally to produce the disease. That told us that Del-1 was very important for the homeostasis of the periodontal tissue.

What was the next investigative step?

We wanted to define the biochemical mechanism that underlies the disease. The obvious place to start was to ask whether Del-1, when present, regulates LFA-1. That's the mechanism that was described in the *Science* paper.

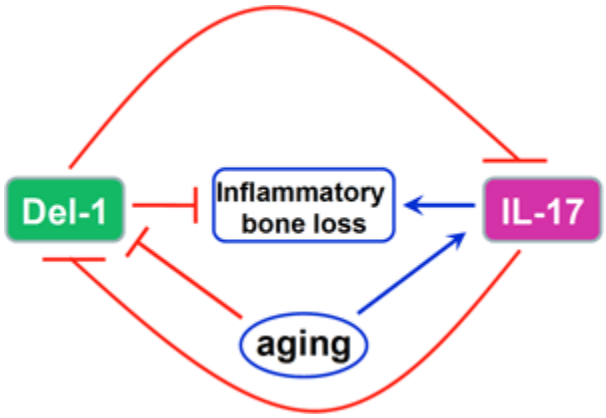
In other words, does the loss of Del-1 allow LFA-1 to run wild, induce excessive inflammation of the gingiva, and trigger spontaneous periodontitis. If not, you would need to continue looking for another still-undefined Del-1 interaction to define the mechanism.

That's right. So we tested double knockout mice that lacked both Del-1 and LFA-1. The idea being,

with both genes gone, the mice would lack the biochemical button that prompts the disease. They should be periodontitis free. And Bingo. That's exactly what we saw. But we wanted to go further than that.

What do you mean?

We saw previously in periodontal tissue that some inflammatory molecules are upregulated in Del-1 deficiency. One stood out like a sore thumb. That was IL-17. It is a signaling molecule that naturally sets in motion the recruitment of neutrophils to a trouble spot. No matter how many times we looked at Del-1 knockouts or their age, IL-17 was always highly upregulated.



Schematic of inverse correlation between Del-1 and IL-17 in aging

Was the upregulation already recognized in periodontitis?

It had been observed. But until now, no causal link had been made either in human or mouse. What made the upregulation of IL-17 potentially interesting is this molecule is a double-edged sword. IL-17 protects us from infection. But it can make life miserable with inflammation. Sound familiar? Periodontal disease is both an infection and an inflammation. That left us wondering whether IL-17 is elevated to combat the infection. Or, whether is it a part of the inflammatory process. To get the answer, we took IL-17 knockout mice and bred them with the Del-1 knockout mice. That created litters of IL-17/Del-1 double knockouts.

The idea being, with no IL-17 or Del-1 in play, the mice should be protected from periodontal disease. Were they?

Completely. The previous LFA1/Del-1 knockouts were relatively protected. The double knockouts were completely protected. There was no periodontal disease to be found. That convinced us that IL-17 was behind the destruction of the gingival tissue. Once we knew this, we headed off into the molecular work and showed that Del-1 and IL-17 are cross-regulated.

What do you mean?

Del-1 inhibits IL-17. In fact, they have a seesaw type of relationship. One goes up; the other goes down. It made perfect sense.

How so?

IL-17 is the master orchestrator of neutophil recruitment. This is well described in the literature. But in our paper, we show for the first time that IL-17 not only orchestrates the recruitment of neutrophils via the various known mechanisms, it also downregulates Del-1. The idea being, when neutrophils

arrive, say, in the gingiva, they encounter no obstruction to their entry into the tissue. If you think about it, this is a good thing. For an acute infection, you want to mobilize neutrophils to kill the bacteria. In a chronic condition, this becomes a problem if the endothelial cells can't control the inflammation to maintain homeostasis.

So homeostasis is really the issue?

That's where my thoughts are right now. But this idea isn't necessarily brand new. A couple of years ago in *Nature Reviews Microbiology*, Dr. Richard Darveau, a colleague at the University of Washington, suggested that periodontal disease is not fundamentally an inflammatory disease. He called it a disruption of homeostasis. The disruption can set in motion a domino effect of infection, inflammation, and tissue destruction. In other words, once homeostasis is disrupted, all that follows is a symptom of that fundamental problem.

What about the distinction between mouse and man. Why are you confident that your mouse data will replicate in people with periodontitis?

Because we have some human data, too, and they're lining up well with our mouse findings. One, we've established that human Del-1 inhibits the transmigration of human neutrophils through a monolayer of human endothelial cells. Thus, human Del-1 has the same function as its murine counterpart. Two, we have shown in human endothelial cells that IL-17 inhibits Del-1 expression. That's exactly what we found in mice. Three, we compared inflamed and healthy tissue from periodontal patients, post surgery. In the inflamed tissue, we found that the expression of IL-17 was highly elevated, while the Del-1 level was very low. In the healthy tissue, it was exactly the opposite. This was the same inverse relationship that we saw in mice. Four, the human and mouse Del-1 have 96 percent homology, or structural similarity. They are essentially the same molecules.

I should add that other human tissues express Del-1. The eyes and the brain produce a lot of it. The lungs also express Del-1, and the gut produces a small amount. Interestingly, the liver and spleen don't express it. So Del-1 is not ubiquitous in the body. Neither is it a periodontal phenomenon. Changes in Del-1 levels may affect other body systems, and research is under way to determine if that's the case.

Let's go back to where you started: the aging process. How do your findings apply to aging and the increased risk for periodontal disease?

My hunch is that a major reason that the elderly are more prone to periodontal inflammation is Del-1 expression decreases with age. In other words, with lower levels of Del-1, older people probably have more difficulty regulating the trafficking of the neutrophils that reach the periodontal tissues. They end up with many more neutrophils than needed, and that triggers destructive, not protective, processes.

Assuming that's correct, how can it be treated?

An obvious place to start would be to apply Del-1 or an active derivative topically as an ingredient in

toothpaste or a mouthrinse. The good news is Del-1 is endogenous to the body. So, there should be no question about its safety. The bad news is Del-1 is a relatively large molecule. That could make it a little unwieldy biochemically to apply in everyday dental products. But the case is far from closed. We could get lucky and discover that the activity of Del-1 is confined to a small part of the molecule. If so, we could make just this small segment recombinantly in the laboratory.

Isolate the active site?

Yes. But right now, I have my doubts that this will be the case. I've seen the structure of Del-1. It has two large domains and several subdomains. My gut feeling is there will be no single active site. The entire molecule may be needed for an additive or synergistic effect. So the sum of the whole is greater than any one individual part. But, hopefully, I may be wrong. This is something we plan to find out in the near future.

Where do you go next to push this project in the direction of the clinic?

I'd like to work with periodontists. We need to make sure that there is good relevance between our mouse findings and the human findings. As just mentioned, we will try to see if we can identify a part of the molecule that is protective against experimental periodontitis. If that's the case, and we are able to produce even higher amounts, then I would like to go into the clinic to do experimental gingivitis to see whether Del-1 can protect against that. If that works, I think the stage is set to go into clinical trials.

Good luck with the research, and thanks for telling us about Del-1.

Glad to do it.