Organoids mimicking celiac disease show new link between gluten and intestinal damage

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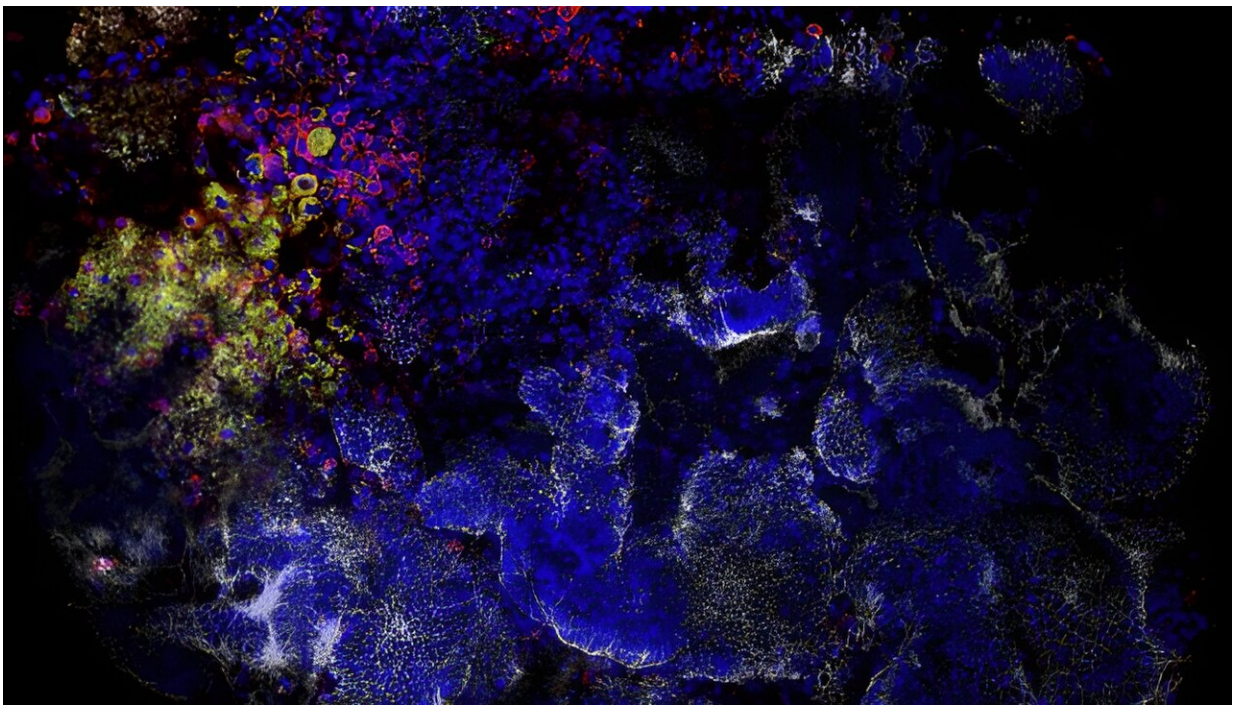

A microscopic image of an intestinal organoid in which the nuclei of the cells are blue, epithelial cells are white, T cells are green and other immune cells are red. Credit: Antonio Santos

Small, laboratory-grown balls of cells made from the intestinal tissue of people with celiac disease have revealed a previously unknown

molecular link between gluten exposure and intestinal damage, according to a study by researchers at Stanford Medicine.

The molecule, called IL-7, has been implicated in other autoimmune diseases including rheumatoid arthritis and multiple sclerosis, but it has never been linked to celiac disease.

The study is the first to describe the use of clumps of cells maintained in a laboratory dish, called organoids, to study autoimmune disease. Unlike previous attempts to model celiac disease in the laboratory, the intestinal organoids grown by the researchers include multiple cell types, including immune cells and the cells that make up the lining of the gut.

The organoids open the door to understanding how different cell types interact in people with the disorder, characterized by an acute sensitivity to gluten, in ways that haven't previously been possible. For example, the researchers found that levels of IL-7 were elevated in tissues from people with active celiac disease and that blocking its activity eliminated the immune reaction to gluten that damages the intestinal lining.

"These organoids provide an accurate reproduction of what happens in people with celiac disease," said Calvin Kuo, MD, Ph.D., professor of medicine and holder of the Maureen Lyles D'Ambrogio Professorship. "If we add gluten to organoids made from intestinal biopsies of people with celiac disease, we see immune activation and killing of the epithelial tissue just like what occurs in patients.

"But now we can probe what's happening in a dish—learning about the disease, developing new drugs and maybe even predicting what drugs will work best for individuals. This has not been possible before."

Kuo is the senior author of the study, which was published on July 24 in *Nature*. Postdoctoral scholar António Santos, Ph.D., is the lead author of

the work.

Prior to this study, there were few ways to directly research celiac disease: One was to use intact intestinal tissue that would die within a day or two of biopsy, or to study rafts of intestinal epithelial cells from patients that didn't contain the many other types of cells, including the immune cells that mediate the disease. Models of celiac disease in laboratory animals like mice don't fully reflect aspects of the disease in humans.

After years of trial, Santos hit on a way to keep biopsied intestinal tissue alive in the lab by suspending them between an air-liquid interface. Bobbing gently on the surface allowed the many cell types—including epithelial cells, immune cells and others—to clump together in balls about one-quarter inch in diameter and grow in the laboratory for weeks or months.

"This is really a game changer for the study of celiac disease," Santos said. "It is a human organoid that contains all of the cells of the small intestine and thrives in a laboratory dish. The only thing close to it would be to experiment directly on people."

About 1% of people worldwide are affected by celiac disease, an autoimmune disorder in which exposure to gluten—a protein found in wheat, barley and rye—triggers the immune system to attack and destroy the mucosal lining of the small intestine.

Common symptoms include bloating, chronic diarrhea, constipation, nausea and gas, but the disease can also occur without intestinal symptoms, which can make diagnosis and monitoring tricky. The only current effective treatment is to eat a strictly gluten-free diet for life.

"Celiac disease is kind of a chameleon," said professor of medicine

Nielsen Fernandez-Becker, MD, Ph.D. Fernandez-Becker directs the Celiac Disease Program at Stanford Health Care. "There are many things we don't know. In particular, while we are pretty good at diagnosing the disease, we don't have a great way of monitoring people.

"Biopsies are invasive, and not always helpful. And often people come to us already on a gluten-free diet, which makes diagnosis difficult and may mean we have to ask them to eat gluten to figure out if they really have celiac disease."

After obtaining informed consent, Fernandez-Becker collected and shared intestinal tissue biopsies from 81 people with celiac disease and 54 people without celiac disease. Of the 81 people with celiac disease, 59 were actively experiencing symptoms, while the remaining 22 had successfully reached remission after sticking to a gluten-free diet.

Santos then exposed organoids made from the biopsied tissue to a component of gluten known to trigger the disorder. Those made from intestinal tissue from people with celiac disease responded dramatically.

Epithelial cells that make up the lining of the gut ramped up their production of a molecule called IL-15 associated with the disorder, the number of immune cells called CD4 T cells that specifically recognize gluten began to increase and other immune cells called B-cells churned out antibodies to a protein called type 2 transglutaminase, or TG2.

TG2 does not normally induce an immune response, and the presence of these autoantibodies is a key hallmark of celiac disease often used to diagnose the disorder. Finally, immune cells called CD8 T cells began to attack and kill the epithelial cells—likely at the behest of their CD4 counterparts.

"The organoids from people with celiac disease were clearly responding

in ways that organoids from healthy people were not," Santos said. "We were able to see many of the key features of the disease, from epithelial cell kill to immune activation and depletion of other cells that make up the villi, or protrusions of the intestinal lining."

Previous studies of celiac disease had implicated two signaling molecules called cytokines. The molecules—IL-2 and IL-15—mediate the signals that CD4 (the immune cells that recognize gluten) and CD8 (the immune cells that kill other cells) T cells use to communicate with one another. But blocking the activity of IL-2 or IL-15 hasn't halted gluten-induced intestinal injury in clinical trials, although attempts are continuing.

Kuo and Santos instead looked at another member of the same cytokine family—IL-7. They found that blocking IL-7 activity in the celiac disease organoids also stopped epithelial cell destruction in response to gluten. Conversely, adding IL-7 strongly induced epithelial cell death in organoids from celiac patients even in the absence of gluten, while organoids from healthy controls were unaffected.

Finally, the researchers showed that IL-7 expression not only increased when gluten was added to celiac disease organoids, but also that IL-7 levels were elevated in biopsies from people with active celiac disease but not in those whose disease was in remission because of a gluten-free diet.

"These results show that IL-7 is a previously unrecognized modulator of celiac disease," Kuo said. "Although more research needs to be done, we believe that the organoids faithfully reflect what is happening in people with celiac disease and that there may be therapeutic opportunities for blocking IL-7 activity."

"None of this could have been done without the patients who agreed to provide biopsy samples, and they did this selflessly, knowing there was

likely no benefit to them," Fernandez-Becker said. "We are incredibly grateful to them."

"We've been working on organoids for nearly two decades," Kuo said. "We've used them to model cancer and infectious disease. Now we have complex systems with immune cells, and we believe this study is just the tip of the iceberg for using organoids to research many autoimmune disorders."

More information: António J. M. Santos et al, A human autoimmune organoid model reveals IL-7 function in coeliac disease, *Nature* (2024). DOI: 10.1038/s41586-024-07716-2

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