

BROKEN

BY BREAD

A VIRUS THAT TURNS GLUTEN INTO KRYPTONITE

BY SHARON TREGASKIS

When you have celiac disease, your immune system goes berserk in the presence of gluten, the protein that gives bread its stretch. The resulting blitzkrieg turns the villi—the microscopic, finger-like protrusions within the small intestine that absorb nutrients and fluids—into the targets of a search-and-destroy mission.

You can't feel anything as the villi flatten and atrophy, but the resulting dysfunction—gas, bloating, diarrhea, constipation—takes its toll. And that's just for starters. As the damage spreads, the symptoms intensify: malnutrition, behavioral and mental health problems, joint and muscle issues, reproductive dysfunction, even increased risk for some forms of cancer.

There is no cure. But for many people with celiac, in the absence of gluten the attacks subside, and eventually the villi can heal—like skin slowly repairing itself after a burn. Yet probably less than 20 percent of people with celiac get an accurate diagnosis.

As for eliminating gluten, that's no easy task with this darling of the food-processing industry. Some things are obvious—bread products and anything that contains wheat or a half-dozen related grains. But gluten also lurks in the shadows—in condiments, spice mixes, thickeners, even the coating that gives french fries their crunch. When you have celiac, eating away from home turns into an exercise in Russian roulette, and reading ingredient lists becomes a matter of survival.

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Approximately one in 130 people worldwide will develop celiac over the course of their lifetimes. "That sounds like long odds," says the University of Pittsburgh's Vira I. Heinz Professor of Pediatrics Terence Dermody, an MD, "but it's not." These days, everyone knows someone who has it.

The puzzling thing, says Dermody, who is chair of pediatrics, is the disparity between the prevalence of the gene variants that confer the risk of developing celiac and the relative rarity of the disease itself.



JOSHUA FRANZOS

Terence Dermody

Between 40 and 50 percent of us have the gene variants for celiac. They're ticking time bombs. Even as we happily make like Eric Carle's very hungry caterpillar—swilling beer and blissfully, ignorantly noshing our way through the Super Bowl buffet—the switch is poised to flip, ready to turn a benign foodstuff into kryptonite.

"So what gives?" says Dermody. "Why isn't celiac more common?"

In April 2017, *Science* published results of a series of experiments by Dermody and colleagues at the University of Chicago that begin to offer an answer: a combination of wrong-time-wrong-place factors that converge around reovirus, a bug so innocuous that most of us have had it and never even noticed. Even its name—an acronym conferred in 1959 by live-oral polio vaccine developer Albert Sabin—evokes its presumptively benign role in human health. Isolated from the respiratory (r) and enteric (e) tracts, or intestines, of human patients, it was an orphan (o) to which scientists at the time couldn't tie a single human disease.

Board certified in infectious disease, Dermody has spent more than three decades studying reoviruses; he is becoming one of the world's leading experts in the field. Reovirus is the fruit fly of virology—ubiquitous, inexpensive to maintain in a laboratory setting, and possessed of a relatively simple genome. That combination makes this virus family the perfect model for basic virology. In the 60 years since reoviruses were first isolated, scientists have built an extensive body of knowledge describing and manipulating their RNA, developing corresponding mouse models, and digging into just how viruses leverage host biology to perpetuate their own genomes. And reoviruses are pretty much harmless.

"I can look in the eyes of the mothers of my students and postdocs and tell them the virus isn't going to hurt them, and they've already been infected," says Dermody. He has supervised more than 90 aspiring physicians and scientists in his laboratories at Harvard, Vanderbilt, and, since January 2016, the University of Pittsburgh.

Although human exposure to reovirus doesn't cause any symptoms of disease or leave a trail of damage in its wake, the virus can't just skate past the immune system. At some point, probably early in life, reovirus makes its way through our guts, and our adaptive immune system generates antibodies that stay with us for life. That's how we know most of us have been infected.

Like a kid learning to read by sounding out new words, the adaptive immune system—housed, among other places, within the lining of our small intestine—learns by exposure, screening for the molecules, known as antigens, that our white blood cells use to distinguish proteins and assess threats.

"We eat really complex things every day," says Dermody, who is also physician-in-chief and scientific director at Children's Hospital of Pittsburgh of UPMC.

"If salmonella comes in, our immune system sees it as a foreign invader, and tries to clear the infection. But that doesn't happen when you eat a potato, or yogurt, or chicken."

That's because the adaptive immune system treats those antigens with a delicate balance of vigilance and tolerance. We encounter novelty throughout our life spans, and we live in a soup of hazards—aerosolized flu particles in the air at work, trace *E. coli* on a piece of spinach, a veritable zoo of microbes in a home-brewed bottle of kombucha.

So instead of launching a reaction in response to every unfamiliar antigen we encounter, the adaptive immune system preserves homeostasis, tolerating commensal microbes and the vast majority of foods we ingest, while mounting a defense against pathogenic invaders. Like the cordoned off entrances at an exclusive Manhattan nightclub, the lining of the intestine has its own protocol for deciding who's waved in, who gets stuck in the queue, and who is sent back to Jersey.

The gut actually sorts out what's coming at it with dedicated virus-detection centers known as Peyer's patches, as well as screening stations where food antigens are processed (the mesenteric lymph nodes and the lamina propria).

That compartmentalized approach "reduces the risk of responding to food antigens in the context of pathogens," says immunologist Bana Jabri. Jabri is an MD/PhD, director of research for the University of Chicago Celiac Disease Center, and Dermody's collaborator and the senior author on the *Science* paper.

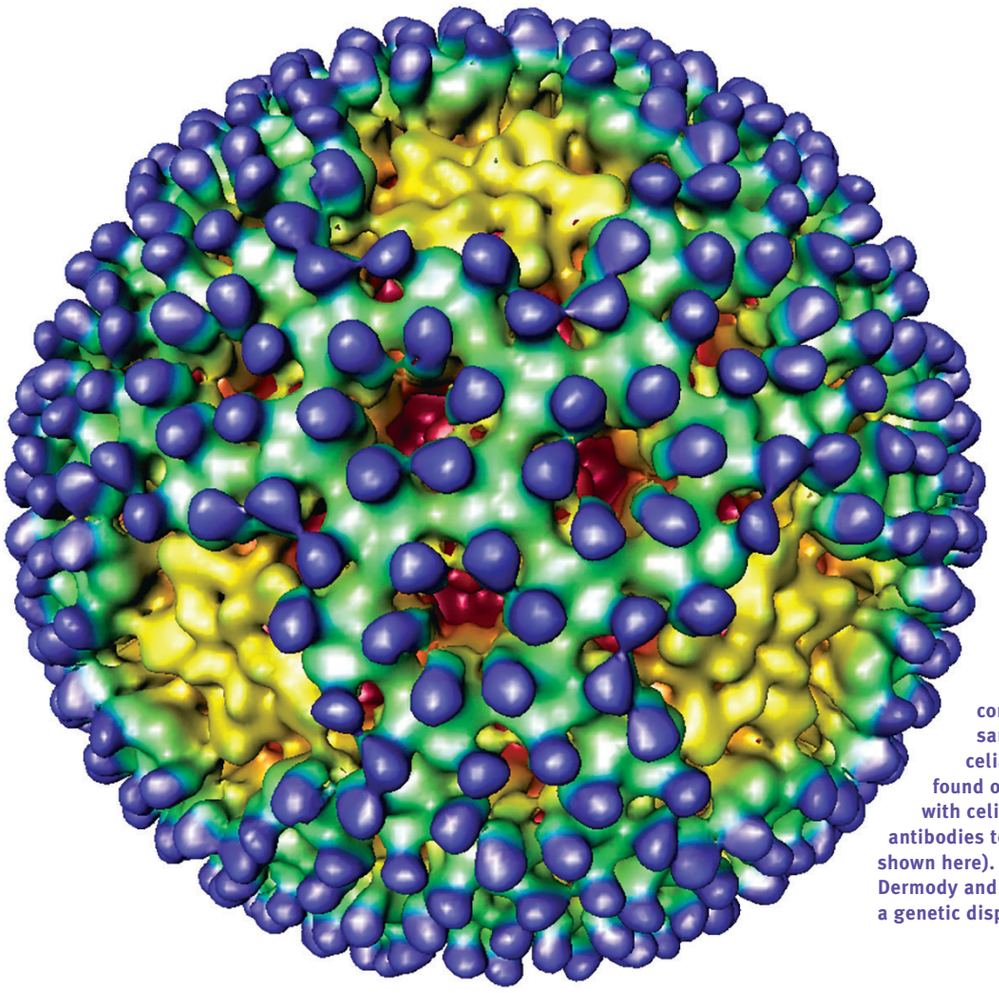
Still somehow, with celiac, the body forgets that gluten has a VIP pass to the intestinal club. And the whole system goes haywire, causing a debilitating inflammatory response.

Scientists have long suspected that viral infection might be to blame.

Yet, "it's really difficult to establish cause and effect, the mechanisms that underlie how viruses could trigger celiac or autoimmunity," says Jabri. "That means it's really important to have a good model system, like the mouse."

And a virus. When Dermody gave an invited lecture at the University of Chicago in 2010, his host, who knew of Jabri's interest in viral triggers, introduced her to Dermody. At the time, he was working with UT Southwestern collaborators to put the

JOURNAL OF VIROLOGY, JULY 2001, 75:14, 6625-6634. "A MONOCLONAL ANTIBODY SPECIFIC FOR REOVIRUS OUTER-CAPSID PROTEIN σ3 INHIBITS σ1-MEDIATED HEMAGGLUTINATION BY STERIC HINDRANCE" EMMA L. NASON, ET AL. REPRODUCED WITH PERMISSION FROM AMERICAN SOCIETY FOR MICROBIOLOGY.



When researchers combed through blood samples of people with celiac against controls, they found one thing linking people with celiac: higher levels of antibodies to reovirus (virus model shown here). This confirmed what Dermody and Jabri found in mice with a genetic disposition to celiac.

finishing touches on a manuscript for *Science* about how viruses exploit the bacteria within the intestinal microbiome to speed their own replication and transmission. Previously, he'd worked with a team detailing the biochemical choreography of reovirus infection and immunity within Peyer's patches and identified host receptors that reoviruses leverage for their own gain. "One of the key elements [virologists] are studying is what's required in order to defend ourselves against a virus and get rid of it," says Jabri. "As an immunologist working on autoimmune disorders, I want to know why the immune system starts recognizing self and other antigens as dangerous."

Together with funding from the National Institutes of Health—a $3.2 million, four-year grant awarded in 2014—they got down to business. Their first task was for Dermody's group to create two genetically engineered reoviruses that would each infect the mouse intestine and precipitate viral antibody production, without causing physical symptoms or tissue damage. Strains labeled T3D-RV and T1L fit the bill. Although T3D-RV-infected mice developed lower reovirus antibody levels than those exposed to T1L, the two strains had a similar effect on Peyer's patches—security was summoned (i.e., antigens appeared), but little else happened. That's what the researchers expected.

Next, Dermody and Jabri turned their attention to food antigens, looking for differences in immune function among mice infected with each strain. Scientists have long used egg protein as a way to study food tolerance and allergies in mice, so the team fed its T3D-RV- and T1L-infected mice the egg protein ovalbumin. Those exposed to T3D-RV were unaffected. But in the T1L-infected mice, immune system signals got crossed, and they promptly developed an egg allergy.

"What really made this a good science day was that we had two closely related strains of reovirus," says Dermody, "one that blocked tolerance and the other that didn't. We could investigate the underlying immune response to food as a dangerous substance, as opposed to an innocuous substance."

In their third set of experiments, the team subbed in a line of mice engineered with a genetic predisposition to celiac and infected them with one of the two reoviruses. Instead of the egg protein, they fed the mice a big dose of gluten. Again, the T3D-RV mice sailed through. The mice infected with T1L experienced an inflammatory response that looked a lot like celiac. Somehow the mouse's food and virus recognition complex had been turned around. Gluten not only fell off the A list—it was suddenly seen, not as food, but as a dangerous viral pathogen. A gut-wrenching case of mistaken identity.

Dermody and Jabri want to explicate the pathways by which T1L triggers intolerance of food antigens.

First, however, they tackled a question too intriguing to ignore: Do *people* with celiac have heightened antibodies to particular viruses?

Jabri and a colleague, University of Chicago gastroenterologist Carol Semrad, had blood samples from patients with celiac, as well as gender- and age-matched controls with and without the celiac genes. The researchers combed through all of the samples measuring titer—the level of antibodies to a given antigen—for reovirus, rotavirus, herpes simplex, and even tetanus. (Basically everyone these days gets a tetanus vaccination, so tetanus served as an extra control to reveal whether folks with celiac just exhibit amplified anti-

body levels across the board.)

People with celiac stood out in only one regard—they had higher reovirus antibody levels than those without a diagnosis, and they were significantly overrepresented among those with very high reovirus titers. Otherwise, their titers were unremarkable. Apparently, certain forms of reovirus can profoundly confuse the biochemical signaling within the part of our adaptive immune system dedicated to food antigens.

The work is far from complete.

"We understand, immunologically, the switch that is thrown that leads food to be tolerated or not," Dermody says, "and we have a clue about reovirus and celiac." But more investigation is required to understand timing and causal elements of the relationship.

Somehow, with celiac, the body forgets that gluten has a VIP pass to the intestinal club.

Dermody and Jabri have studies under way to test whether a reovirus vaccination can keep mice with the celiac gene from developing the disease. If it works, they'll have made the case that for people at high risk, a vaccine might prevent full-blown celiac. They're also designing a prospective study to follow children with a genetic predisposition, monitoring their viral antibodies, weaning, and solid food introduction for better clues about when and how celiac manifests. The work also has the potential to reveal other viruses that can derange the same delicate signaling pathways reovirus affects. Meanwhile, both Dermody and Jabri have a host of questions about the role of the microbiome, as well as the effect of the timing of a viral infection on subsequent immunity dysfunction.

Michele Dorfsman, an MD associate professor of emergency medicine at Pitt, and her children were diagnosed with celiac in 2015, when her youngest was 9 and the oldest was 14. Various digestive issues had plagued the four for as long as they could remember. Then Maria, the youngest, developed severe tummy aches and anxiety. She started seeing a therapist, and her pediatrician treated her for constipation. Her symptoms escalated, however, and she was finally admitted to the hospital. As part of her inpatient bloodwork, the lab checked for gluten antibodies. A biopsy of her villi confirmed celiac. "Now that I quit eating gluten," says the sixth grader, "I feel amazing. I feel really happy, not sad anymore."

The transformation was incredible, says Dorfsman. "Maria jokes that she had to go to a therapist because of her celiac."

At the time, however, it was no joking matter. Celiac genes run in families, so Dorfsman and the older children were screened, as well. The elder, Elena, had a positive biopsy though her endoscopy looked normal. Fourteen-year-old Oscar, however, had ulcers. The severity of his presentation also helped to explain a mystery that had confounded the family since his infancy. "When Oscar was 1, he weighed 22 pounds," says Dorfsman, who notes that her son hovered in the third percentile for height and weight throughout his childhood. "When he was 2, he weighed 20 pounds." A failure-to-thrive workup at the time yielded no insight.

Then the family eliminated gluten. Oscar grew four inches in two months, and Dorfsman, who is the program director for Pitt's emergency medicine residency, discovered that her very long list of food intolerances evaporated. The renewed ability to enjoy avocado, broccoli, cantaloupe, cauliflower, pineapple, and zucchini has more than made up for the pizza she foregoes at resident events.

"My GI tract was just so messed up that I was reacting to a lot of different things," she says.

Dorfsman's family says the boost in overall health has been well worth the hassles of going gluten free.

That said, each would happily have opted for a vaccine, if one had been available, to preserve their gluten tolerance.

"Couscous," says Maria.

"Chicken nuggets," adds Elena.

"Pizza and hamburgers with real buns," says Oscar. "Sourdough bread. Baguettes. Bread bowls."

As passionate as Terry Dermody is about exploring reovirus, he's equally intent on training aspiring scientists. With ever increasing numbers of his trainees launching their own labs—often leveraging the projects they started under his tutelage—the field has been getting crowded. "I wanted my postdocs to be able to take their reovirus scholarship and use it as the foundation for their own independent careers," he says. "I wanted to be out of their way." So even as the work with Jabri continues, Dermody is increasingly focused on chikungunya, a mosquito-borne virus common in Africa, Asia, and the Indian subcontinent.

Most people clear chikungunya. But a select few develop severe, lifelong arthritis. Understanding how a blood-borne virus makes its way to the joints could offer insight about how viral invaders circumvent human host barriers to affect a particular system or organ, whether the liver (as in hepatitis), the brain (encephalitis), or the joints (chikungunya). "What's the beacon calling the virus to that spot?" Dermody wonders. "And once it lands, how does it initiate an infectious cycle and destroy the cell?"

A rewarding career, Dermody frequently tells students, is at the intersection of three circles inscribing personal talents, personal passions, and things the world needs. Find the space where those three overlap, he says, and you can change the world.

Yet for Dermody, who leads a major Pitt department as well as both the clinical and research programs at Children's, the idea that his basic inquiries into the biology of an innocuous virus could actually reduce human suffering in his own lifetime is still a thrill: "I couldn't have imagined in the spring of 2010 that we would ever get to the point where we might alleviate a condition that is prevalent in one in 130 Americans.

"To be able to help in that way, with our very basic research studies, learn new things about pathogenesis and preventatives, is pretty gratifying." ■