[MUSIC PLAYING]

TERENCE S DERMODY: Good morning, everyone, and Happy New Year. I hope everyone had a wonderful holiday and was able to enjoy a little bit of time with family and those who are close to you. And welcome back.

So I've given talks to you all over the past 2 and 1/2 years about a number of topics, but I've never had the chance to tell you about research that we do in our lab. And I'm really excited this morning to tell you about one project in our lab that focuses on viral infections as a potential trigger for celiac disease, a very common gastrointestinal disorder.

And so it's Grand Rounds, so I first need to start with some objectives. And they are to first understand the clinical manifestations and pathogenesis of celiac disease, and then we'll appreciate how viruses can evoke blockade of immunological tolerance to newly introduced food antigens. And then we'll define how viruses differ in the capacity to block food antigen tolerance.

And again, so it's Grand Rounds, so I have to share with you my financial disclosures. First of all, we receive funding from the National Institutes of Health. And I want to thank all of you, as we start a brand new tax year, because when you pay your taxes, it goes right to support research that's done in our laboratory. And from really, the bottom of my heart, and all of our colleagues, we thank you for that support.

There'll be no discussion of off label pharmaceutical use. As it turns out, gluten is not a pharmaceutical. And so we'll talk about that, but it's not really an off label topic.

And we would love to develop a vaccine to prevent celiac disease. We would love to do so. And you'll hear about some strategies forward that we might be able to do that.

But now let's learn a little bit about celiac disease. First of all, this is an illness that's an autoimmune illness. And it's characterized by the loss of immunological tolerance to gluten. Gluten is a complex protein found in wheat and rye and barley, primarily.

The characteristics are really as Madison described them-- diarrhea and malabsorption. And the protein loss producing many of the symptoms that she described. And occasionally, there are other autoimmune manifestations to celiac.

It's a fairly frequent illness with an epidemiology of about one in 133 in the United States. And it does have genetic risks. And those risks are the HLA DQ2 or DQ8 alleles.

These are MHC class 2 molecules. And they present little bits of gluten-- gluten peptide, to CD4 positive T cells to orchestrate either tolerance or to orchestrate inflammatory attack, depending on the person.

Now, it's interesting that so many people carry one or both of these alleles-- 30% to 45%. But yet the prevalence of the illness is about one in 133. So clearly, there must be other genetic and environmental factors that influence the development of celiac disease, because it certainly is not as common as the genetic risk alleles.

Now, the action, the pathology, really is at the level of the intestinal villi. And celiac is an interesting autoimmune disease because the antigen is not really a self antigen. The antigen is a food antigen.

And when those with celiac ingest gluten, then these anti-gluten inflammatory TH1, T helper 1 cells-- they're marked by a transcription factor called T-bet-- infiltrate the lamina propria. And what they can do is release cytokines and other substances that injure the intestinal epithelial cells, these cells that line the villi, and they destroy them.

And so the villi in a normal situation are long, finger-like projections that give us lots of surface area for absorbing nutrients, are all blunted. And this villus atrophy, as it's called, is the signature pathological hallmark of celiac disease-- villus atrophy.

So what about celiac disease at our hospital? And I thank Leah for providing all of this information.

Our GI division cares for about 550 children with celiac disease. The diagnosis is made by screening labs, the presence of those HLA markers, and also auto-antibodies that are elicited in people with celiac disease that see specific enzymes that can help with the diagnosis. But the diagnosis is established by the demonstration of that pathological hallmark of villus atrophy on an endoscopy procedure.

Management is multidisciplinary, as you can imagine. That involves physicians, dieticians and nursing support. It is really hard to maintain a gluten free diet, and really hard to maintain a gluten free diet for life, which is really the only therapeutic option we have for celiac disease.

And of course, we need to follow these people carefully. Early on, malabsorption can lead to all kinds of difficulties that manifest as alterations in growth and development. And untreated celiac can have significant complications, which include a variety of malignancies in the GI tract, as well as iron deficiency, anemia and other manifestations of [INAUDIBLE].

OK. So let's talk a little bit about the pathogenesis of celiac disease as a precursor to trying to understand how it's triggered, why so many have the genetic risk alleles, but yet so few actually manifest the disease. So that's what we're going to try and get into.

So gluten is a big, complex protein that's actually difficult for our bodies to digest. And so peptides that are generated from the digestion of gluten, long strings of amino acids, are taken up by dendritic cells that normally filter antigen in the lamina propria. That's really what they do.

And in most people, those peptides are presented in the context of MHC class 2 molecules, to CD4 positive T cells that are marked by this transcription factor, FOXP3. These are regulatory T cells, and they serve as a break, essentially, on immune response. So no specific pathological immune response is generated to gluten peptides in the vast majority of us. So pizza and beer and so forth, pretty much everything you would find at a Super Bowl party, are safe.

But the situation is different in those with celiac disease. For reasons we don't understand with certainty, instead of these dendritic cells presenting peptide without any kind of a danger signal to elicit regulatory T cells, they lead to the generation of these inflammatory dendritic cells. And these inflammatory dendritic cells release different types of cytokines, like IL12 and IL23, and these are signaling molecules to CD4 positive T cells that lead to the development of anti-gluten T helper 1, or TH1 CD4 positive T cells.

Now this is the kind of immune response that you would expect to occur if the antigens that were being presented by these dendritic cells were derived from microbial pathogens-- salmonella, or rotavirus, some kind of a germ that infected the intestine would elicit the differentiation of T cells to make antigen-specific inflammatory T cells in the context of danger.

But this happens in celiac disease, as best we know, no danger whatsoever. Yet these pathological T cells that recognize gluten peptides are formed. And then, at the same time, the regulatory T cells that would normally suppress these antigen-specific inflammatory T cells are likewise diminished. So they're suppressed.

So the cells that normally put a brake on the immune response are inhibited. And so now we have a liberated inflammatory response. It's directed to gluten, of all things.

These T cells can then migrate back-- all of these events, by the way, occur in the mesenteric lymph nodes. And then these T cells, once fully activated and primed, can migrate back to the lamina propria. And when a person is exposed to gluten, they can elaborate injurious cytokines like interferon gamma and others that lead to the damage to these intestinal enterocytes that ultimately results in the villus atrophy.

So the key question, truly, in celiac pathogenesis, is what's the nature of this signal that leads to a normally toleragenic response by dendritic cells, where the immune system tolerates these gluten peptides, so we don't get sick when we eat pizza and drink beer, what causes these dendritic cells to become inflammatory, leading ultimately to the elaboration of these injurious TH1 inflammatory T cells?

Well, viruses are maligned for many, many things. As it turns out, many of you in this room have maligned a virus for one reason or another. And in many cases, that is reasonable. The viruses are in fact responsible.

But it turns out that an important danger signal that's elicited by infection is a cytokine called type 1 interferon, actually a family of cytokines that are all type 1 interferons. And viruses lead to the elaboration of type 1 interferon.

So it's not surprising that those studying celiac disease would think that potentially, by virtue of inducing type 1 interferons, that maybe this is the danger signal that leads to the development of celiac if a person is exposed to gluten at the same time a virus infects an individual.

And it turns out that viral infections have been associated with celiac disease in epidemiological studies-- some strong, some not as strong. And the viruses that have been implicated are adenovirus, enterovirus, hepatitis C virus, and rotavirus.

So that provided at least some hint of a signal that viruses might trigger the development of celiac, with the idea that maybe type 1 interferons would be the cytokine mechanism that would lead to this danger.

Well, it was about at this time that I first met Bana Jabri, an immunologist at the University of Chicago. It was in the spring of 2010. I was there to give a seminar on our reovirus work. And Bana asked me if I had thought ever about whether reovirus might serve in some way as a experimental system that we could explore whether viruses blocked tolerance to food antigens.

And I must say, I hadn't really conceived of that idea as I was sitting in Bana's office after my seminar. But it sounded like an interesting strategy, one we ought to try. And here we are, now almost nine years later, and I'll tell you about this collaborative effort that's led to this link between reovirus and celiac disease.

OK. So first, just a couple of words about reovirus, one of the two viruses that we study in our laboratory. These are beautiful viruses. This is a cryoelectron microscope image reconstruction of a reovirus [INAUDIBLE]. And I've rendered it in the actual colors of the virus.

So many of these viruses that you see in these grainy EMs are not attractive. But reoviruses, in fact, are.

They package up 10 segments of double stranded RNA as a genome, so it's almost like they have little chromosomes, these viruses, into two protein shells. And you can see the outer shell here with these spike-like projections of the attachment protein that lead to the binding of receptors. These viruses replicate in the cytoplasm, and childhood infections are exceedingly common.

Virtually everybody in this room has been infected with each of the three reovirus serotypes. And most of us were infected before we went to school. They're used in clinical trials as oncolytics, and they're related to the orbiviruses, which are pathogens of livestock, and the rotaviruses, which are a common cause of viral gastroenteritis.

Reoviruses got their name from Albert Sabin, of all people. And Albert Sabin called them REOs as an acronym for Respiratory Enteric Orphan virus.

And the reason Sabin called them orphans is because although infections are very common, disease is not. Disease is actually very infrequent after infection with reovirus. And as it turns out, if you're a virus, you have to really produce a disease to have a parent. And so no disease, you're an orphan. And so that's how REO got their name as Respiratory Enteric Orphans.

Now, I mentioned to you that most people acquire reovirus infection early in childhood. And this is evidence to support that contention.

This is a study that we did in Nashville many years ago. John Williams actually helped with this study. We determined the antibody responses to reovirus in cohorts of children that we had followed in a big vaccine practice over the years.

And most kids who were born had evidence of reovirus antibodies, all passively acquired from their mothers. We know that because by the age of 12 months, all of that antibody was gone.

And then you can see a steady increase in the acquisition of reovirus antibodies, such that by the ages of 5 to 12, a little over half of our sample had become infected with reovirus, none of whom we could really tie to a specific illness. And we found this fascinating because of course, the prime age of celiac disease onset, classical celiac disease like Madison's case, is between the ages of one and four, right at the time that reovirus antibodies are being acquired, as evidence of reovirus infection.

So it turns out there are a lot of reovirus strains. Oh my gosh. A lot, a lot, a lot.

But you know, we're simple people. And we study, primarily, two. One is called T1L and the other is called T3D.

Both of these viruses actually were isolated by Sabin, T1L from a boy called Lang, and T3D from a boy called Dearing. And that's how they got their names.

Now, they differ in a lot of interesting biological properties, and we thought they would be interesting to study with this idea of whether they influence a tolerance to a newly introduced food antigen. But it turns out that T3D, the parent virus, doesn't infect the intestine very well.

So we use genetic reassortment to replace two T3D genes with two T1L genes. And those genes are L2, NS1, and it doesn't matter that much in terms of the functions of these genes. Just know that when we replace those T3D genes with T1L alleles in those genes, now this virus called T3DRV, for [INAUDIBLE] virus, is capable of infecting the intestine. I'll show you that data in just a moment.

And before I say another word, I just have to acknowledge that every experiment that I'm going to show you is a synergistic experiment done in Chicago and Bana Jabri's lab, and also done initially in our lab at Vanderbilt, and now in our lab in Pittsburgh. We're sort of like a lichen, you know, the combination of the alga and the fungus. Bana is the alga. [INAUDIBLE] we are.

And so this project would not have been possible if we didn't have Bana's immunological expertise. And we added our interest in reovirus to drive the project forward.

So let me just show you, first, that these viruses are equivalently fit and they replicate well. This is the science part of the presentation now, OK? We've got about half a dozen slides. We're all going to stay together. It's all good.

So these are the two viruses, T1L and T3DRV. And these viruses were used to infect an intestinal cell line called [INAUDIBLE] cells.

And all we've done is just determine the viral titer, or the amount of virus, the viral load if you will, over a time course. And both of these virus produce equivalent viral loads and replicate with equivalent kinetics. They're both fit in cell culture.

And then we infected mice with these virus. Each dot represents a single mouse. And then euthanized the animals at 24 hours after infection, and then isolated all the parts in the abdomen that we could identify, the duodenum and jejunum, ileum, Peyer's patch, the mesenteric lymph node and the spleen, and we determined how much virus is at all of these sites.

And you can appreciate the red dots for T1L and the blue dots for T3DRV, that the viral loads are pretty comparable. Pretty comparable, at least at this 24 hour time point.

So we thought now, we had viruses that were equally replicative. They could infect the mouse. And we could ask the question, do either of these viruses, when introduced at the same time a new food is introduced into a mouse, block the tolerance that normally would occur in a mouse exposed to a new food antigen.

So this is the protocol we used for these experiments. So these are the mice diagrammed up at the top of the slide. And on the very first day of the experiment, we introduced into these mice CD45.1 positive CD4 positive T cells that all recognize a peptide derived from the hen egg protein, ovalbumin.

Immunologists love ovalbumin. I don't know why. But they love that protein. And they have generated over the years tons and tons of tools to study every aspect of the immune response to ovalbumin.

So now these mice, once we've introduced T cells that recognize the ovalbumin peptide, their T cells will now be able to respond if ovalbumin is introduced into the mouse. So it just gives us an experimental system that we can study for the development of either inflammatory T cells or the regulatory T cells.

So once we introduce these naive-- they haven't responded yet to antigen, but they would recognize it if antigen was introduced-- into the mouse, we wait a day, infect them with either the virus strains T1L or T3DRV. We do this orally. And then we wait six days while we're feeding ovalbumin in the water.

This is the first time these animals have seen this food antigen. So the virus has gone in. Now we put in the new food antigen. We wait six days, we euthanize the animals, and then we can remove various parts where there might be T lymphocytes, and ask, what is the nature, or the phenotype, of these T lymphocytes.

We use flow cytometry for these experiments. And you all, thankfully, this is the only flow cytometry plot you're going to see in this entire talk. OK. Dots from now on.

And so, but this will tell us whether these animals are developing an immunological or inflammatory response to the new food ovalbumin, or a regulatory suppressive response to this new food. OK. You with us?

All right. So, remember, in the normal circumstance, a new food antigen like ovalbumin would be taken into the lamina propria, taken up by dendritic cells. Those ovalbumin peptides, OVA peptides, would be presented. And they would lead to the differentiation of CD4 positive T cells, naive T cells, to make these regulatory T cells. And they're marked by this transcription factor FOXP3. That's how we know they're regulatory T cells.

The question is, what happens when a virus is introduced at the very same time as the new food. So that's the question.

So first piece of data. So these animals were either mock infected or infected with T1L, and then fed ovalbumin. A week later, they were euthanized. And then we asked what percentage of all the T cells that we could identify in these animals now are inflammatory.

And you can appreciate that in the animals inoculated with T1L, there's about a threefold increase in the number of these T-bet positive, CD4 positive T cells. These are the inflammatory TH1 cells. And there's a concomitant decrease in comparison to mock in these regulatory T cells. These are marked by the FOXP3 transcription factor.

So inflammatory T cells up, regulatory T cells down. This is an immunological hallmark of blockade of tolerance. These animals have developed an immune response as if the ovalbumin was a virus, or salmonella, or some dangerous microbe. But it's not. It's ovalbumin. It's a food.

So the situation with T3DRV was different in that in comparison to T1L, the number of these inflammatory TH1 cells was diminished in T3DRV infected animals. And the T regulatory cells were increased in comparison to T1L. So T1L and T3DRV differ in their capacity to block this tolerance.

So we wondered why. And we thought, well, type 1 interferons, as I told you at the beginning, can be a danger signal for dendritic cells, leading to the development of inflammatory T cells. So sure enough, when we look for genes that are induced to be expressed by interferons, T1L induces much higher levels of interferon gene expression than does T3DRV.

So we really thought that we had the missing link. So virus, interferon, and that would then lead to the development of these inflammatory dendritic cells that would lead to inflammatory TH1 cells.

So we tested this using mice that lack the interferon receptor. They can't respond to interferon. So even though they make it, they can't respond to it. And we thought they would be blocked from tolerance.

So here in wild type mice, you can see that again, these are the inflammatory T cells. They go up in animals infected with T1L. But they also go up in the interferon receptor knockout mice.

So clearly, interferons are not required for this effect. So that was a puzzling result. Even though the viruses differ in their capacity to elaborate interferon, the capacity for an animal to respond to interferon didn't affect the development of these inflammatory TH1 cells.

So that then led us to target another transcription factor. And you only need to hold on just another couple slides. Just another couple slides.

We had done a lot of RNA sequencing profiling in these mice infected with T1L and T3DRV to get some clues about how they're different, how this host is differing when infected with these two different viruses, one that blocks tolerance, and another that doesn't.

And so we came across IRF1. First of all, IRF1 is elaborated in dendritic cells. And it's known to be essential for the development of these TH1 so-called inflammatory T cells.

We found it was activated by T1L, but not T3DRV in both wild type mice and, interestingly enough, mice that lacked the interferon receptor. Hm.

So clearly, there's some type of a danger signal that can lead to the development of IRF1. And really intriguingly, in small case studies, IRF1 was found to be upregulated in the mucosa of children with celiac disease, bringing us right back to the issue at hand that started this work in the first place.

So we did a similar experiment using IRF1 knockout mice, so mice that lacked IRF1. These are the wild types. And they lose tolerance. And these are the knockout mice, mice that cannot express IRF1. And in these animals, tolerance is blocked.

So now we know that IRF1 is required by dendritic cells, eventually leading to the development of these inflammatory TH1 T cells that ultimately can damage the intestinal villi.

Now, I don't have time to tell you about a lot of experiments that we did with gluten, but it turns out, if you feed mice gluten, they're not going to develop any type of an immune response to gluten, because they don't express either the human DQ2 or DQ8 MHC alleles.

But if you do these experiments in mice that have been engineered to express the human MHC DQ2 or DQ8, feed them gluten, and infect them with type 1 Lang reovirus, T1L reovirus, tolerance to gluten is blocked.

So the same phenomena that occur with ovalbumin also occur with gluten in the right genetic background. So that was really exciting.

Just a couple of last things to just share with you. First, we wondered a lot, how did these viruses differ in their capacity to block tolerance. T1L does it when it's introduced with ovalbumin. T3DRV does not.

Now, I showed you that at the 24 hour time point, the viral loads of these two viruses were pretty comparable. They both infect the intestine pretty comparably. But if we examined at 48 and 72 hours after infection, well, there was a striking difference in that there was a lot more T1L reovirus than T3DRV reovirus in these infected mice. And we wondered why that might be.

So god bless Judy Brown, who was a graduate student with me, counted all cells that are undergoing a non-inflammatory mechanism of cell death called apoptosis. There are lots of biochemical markers of apoptosis. The one we used was a cleaved, or activated form of the protease Caspase-3. And these are all intestinal villi that have activated Caspase-3. You can appreciate the stain at the tips of these villi.

And in comparison to T1L, T3DRV induces a lot more non-inflammatory cell death than does T1L, is cleared more rapidly, and so doesn't maintain a prolonged replication cycle with inflammatory danger signals that we think are required for, ultimately, the activation of IRF1 in these dendritic cells, and then the proliferation of TH1 T cells.

So putting all of this together, in comparison to T3DRV, which induces this non-inflammatory mechanism of cell death called apoptosis, and is cleared rapidly, these would be apoptotic cells sloughed into the intestinal lumen, T1L infects efficiently. It does induce high levels of interferon. And we know that the interferon is required for the inhibition of these regulatory T cells.

But what leads to the danger in the dendritic cells and the activation of this transcription factor IRF1? We don't know. So that's a mystery.

But ultimately, these dendritic cells, primed by danger signals, lead to the differentiation of naive CD4 positive T cells to make inflammatory TH1 cells. They leave the mesenteric lymph node, migrate into the lamina propria, and now they're fully licensed, these CD4 positive T cells, to elaborate inflammatory cytokines that mediate the injury to the enterocytes that ultimately produce the villus atrophy.

Now, one last question to try and answer. Is reovirus a trigger for celiac disease in humans? And judging by the looks on your faces, you share some of the skepticism, it seems to me, as the people in this photograph about this question.

And we certainly don't have this answer with certainty. But a study that we did with Bana, we took age and gender-matched controls in persons with celiac disease-- those who have active illness and those who are maintained on a gluten free diet. And we determined whether or not they had antibodies in their bloodstream against reovirus.

And you can appreciate from these data, just at the threshold of statistical significance, that those with celiac disease had more frequent detection of reovirus antibodies. And their antibody levels are higher.

And I know what you're thinking. This is an autoimmune disease, and they have activated immune responses. So wouldn't this be true with any antigen to test?

And actually, that's not the case. So we also tested for rotavirus. I show you the data here. We tested for tetanus and herpes simplex virus, and found no differences in the antibody responses in the controls in those persons with celiac disease.

So a clue in this retrospective cohort study that something is different about the way that persons with celiac disease react to reovirus. That's all we can really say from such an experiment.

The last just a couple of points to leave you with-- we published our first results on this project about a year and a half ago. And I must say, we elicited a lot of attention. The study was picked up by the press and we got a lot of email from colleagues and from those with celiac disease, inquiring about the study.

And I have to say, this was really a new experience for us. We were really comfortable laboring in obscurity, working on a virus that most people confuse for a large city in South America. And so imagine my surprise when I got this note from a woman called C Dermody.

C writes, I just read your article about the possibility of a reovirus T1L as responsible for celiac disease, at least in mice. Smart woman, C Dermody. I was diagnosed only three years ago with celiac disease. And she goes on to tell her story, volunteering samples if they would be of use to our research.

But she also writes, I was interested in your research because of your name. We share the same surname, and I am keen on exploring the family history. So I sent this note to my dad, who is the keeper of the Dermody genealogy.

And my dad and C Dermody corresponded a number of times. And other than finding that our families immigrated from Ireland in the middle part of the 19th century, when about a third of the country immigrated from Ireland, we couldn't put our Dermodys together. But I was, I have to say, incredibly grateful that the humble reoviruses had brought the world just a little bit closer together.

OK. Let me leave you with a couple of takeaways. First, celiac disease is an autoimmune disorder. But it's triggered by immune responses against a dietary antigen-- not against a self antigen now, but against something in the diet, gluten common, and wheat, rye, and barley.

The reovirus strains that we study a lot, T1L and T3DRV, differ in the capacity to abrogate tolerance to food antigens. Fascinating. And we think that this is explained because T3DRV induces apoptosis more efficiently than T1L, which may limit replication and tolerance loss. Apoptosis not being an inflammatory mode of cell death, not expected to elaborate those danger signals that lead dendritic cells to react to whatever antigen they are being exposed to.

And I have to say, we're incredibly excited about this project. There's so much work to do. First, we don't understand the mechanism by which the virus leads to blockade of tolerance. What is the nature of these danger signals? If it's not type 1 interferons, then what is it? What is the virus doing that elaborates that kind of a response?

The second, we wonder about other environmental factors. Reovirus infections are common. The genetic risks are common. Still, celiac is rare. And of course, we're very interested in exploring whether alterations in the microbiome might influence celiac disease development, especially all the antibiotics we use in the first couple of years of life, and whether that might influence the way that an immune response might react to a food antigen.

And third, we wonder whether the immunization against viruses that could be potential triggers for celiac, at a time far ahead of exposure to gluten, might prevent the development of celiac disease in those at risk. So this is some of the avenues of research that we're currently exploring.

But perhaps most excitingly, we wonder if finally, reovirus has a parent. And maybe that parent is celiac disease.

I want to just close up and thank some folks. A number of people who work on the celiac project in our lab-- the leader now of that work is Pam Brigleb, who worked closely with Gwen Taylor and Kelly Urbanek, although others have contributed to the work.

And the leader of this work, until she graduated last year, was Judy Brown, who is now teaching at Trevecca Nazarene in Nashville. None of the work could be done without Bana and her team in Chicago. And we were very successful in recruiting Reinhard Hinterleitner to the University of Pittsburgh, in the Department of Immunology. So he's moved from Chicago to Pittsburgh, and will continue to work with us.

We have other collaborators who have helped in various aspects of the study. We're grateful to the NIH and the Heinz Endowments for supporting our work.

This is a photograph of our lab, taken last summer. I'm so grateful to have the opportunity to work with these people-- the smartest, hardest working, kindest people in the world. We get to work together to try and figure out how viruses cause disease.

And I'm incredibly indebted to Madison and Michelle, who told you a little bit about their story with celiac. And I'd be happy to answer questions. And I know Madison and Michelle would too. And if you would come and join me on the stage, we'll be glad to try. Thanks very much.

[APPLAUSE]