# DR. DETLEF SCHUPPAN: Innovating Unprecedented Treatments for Celiac Disease

BY MARINA ILYAS, JACOB MARTIN, ESTHER LIM

Detlef Schuppan, MD, PhD, is a professor of Medicine, Gastroenterology, and Hepatology at the Medical Center of the Johannes Gutenberg University and Beth Israel Deaconess Medical Center, Harvard Medical School, and founding director of the Institute for Translational Immunology. He is recognized as a leading expert in celiac disease and his research into celiac disease, fibrotic diseases, cancer and autoimmunity has led to numerous developments in the field. He discovered tissue transglutaminase as an autoantigen in celiac disease in 1997, which led to a paradigm shift in celiac disease research and the development of a highly reliable diagnostic test. In this interview, we hear his perspectives on celiac disease treatment and learn about exciting progress in the field.

**BSJ**: What is celiac disease, and how does it affect the human body?

**DS**: Celiac disease is a nutritional disease that has a fairly well-defined genetic basis. It is an immunological intolerance to gluten proteins in wheat, barley, rye, and related cereals, and these gluten proteins represent about 90% of the cereal proteins present. Gluten is digested to a smaller extent than other food proteins, so everyone has fragments of somewhat intact gluten peptides that reach the small intestine. Part of this is taken up by the gut mucosa, the lining of the gut containing the lamina propria, a connective tissue lining that harbours the largest immune system of the body. Normally, little bits of certain nutrients, like the gluten peptides, get into the lamina propria of the gut wall where the immune system senses them but still maintains a level of active immunosuppression. This means that the gut is primed to have tolerance for foods, which is important for the maintenance of the organism. Hence, normal people without celiac disease will not have an adverse response to gluten.

However, it is much harder for people with celiac disease to be tolerant to gluten. Due to a certain genetic predisposition, their bodies recognize gluten peptides as something bad that has to be fended off. After ingestion of gluten, an immune reaction is triggered in the upper small intestine, which leads to intestinal inflammation and the classical signs of celiac disease. When you take biopsies, you see various degrees of atrophy of the villi, which are finger-like protrusions in the small intestine that are important for nutrient uptake. Consequently, their intestines cannot adequately absorb nutrients like minerals, vitamins, and amino acids, which can result in malnutrition, anemia or osteoporosis. This can even lead to intestinal cancer in adults and growth problems in children.

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Adults diagnosed with late-onset celiac disease often also suffer from other intestinal problems,

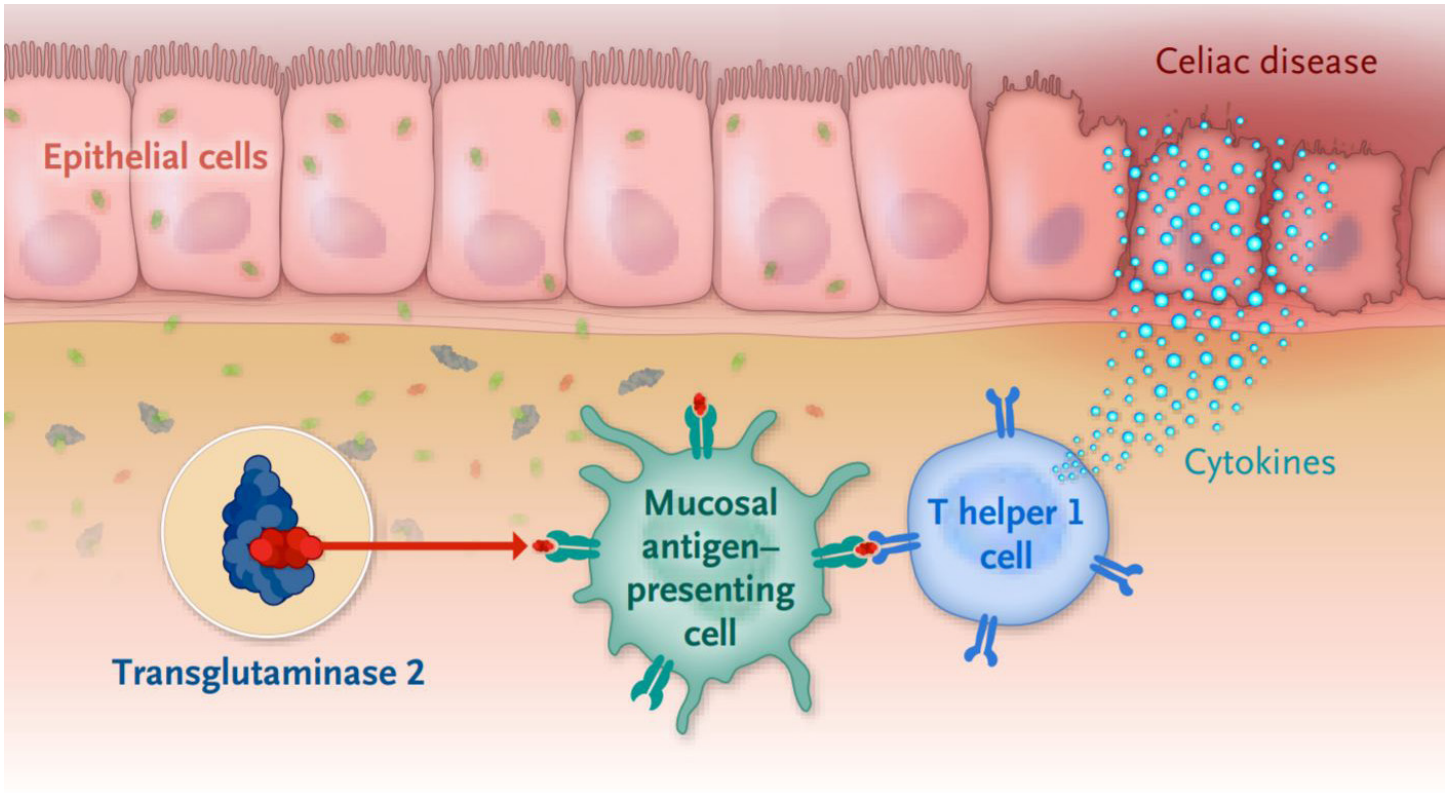



***Figure 1: Atrophy of villi in small intestine.*** *T-cell activation and the subsequent release of cytokines leads to inflammation of the epithelial cells, and shortening of the villi.*

joint pain, difficulty in concentration, and associated autoimmune diseases (from multiple sclerosis to rheumatoid arthritis to thyroid diseases) and not necessarily just severe diarrhea.

**BSJ**: What is the genetic basis of the disease?

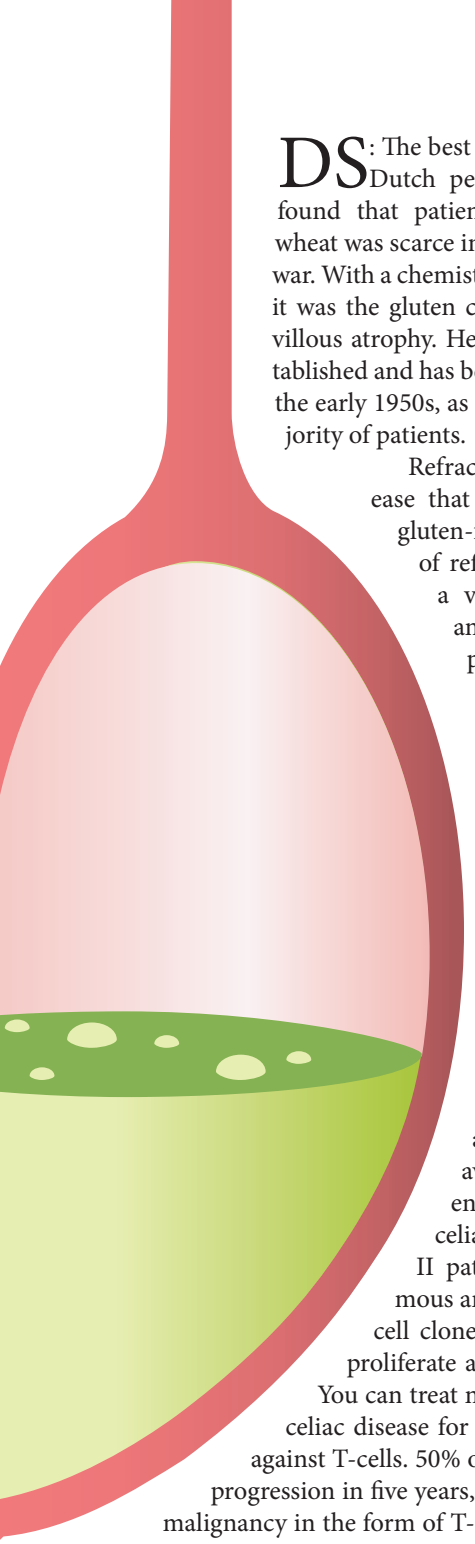
**DS**: The genetic background of celiac disease is in DQ molecules. They are immunological molecules that present gluten to T-cells, thus activating the T-cells. Human Lymphocyte Antigen (HLA)-DQ2 and -DQ8 molecules are necessary but not sufficient genetic predispositions for celiac disease. What that means is that everyone who has celiac disease has HLA-DQ2 and -DQ8, but not everyone with HLA-DQ2 and -DQ8 has celiac disease. About 30-40% of most populations have HLA-DQ2 or -DQ8, but only a small percentage of this group develops celiac disease. If you belong to the 60-70% who do not have HLA-DQ2 and -DQ8, you will not develop celiac disease.

There are also many other extrinsic factors that may lead to celiac disease and its symptoms. The gut microbiome plays a role, and antibiotic treatments can alter the microbiome in early childhood. Certain intestinal infections by viruses can trigger a higher sensitivity to gluten. Non-steroidal and anti-inflammatory medications, like Advil, if consumed in large amounts, can disturb the intestinal barrier and make it more sensitive to anything that comes in.

**BSJ**: What are the current methods to diagnose celiac disease?

**DS**: We have developed a non-invasive autoantibody blood test that can diagnose active celiac disease on a population level that is now used worldwide. Autoantibodies are antibodies that are directed against the body's own proteins, and more rarely, non-protein molecules. In celiac disease, you have a very specific autoantibody which is directed against an enzyme called tissue transglutaminase (TG2) that is ubiquitous in the body and also present in the gut lining. We discovered TG2 as the celiac autoantigen in 1997. If you do upper endoscopy and take small biopsies, you are able to see the intestinal lesions typical of celiac disease, namely villous atrophy, crypt hyperplasia, and lymphocytic infiltration. If the autoantibody test is positive and you get confirmation of intestinal lesions by biopsy, you can confirm that the person has celiac disease. For population studies, it is enough to just do the blood test, which is cheap and quickly done, and then confirm the diagnosis by upper endoscopy and biopsy.

**BSJ**: Currently, what are the most viable and cost-effective options for treatment in mild to moderate cases of celiac disease? What about for cases of refractory celiac disease?



DS: The best treatment is a gluten-free diet. A Dutch pediatrician, Willem-Karel Dicke, found that patients' conditions improved when wheat was scarce in supply during the second world war. With a chemist, van de Kamer, Dicke found that it was the gluten component of wheat that caused villous atrophy. Hence, the gluten-free diet was established and has been the mainstay of therapy since the early 1950s, as it was highly effective in the majority of patients.

Refractory celiac disease is celiac disease that is not responsive to the strict gluten-free diet, and there are two types of refractory celiac disease. Type I is a very high sensitivity to minute amounts of gluten. In gluten-free products, a minor amount of 20 milligrams per kilogram is allowed. However, patients with Type I refractory celiac disease have ongoing complaints and elevated levels of antibodies in the blood and will have an immune reaction to even a few milligrams of gluten per day, sometimes even leading to symptoms like severe diarrhea. Most of these type I patients will never develop malignancy, but it is still difficult to remedy because minute amounts of gluten cannot be avoided even in strict gluten-free environments. Type II refractory celiac disease is pretty rare, and type II patients have signs of an autonomous and malignant process of immune cell clones which, like in cancer, start to proliferate and cause inflammatory damage. You can treat milder forms of type II refractory celiac disease for a long time with drugs directed against T-cells. 50% of patients will not have a disease progression in five years, while 50% will progress to overt malignancy in the form of T-cell lymphoma of the gut. This is very difficult to treat. It usually leads to death within a few months with the only possible treatment being a bone marrow transplant.

BSJ: You recently published a study on the efficacy of a transglutaminase inhibitor, ZED1227, at reducing small intestinal damage in celiac patients. Why was there a need for new treatment options for celiac disease, in addition to a gluten-free diet?

DS: The reason is that the gluten-free diet is difficult to maintain in everyday life. It will be very important for people with celiac disease to be able to have a standby medication, which would protect them from the ingestion of minor amounts of gluten that is usually unavoidable in social settings.

There have been trials for enzymes that you can take with your meal that degrade the rest of the undigested gluten peptides that cause an immune reaction. These enzymes can be quite efficient in vitro or in some well-controlled in vivo scenarios. But in real life, they were not very efficient, due to mechanical problems of mixing with the ingested food and also because the reactions are pH-dependent. Some very efficient gluten-degrading enzymes have been developed but they are not yet sufficient to completely get rid of the gluten. Up to now, these approaches have not been successful.

BSJ: How is transglutaminase involved in the pathogenesis of celiac disease? How did you identify this pathway?

DS: Tissue transglutaminase (TG2) is the celiac disease autoantigen and an enzyme that modifies the gluten peptides that enter the gut by changing their biophysical properties via induction of a negative charge in the immunogenic (HLA-DQ2/8-presented) gluten peptides. When certain neutral glutamine residues in the gluten peptides are modified into acidic glutamic residues by the action of intestinal TG2, it changes their properties with regard to the immune system, allowing increased binding to HLA-DQ2 and -DQ8 molecules on immune cells in the gut, which in turn, causes increased T-cell activation. Hence, we decided to develop a drug based on inhibiting TG2 to prevent this potentiation of the immune response. Since TG2 is centrally involved in the pathogenic process of celiac disease in almost all cases where the patient has elevated TG-2 autoantibodies, such treatment should be effective to very specifically attenuate the inflammatory response to gluten.

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The idea was that if we have an inhibitor of TG2, we can prevent its reaction with the gluten peptides and thus their immunogenic activation also in vivo. Before the identification of TG2, it was unclear what the autoantigen of celiac disease was, and many renowned research groups tried to find the assumed extracellular

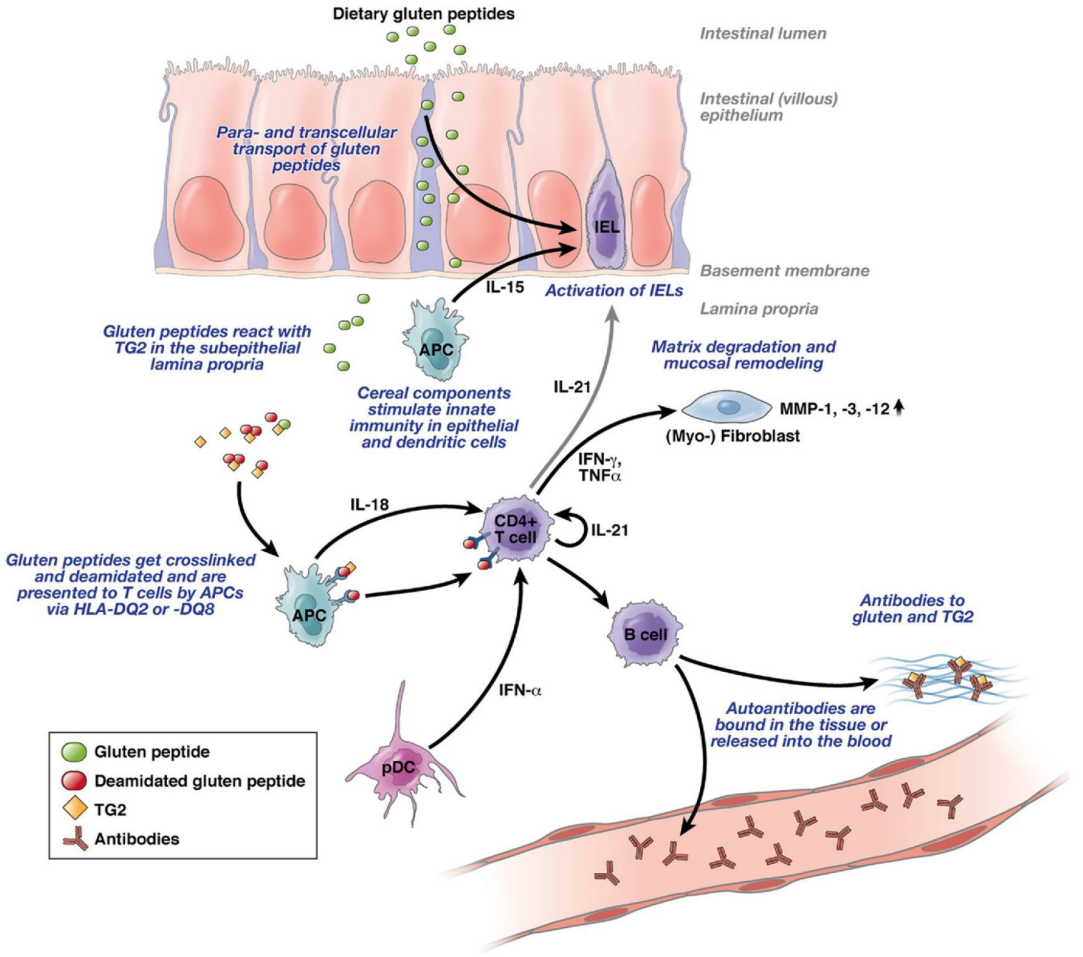


***Figure 2: Pathogenesis of Celiac Disease.*** *Gluten peptides are transported to the subepithelial lamina propria where they react with TG2. The deamidated peptides are presented to T-cells via HLA-DQ2 or HLA-DQ8, initiating an immune response against the mucosa of the small intestine.*

matrix component to which the autoantibodies were directed. I was quite knowledgeable about the extracellular matrix (connective tissue) components by that time because I did my PhD thesis about identification and sequence analysis of basement membrane proteins. I thought it would be possible to find and identify the matrix component that reacts with these autoantibodies. After some erroneous pathways, we used radiolabeled cell culture from fibroblastic cells and managed to immunoprecipitate TG2 with patients' autoantibodies, and thus identify it as the autoantigen of celiac disease. Unexpectedly, TG2 is not a matrix protein, but it can associate with the matrix. When it is secreted from cells, it can bind to fibronectin in the matrix and this causes the pattern that you see with the autoantibodies on tissue sections. That is how our research into TG2 in celiac disease started. We published our findings about TG2 as the autoantigen of celiac disease in Nature Medicine in 1997, and this propelled many research groups to see how TG2 could be linked to the pathogenesis of gluten in driving celiac disease. My colleagues in Norway and the Netherlands found that TG2 can deamidate with gluten peptides in vitro. Since then, thousands of papers have exploded in terms of the pathogenesis of celiac disease. When I relocated from the US to Germany in 2011, I worked with a clinical developer and a company specialized on transglutaminases to develop a molecule that specifically targets TG2 and eventually, to produce a clinical drug.

**BSJ**: In your study, patients received varying doses of ZED1227 as treatment for celiac disease. What were the most significant findings of your study?

**DS**: Our TG2 inhibitor drug is targeted mainly to the intestine. It does not significantly go into the circulation and is very specific to this process of celiac disease. We could not be 100% sure if it would work, so it was a bit courageous to do this. In the so-called phase 1 testing more than 100 healthy subjects were given increasing doses of the drug up to a one week period. Fortunately, they had no side effects. Before that, we did many animal experiments for safety and tolerability, and there were no genetic or other adverse changes observed. In our recent phase two clinical study we did endoscopies on the 160 volunteer celiac patients, who were in remission on the gluten free diet, before and after a daily challenge with three grams of gluten (20% of a usual daily dose) in the form of a cookie for six weeks. Biopsies were examined and assessed for villous atrophy and inflammation by an expert pathologist. Such exact assessment of the biopsies is a complex process because you have to correctly orient the sections to measure the villus height. The blinded evaluation showed that only one quarter of the patients had worse outcomes, while all the others were similar in terms of retained villus height. Only one quarter of the patients were on the placebo pill and the other three quarters got three

different doses of the drug, so this indicated that the drug could work. Upon unblinding, it was then confirmed that the retained villi heights observed in patients were indeed due to ZED1227, and we were extremely happy because, in a way, it became the first drug with proven efficacy.

"it was then confirmed that the retained villi heights observed in patients were indeed due to ZED1227"

**BSJ**: In 2009, you proposed that TG2 inhibitors and DQ2 blocking peptides could potentially be used to prevent inflammation in celiac disease since the deamination of gluten peptides by TG2 and the subsequent presentation by HLA-DQ2/8 are the processes that initiate the adaptive immune response. Now, you have actualized this possibility in your clinical trial in 2021. Could you share with us your thoughts on your research journey in celiac disease?

**DS**: As you can see, it took a long time, from the discovery of the enzyme and the input of many other researchers who further explored the mechanisms of this enzyme, to developing this first clinically-proven drug. We discovered the enzyme in 1996, published our findings in 1997, established the clinically highly useful antibody assay in 1998, and finally successfully concluded our phase 2 trial in 2021. That took 25 years. I think if you work in research, you would know that the process of completing an experiment and confirming those results can take many years. There are many failures, repetitions, and confirmations, and it is even more difficult if you want to get it published in a good journal. You have review processes that can last for two years, usually requiring many more experiments to be done. Research, and especially translational trajectories, are very different from what many people might think.

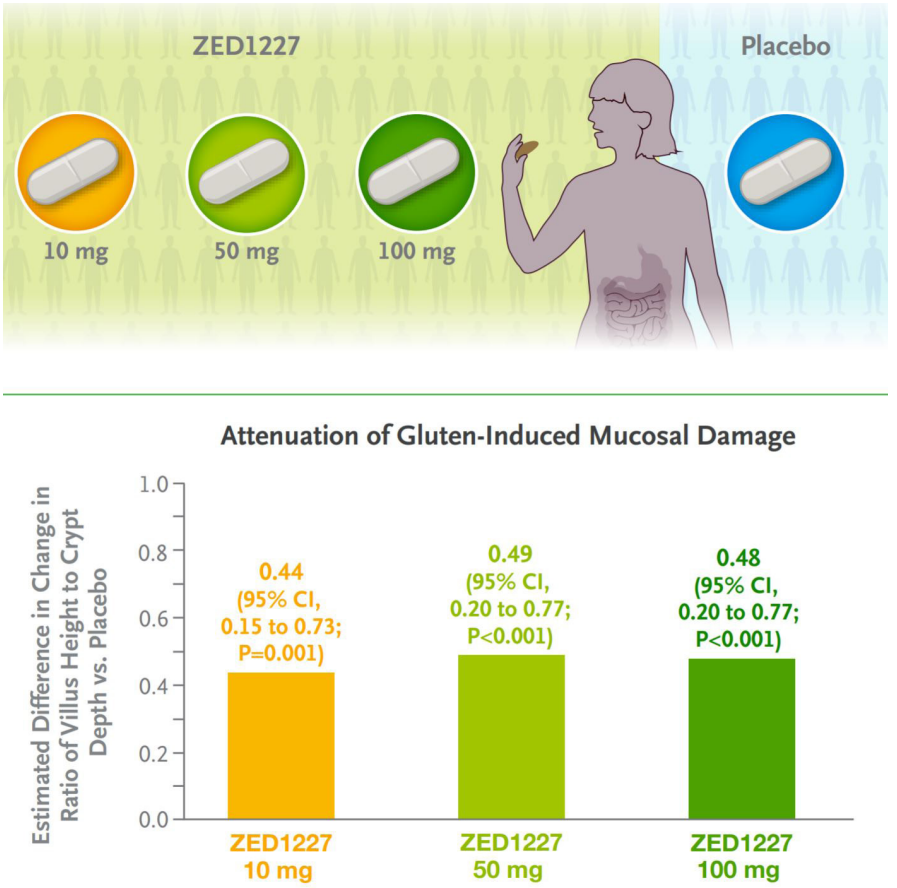


***Figure 3: Reduction of Gluten-Induced Mucosal Damage**. The three different doses of the transglutaminase-inhibiting drug, ZED1227, led to a decrease in gluten-induced mucosal damage.*

**BSJ: What are some projects you are currently working on in your lab? What is the future direction of your research in the field of celiac disease?**

DS: We have several areas now in the lab that are quite interesting and also promising. We have various projects and clinical studies on autoimmune diseases. Regarding the role of nutrition and especially wheat, we have just finalized a multiple sclerosis clinical study with and without wheat. We also just completed a study on primary sclerosing cholangitis and familial Mediterranean fever and started studying rheumatoid arthritis and lupus, all being autoimmune disease that are apparently exacerbated by wheat. Here the trigger is not gluten but the amylase-trypsin inhibitor proteins of gluten-containing grains, a discovery that we made in my group in Boston, first published in the Journal of Experimental Medicine in 2012. Another interesting area is what we call atypical or type 2 food allergies. That is the novel entity that is the primary contributor to irritable bowel syndrome (IBS), which is very prevalent in many populations. There is the connection between the brain and IBS, between the brain and the gut, and vice versa. Whatever you have in the gut (food components and microbiota as well as microbial metabolites) influences your well-being dramatically, so we are very interested in the immunological and metabolic communication between the gut and the periphery, and we have several projects on this. Another very big area is fibrotic diseases where we work with blood markers of fibrosis progression, which is well reflected in certain serum markers and on the development of antifibrotic therapies, in view of organ scarring (fibrosis) being responsible for roughly 50% of chronic diseases worldwide. We are also working on novel therapies for solid cancers, such as anti-cancer therapeutics that address both cancer cells and the immune system around the cancer to convert the usual cancer-tolerant local immune response to an active anti-cancer immune response. If we combine such therapies alongside direct cancer cell growth-inhibiting drugs, we can obtain very good effects.

As for celiac disease and medical research, I believe that there is still a good amount of positive challenges and possibilities for good translational research, which have always been my vocation, being both a basic scientist as well as a clinician. I think the very important thing for the future is to maintain interest in the life sciences, natural sciences, and medicine, and have people who are intrinsically motivated to contribute to the field. I consider this a priority for myself and a prominent task for senior scientists that we owe to the next generation and to society.

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