Alessio Fasano, MD: How a Cholera Researcher Became an Expert on Autoimmune Disease

Interview by Craig Gustafson

Facilitated by the 2018 Institute for Functional Medicine Annual International Conference, to be held May 31 through June 2, 2018, in Hollywood, Florida. For more information, please visit http://www.ifm.org/.

Alessio Fasano, MD, *is the W. Allan Walker Chair of Pediatric Gastroenterology and Nutrition and chief of the Division of Pediatric Gastroenterology and Nutrition at MassGeneral Hospital for Children. His visionary research, which established the rate of celiac disease at 1 in 133 people, led to the awareness of celiac disease as a growing public health problem in the United States. Dr Fasano founded the Center for Celiac Research and Treatment in 1996, where he treats adults and children for gluten-related disorders. He is a passionate advocate for collaboration in research and clinical work; has authored the book* Gluten Freedom*, which dispels confusion about gluten and how it can affect health; and is widely sought as an expert in celiac disease, intestinal permeability, and autoimmune disorders.*

***Integrative Medicine: A Clinician's Journal* (*IMCJ*):** Your most widely known work is related to celiac disease and autoimmune disease. How did you get interested in this research?

Dr Fasano: Originally, I was not interested at all. In the early days, I was really focused on diarrheal diseases, believe it or not, particularly infective diarrhea diseases like cholera. Being a pediatric gastroenterologist, I focused on what is the most impactful science I can do to make a difference. We still lose 4 or 5 million kids a year to diarrheal disease. It seems to be insane, but that is the truth. So, that is what I was focusing on, to understand how pathogens cross-talk with us to achieve whatever goal they need to achieve. As a byproduct of that interaction, you got sick with diarrheal disease, so you die from dehydration.

The deeper I delved into these kind of studies, the more I realized that the way that bacteria talk with us is through tools that are there for other purposes. They just stumbled into this cross-talk, so to speak, hijacking a mechanism that the host uses for different purposes, in order to achieve their lifestyle goals.

That led me to believe, that among the other mechanisms they use, bacterial interaction with their host may affect some of the key functions of the gut that have been overlooked for many years, including making the gut more permeable to substances from the environment. So the bottom line is, increased gut permeability leading to increase trafficking of macromolecules is one of the common bioproducts of the enteric pathogens-host interaction.

Just by serendipity, at the same time, researchers were beginning to understand some of the mechanisms that lead to autoimmunity. And definitely, the breaking of tolerance comes from the environment—the triggers of autoimmunity—is what really leads you to develop this disease if you are genetically predisposed. But, also, these components have been overlooked for many years. The major focus was—and still is I should say—on how and why the immune system got aggressive against itself and killed your tissues. That is the hallmark of autoimmunity. But how the entire process starts, why the immune system becomes so belligerent, and what it is reacting to has always been a black box, so to speak. Only in recent years has attention to antigen trafficking—in other words, the small unit that comes from outside to inside our bodies through increased permeability—been on the radar screen of scientists that are interested in why you start this entire process that leads to autoimmunity.

The bottom line is, by studying how bacteria make the intestine become more permeable, I ended up studying the mechanism that starts the cascade of events leading to autoimmunity. Those 2 power roads crossed at a certain point when I started to focus on another diarrheal disease that was an interest of mine, and that was celiac disease. This ended up being categorized as an autoimmune disease, and here you are.

So the 2 worlds that appeared not to be connected to each other end up having a common denominator. And that is the reason why I am so interested in autoimmune disease, particularly the early steps that lead to developing autoimmune diseases. Nevertheless, these studies of the cross-talk of bacteria and how they communicate with us, that I started many years ago, now translate in not just a single bacterium, like *Vibrio cholerae* that makes you sick, but an entire community which we call the *microbiome*. With the understanding that the microbiome plays a major role in many autoimmune diseases, you now appreciate that the focus of 30-plus years of science has come to fruition in terms of understanding why people develop autoimmunity.

***IMCJ*:** How did you and your team make the link from celiac disease to intestinal permeability and the discovery of zonulin as the gatekeeper for that process?

Dr Fasano: Once again, this was another act of serendipity. I was studying, as I was telling you, diarrheal diseases, and

mainly I was working on cholera. In the early 90s, I discovered another toxin, zonula occludens toxin—or ZOT—that *Vibrio cholerae* uses to make the intestine more permeable. In studying the details of how this toxin works, we realized the machinery that this toxin uses are way too sophisticated to be there just to get sick from cholera.

We reasoned that this toxin was probably mimicking a molecule that was doing this for a living—one that we produce to modulate gut permeability. After a series of experiments, trying and trying again, to look for this molecule, we eventually discovered this molecule, named *zonulin*, that was present in the gut of human beings whose function is indeed to control gut permeability.

Of course, at that point, we reasoned, like for anything that has a physiological function, what the problem would be when you push it to the extreme and you propel from physiology to pathology. As I mentioned before, autoimmune diseases are considered conditions in which you lose the capability to keep these enemies at bay and prevent autoimmunity from occurring. Because celiac is the only autoimmune disease for which we know the trigger—that is, gluten—it was only logical for us to look first and foremost at celiac disease and ask the question: "Is the zonulin pathway involved?" If it is, do we have any evidence that these people have an increased amount of zonulin, and therefore an exaggerated permeability, compared to normal people? The answer turns out to be most definitely, yes. They produce 10 times more zonulin than normal people do and, consequently, their intestine is more permeable even before the intestine is damaged by the autoimmune process. So the reason we choose to study celiac is because it is the only autoimmune condition that you can turn off—no gluten in the diet—or on—reintroducing gluten in the diet—at will.

The second question for us was: "If you put these people on a gluten-free diet, which is the treatment for celiac disease, what happens to the zonulin pathway?" And the answer is that the levels of zonulin in the serum come down. So that is how we eventually ended up linking the discovery of zonulin with autoimmunity, using celiac disease as a paradigm.

***IMCJ*:** So then once you had that in hand, were you able to bend that knowledge into some application with clinical potential?

Dr Fasano: Well yes. And again, there was a third act of serendipity. When we started to look into the similarities between the toxin elaborated by *Vibrio cholera* that increases gut permeability that we called ZOT, and zonulin, we found a fragment that overlaps the 2. So, we reasoned that they probably use the same machinery and target the same receptor. We made a synthetic peptide to prove ZOT and zonulin do work the same way, and that appears to be the case.

This peptide seems to block the action of zonulin and prevent gut permeability from occurring. By the time we discovered that zonulin was linked, not only to celiac disease, but to a variety of autoimmune diseases, this peptide represented to us a possible tool to eventually treat autoimmune diseases. Keep in mind that we do not have anything right now in terms of an efficient treatment for autoimmunity. The idea being, if we use this peptide and we keep gluten at bay for celiac disease, then maybe we can eventually have another way to treat celiac disease, other than a gluten-free diet.

At the beginning, we did some preclinical studies that seemed to suggest that this peptide will eventually do that kind of job correctly. Long story short, in 2005 we were able to give this molecule orally to volunteers, first and foremost to prove that it is safe. And it turns out to be safe. Then we started to work on people with celiac disease, taking advantage of the fact that you can turn on and off the autoimmune condition at will by giving gluten in the diet or eliminating it. In double-blind fashion, we gave people with controlled celiac disease, who otherwise were doing well on a gluten-free diet, either gluten by itself—making them sick and increasing gut permeability—or gluten plus this peptide. We verified that this peptide may eventually prevent the detrimental effects of gluten on people with celiac disease by preventing their increased gut permeability and therefore keeping gluten out of the body.

Fast-forward to now, where this peptide is being given to almost 1000 people. It turns out to be extremely safe and hopefully soon we will enter a phase 3 trial. That is the last step before commercialization. That was an unexpected outcome of our research.

***IMCJ*:** Where has your research in the area led you since then?

Dr Fasano: We used celiac disease as a paradigm for autoimmune diseases, but we are very interested in other conditions for which there are no treatments at all: type 1 diabetes, multiple sclerosis, rheumatoid arthritis, and so on. The total focus of our research right now is to understand what it takes for somebody who is genetically predisposed to develop an autoimmune problem, and to eventually have this zonulin pathway go out of control and increase antigen trafficking that will lead to autoimmunity. So, the 2 major goals we have are, first, to personalize medicine so we can stratify the population of people with these autoimmune diseases and find the ones who have the zonulin pathway more involved, so they can benefit from this peptide treatment and maybe eventually ameliorate the autoimmune process.

The second, and much more ambitious, goal is to test whether or not we can prevent autoimmune disease by studying the early steps that lead to loss of tolerance. To do that, of course, you need prospective studies from birth. Once again, celiac disease came to the rescue We decided to focus on infant research for celiac disease because they often have another family member who is affected by celiac disease, and therefore they have a much higher risk compared with the general population. We follow them

from birth and we monitor everything. We monitor Mom's lifestyle, the way that these babies are born, if they were breastfed or bottle fed, their infections, their antibiotic use, the vaccination calendar, if there are pets at home, other siblings … you name it. The goal being the timeline of the chain of events that leads to loss of tolerance and development of celiac disease.

We do this by monitoring the genome of these people, the microbiome, and how a microbiome imbalance, if we detect that, will affect the zonulin pathway and therefore increase gut permeability. In the meantime, we found that one of the strongest stimuli for zonulin release and upregulation is indeed gut dysbiosis. We monitor the metabolic profiling. Now, we will have a "crystal ball" to see all this coming together. If the storm is coming to the doorstep, you know that the next thing that happens is this kid will break tolerance to gluten, leading to the onset of autoimmunity. At that point, we can eventually intervene to stop this chain of events. Of course, we cannot change the genome of the kid because genetic editing is very complicated, particularly in autoimmune diseases. But we can definitely change other "ingredients" of the autoimmune recipe, like microbiome composition, and correct the dysbiosis so that the zonulin pathway is not activated and the child stays healthy.

***IMCJ*:** Intestinal permeability is a key factor in your research, but you have said that you are not a fan of the term *leaky gut*. Would you explain that position?

Dr Fasano: You should have noted that we are deep into our interview, and I haven't even once mentioned *leaky gut*. The reason why I don't is because, you are right, I don't like it. I don't like it because it has been used and abused over the years to explain some phenomena that sometimes turn out to be correct, but others definitely are not defendable as being linked to the loss of barrier function. During these years, interestingly enough, there has been a very polarized discussion on this matter. The believers are mainly, not exclusively, but mainly in the domain of complementary medicine. They really believe that leaky gut is at the core of many human diseases—to the point they made it a generalized concept. If you have a leaky gut, you will develop disease—period. The nonbelievers who were mainly, not exclusively, but mainly among conventional evidence-based medicine, said, "There is absolutely nothing true about this; it is not scientifically defendable." Like everything else, I don't believe that the truth lies only in 1 camp. In the real world, we do not have either black or white, rather we have shades of gray. The truth is something in between.

So, with molecular biology and human genomics building up the evidence that our interface barriers between us and our surrounding the environment, including lung barrier, gut barrier, and the blood-brain barrier, another major barrier between vessels' walls and the brain—are extremely dynamic, not static structures. So this antigen traffic is modulated all the time. With the evidence that there are structures that can be modulated, and the fact that many autoimmune diseases are linked to the presence of genes that control this antigen trafficking, even the conventional, evidence-based medicine world came to the conclusion that gut permeability is indeed a major element, along with many others, involved in the pathogenesis of autoimmunity. At the same time, some of the complementary medicine people trumpet this news and say, "I told you so many years ago, but you didn't want to listen."

But once again, there now exists a common ground, in between the extreme position of conventional, evidence-based medicine and the extreme point of view of complementary medicine. They see bona fide proof of the fact that this antigen trafficking business is important. Of course, the extremes are still there: the ones who still claim that leaky gut is a major deal for everybody, and the ones who still deny that this gut permeability has anything to do with autoimmunity.

***IMCJ*:** At the Institute for Functional Medicine's Annual International Conference, you will speak on Thursday May 31, 2018, and your topic is "Autoimmunity and the Interplay of Genes and Environment." Where does this discussion begin?

Dr Fasano: The discussion begins with the fact that we have to admit our ignorance about many things that pertain to human biology, including if and how and why people get sick. In other words, to review the state of the art of our current knowledge and see what kind of progress we have made toward the next step in understanding the pillars that lead to autoimmune disease. Looking at the overall program, my goal is to set the stage for the rest of the speakers and communicate to the audience why the program of the conference has being engineered this way.

Without giving up too much, here, there are several aspects that need to be revisited. The 5 pillars to develop chronic inflammatory disease, including autoimmunity, seems to be crystallized in (1) who you are genetically speaking, so your genome; (2) what kind of environmental trigger may eventually be at play to develop autoimmunity; (3) what is wrong with the immune system that turns on and does not turn off anymore, and why it unleashes inflammation against its own body; (4) why the gut barrier and other barriers are jeopardized, so that antigen trafficking becomes involved; and (5) the composition of the microbiome that eventually will move you, epigenetically speaking, from a genetic predisposition to developing autoimmunity to clinical actuality. Each of these 5 pillars will be covered by many of the speakers who make up the program for the meeting. And so, mine will be a 10 000-foot overview of what the state of the art of our current knowledge of the pathogenicity of autoimmune diseases is and where the science will go from here.