Does IBD Result From Our Genome's Influence On Immune Responses To Our Gut Microbiome?

Amirah Momen


Dr. Kenneth Croitoru

Dr. Kenneth Croitoru completed medical school at McGill University in 1981. From there he went on to train as an internist before further subspecializing in gastroenterology. He then went on to Postdoctoral training as a Medical Research Council of Canada Research Fellow in Mucosal Immunology at McMaster University. In 1992, Dr. Croitoru joined the Division of Gastroenterology at McMaster University where he became Training Program Director and Associate Director of the Division of Gastroenterology. Dr. Croitoru has been the recipient of extensive research funding from CIHR, The Canadian Association of Gastroenterology, and was awarded a 5-year Canadian Crohn's and Colitis Foundation (CCFC) IBD Research Scientist Award. Dr. Croitoru has served as Chair of the CCFC Medical Advisory Board and was instrumental in the development of the CCFC IBD Research Institute, where he served as Chair of both the Medical Advisory Board and the Executive Committee. In 2008, Dr. Croitoru joined Mt. Sinai Hospital at the Zane Cohen Centre for Digestive Diseases as a Clinician Scientist in the Division of Gastroenterology. He is also a full Professor of Medicine at the University of Toronto. Dr. Croitoru's research interests include investigating the role of both effector and regulatory T cells in the pathophysiology of intestinal inflammation in Inflammatory Bowel Disease (IBD). He is the lead investigator of the GEM project, a multi-million dollar study examining the genetic basis of IBD. Dr. Croitoru is featured in this edition of the UTMJ alongside his colleague, Dr. Dana Philpott. Together they have won a 15-million dollar Canadian Foundation for Innovation (CFI) Leading Edge fund which they have used to establish the University of Toronto Host-Microbiome Research Network (HMRN).

UTMJ: To start off, can you tell us a bit about how you became interested in gastroenterology and GI mucosal immunology research? What was that journey like?

KC: When I was doing internal medicine, I had [already] done an undergraduate degree in immunology, biochemistry and physiology, which was an unusual undergraduate combination of degrees. After medical school and internal medicine, I was looking at what careers would interest me and I realized that I wanted to do research, that I wanted a specialty where I would also have technical skills that I needed to develop; that led me to gastroenterology. Research was part of that thought process. When you sort of put it all on the table, it had to be a specialty where there was interesting research, it had to be a specialty that was interesting clinically. [Gastroenterology] had a bit of a surgical spin and sort of built on some of my interests from undergrad. So, gastroenterology and, specifically, inflammatory bowel disease seemed to start fitting those needs.

After I finished clinical training, I decided to do a Postdoctoral Research Fellowship in mucosal immunology. Mucosal immunology has, over the years, evolved to really focus on how the immune system of the mucosa interacts with the environment, and one of the strongest environmental interactions is that with our gut microbiome. So, understanding how that immune system responds to bacteria which are foreign, but how it doesn't allow for constant immune activation and inflammation really became an interesting question, and this whole area of immune tolerance to gut bacteria and even to food antigens and how it controls that became interesting to me. And then the whole notion that in order to understand that interaction you had to be able to look at both immune response but also at the character and the nature of the gut bacteria in that whole ecological niche – really, that has only been possible in the last five years with next-generation sequencing. The ability to sequence all of the bacterial DNA to understand what's there and not rely on culturing bacteria [has been revolutionary], because our culturing technology is very archaic …

UTMJ: Some of your work looks at how T cells maintain homeostasis in our gut and how dysfunction of their immune response can result in mucosal damage. Can you describe your primary research question(s) and how your research is designed to answer it?

KC: So, there are many ways to do this. One is using mouse models where you can manipulate the system,

take T cells out of a mouse that you have exposed to something or have kept germ-free. The other way is to look in the model of IBD; that is, actually [examining] the disease in people, in patients. One of the studies we're doing that is really trying to get at the heart of the cause of inflammatory bowel disease is this project called the GEM project. It's a very expensive, very long undertaking…I mean, we've been doing this since 2008, but the idea is that if you really want to understand what triggers the disease, you have to look at people before they get the disease. So, if you stop and think about that, how do you do that? You get a large cohort of people who are at high risk of developing the disease, you study them all, and as the people develop the disease you go back and say: 'aha, we have samples from when you were healthy, let's see what has changed!' This – will, hopefully – allow us to look at what is actually triggering, or causing their disease. If you don't do that, then it's hard to really cure this disease. These are complicated diseases where we know there's a genetic predisposition and we know there's an environmental trigger of some kind, and somehow that leads to an abnormal immune response.

This study is very much like the Framingham study, which you guys have learned about. That was a large cohort of normal people in a given geographic area, and this allowed us to identify those risks that contribute to the development of stroke and high blood pressure and coronary artery disease. The idea with the GEM study is similar, except that to do a population-based prospective cohort study in inflammatory bowel disease would be very expensive because you'd have to take the whole city of Toronto, for example, and in that expect to see…well, the current prevalence of the disease is about 1 in 150 in the country, so you'd have to see a lot of people before you'd see new disease developing. So, developing this high-risk cohort of healthy first-degree relatives [of individuals with IBD] allows us to look at this question.

The question we have is: Is there something that's abnormal in the gut microbiome of these people before they [present with] disease that tells you they're at risk of developing disease? If you identify that, then maybe you can change that somehow through diet, through giving them probiotics (for lack of a better term), giving them antibiotics…maybe even one day vaccinating them against a certain bacteria. So, this sort of approach allows us to then say 'Okay, let's define what changes in the bacteria and how we may stimulate the new response differently'. Then you go back to the mouse model and you say 'Okay, here's the bacteria, here's the immune response; can you remodel this in a mouse?' and now you can manipulate it to look for ways to actually treat it, drug it, develop either specific drugs to targets, or those other things that I just mentioned, [like] change the bacteria, give probiotics, give antibiotics or even vaccinate or boost the immune response.

UTMJ: So, this is a very large study that's long-term –

KC: The clinical part of this is huge. In the prospective cohort study, we have 3200 subjects. We've been doing this since 2008, but really more important is that we have 35 subjects who have developed disease, who have converted under our nose. We're projecting to see 70 new subjects [converting to having IBD] in the next 2 years. We're starting to do the analysis, sequencing their genetics, sequencing their gut microbiome and looking at how these associate with each other. The first-degree relatives that make up our study sample are either siblings or offspring [of individuals with IBD]. We guessed that 0.3 percent per year [would convert] based on what has been published, but no one actually knew what that number is, so that's what we're showing – we're tracking at exactly that number and it isn't much different for siblings than for offspring.

UTMJ: You mentioned that there could be potential therapeutic outcomes for this research. Do you set out with the goal of developing therapeutic interventions, or do you approach your research more focused on understanding the underlying cause of IBD?

KC: The hope is that there will be specific therapeutic potential from what we learn in this study. You can't get to the point of therapeutic interventions or changing disease or curing disease until you understand pathogenesis; you have to understand what's involved in causing this disease. [In] the first instance, we're still over on this side of the coin, which is trying to just understand what's going on, what triggers this disease in someone, why it is that their sibling doesn't [develop the disease]. There's only a 50% concordance rate for IBD in monozygotic twins; what makes one different from the other? Clearly it's not the genetics only. So, once you can understand that, then you have a handle on where to look to see how you can intervene. Our current funding is for another two or three years... in the meantime we're looking for other funding… all to keep this study going until we can take this and translate it into actual therapy. That's the challenge for clinician-scientists: what makes us different from PhD scientists (although even PhD scientists are trying to understand the same things) [is that] we have a better understanding of the clinical issue, the context, the patient, what we need, what treatments would work in terms of being acceptable to the patient. This study is quite a multi-disciplinary study; we have people on this project who are soil microbiologists, epidemiologists, geneticists, statisti-

cians (a whole army of statisticians) because no one person brings all of those skills together. Nonetheless, the clinician-scientist is important in trying to help direct that through the context of the patient, bringing that classic bed-to-bench-side-to-bed [into fruition]. I think everybody would like to take the findings and make a drug or make a therapy that works.

UTMJ: Have you thought about how to integrate current innovations in immune-therapeutic developments into your work or clinical practice? For example, with some cancers there have been efforts to engineer T cells to target cancer cells–

KC: Yeah. There are T cells that kill and T cells that regulate those killers. So, why is [it] that T cells don't kill everybody's cancers? Well, maybe they do to maintain or prevent cancer development, but once cancer gets going, the T cells, or the immune response, fails if effector T cells are not effective, or if regulatory T cells prevent them from working. So, either you try to increase effector T cells or decrease the regulatory T cells. In IBD, it's the opposite: we want to decrease the T cell response, or the immune response. So, if you have a way of increasing regulatory T cells you may be able to control that inflammatory response regardless of whatever the trigger is, or without knowing what the target is, because today we don't know what the target is. We did a study examining various models of colitis in a mouse and we had shown how, with an antigen, you can manipulate regulatory cells to become more effective. Then we went and did a study based on work done in a multiple sclerosis model … there is a drug that is used in transplantation called OKT3, an anti-T cell antibody, and it's used to prevent transplant rejection. So that mouse study in the MS model used the same antibody and fed it to the mouse and showed that you can prevent the multiple sclerosis model from developing. We went back to our colitis model and showed that you can do a similar sort of thing, you can feed the mouse this antibody, increase the T regulatory cell function and prevent the colitis model [from developing]. A lot of people are looking at how you can manipulate the T cell response. There's the immune side of things, trying to manipulate that in people, and then there's the target side of things, which is trying to identify the target better or modulate the target if it is indeed the intestinal gut bacteria.

UTMJ: So, that research is separate from the GEM project, right?

KC: I have a basic science lab at the Medical Sciences Building in the immunology lab area (I'm cross-appointed to immunology) where I do a lot of this fundamental T cell biology research. Then I have a clinical research program which is here at Mt. Sinai at the Lunenfeld [the Lunenfeld-Tanenbaum Research Institute] which is the home of the GEM project, which is this global prospective cohort study examining at-risk individuals. [That] allows us to create this bridge between the basic science people and the clinical science people, and that's how we got into the microbiome arena. So Dana [Philpott]* and I applied for this huge 15-million dollar Canadian Foundation for Innovation (CFI) Grant: it was for a host-microbiome network to set up the infrastructure, to buy equipment, to be able to do this in-human study and in-mouse study and back and forth.

**Editor's Note: An interview with Dr. Philpott is also featured in this issue.*

UTMJ: Can you describe how you are using second-generation genetic sequencing methods to look at how host genetics interact, or associate, with the gut microbiome to result in IBD? Are you then using that information to manipulate and study mouse models of IBD?

KC: We have data from the GEM project, a large cohort where we have done exome chip sequencing [on people], and we have sequenced their gut bacteria, and what we want to know is whether the microbiome is influenced, or modulated, by the host genetics. No one has really been able to show that up until now. There was a recent paper by Ruth Ley at Cornell where she showed that the microbiome is heritable. We actually have similar data from our healthy first-degree relative cohort that the microbiome shows heritability; when you can look at specific taxa of the microbiome. We are now in the process of doing a GWAS [genome-wide association study] and we have a number of signals of regions [of the genome] that show very high statistically significant association with various taxa in the microbiome. So, this is now going to be one of the first studies with data [showing] that there's actually genetic control of the microbiome. So, now you have this scenario where if you have abnormal genes, like the ones associated with Crohn's disease, that are genes that we know are involved in host-microbe interaction, like this gene called Nod-2. [Then] we can start to ask the question: Do Nod-2 mutations influence the microbiome? Our data suggests that the answer is, it doesn't.

UTMJ: [Laughs] I was not expecting that ending….

KC: Well, isn't science interesting? Dana has data in the mouse model that shows the same thing [as our data]. In the human, we're not able to show a Nod-2 influence on the gut microbiome. We're still look-

ing. Maybe we're missing it. These are really large dataset type experiments [where the] statistical probability of you missing something is always great, and [the] statistical probability of you finding something real is also uncertain. You need big numbers, big data, and big computers to play with it.

UTMJ: In closing, what do you predict will be the next big innovation in your field of research or clinical practice? What are you most excited to see moving forward?

KC: Defining the microbial profile that is triggering this disease. If we can show that, then all of this effort and money is worth it. But, that may take time. If you look at the Framingham study, that was started in 1950 and it's still going on today. People are still looking at that cohort as it evolves, and I think the same will be said for this type of a cohort and study because it will continue to teach us about the disease. Even if we identified a microbiome profile that is very high risk for you developing a disease, you still have to prove that; you still have to then show how you're going to try to manipulate it to make a difference. So, I think this [GEM Project] is probably the only study of its kind in the world. This is probably one of the most exciting studies; it's the most high-risk because God knows what it's going to lead us to, and it's costly, but it's that kind of research that really is outside of the norm. Most disease-related research is case-control studies and [that is] by necessity because that's what any one researcher can handle. But to really get at the question, you have to go beyond that. You may go back to case-control studies to verify things, but it's really going to be tough … unless it's so obvious of a disease, like with UTIs caused by bacterial infection and you see the bacteria … something like that. This [IBD] isn't a simple disease.

UTMJ: Why not?

KC: Because we know the genetics are complex, it's not a monogenic disease, and we know the trigger is not a single pathogen, because we've looked almost as long as the Framingham study for a single virus or single bacteria. It's not *Helicobacter pylori*, which we thought 'Wow, that would be neat! [if it was like] peptic ulcer disease', which we now know is caused by one bacteria; IBD is not that. If it is a bacterium, it's going to be a rare bacterium, or a bacterium that is gone by the time you see the patient with the illness. We think it's a change in the community of bacteria and how that community interacts with each other. That's a hard thing to define. So, it's a complex genetic, complex microbial, and complex immune response to all of that. We're in this for a while ….