July 2009 Issue | Kristina Harris, PhD University of Maryland School of Medicine

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Welcome to *Functional Medicine Update* for July 2009. We were very fortunate last month to start a two-part series on gluten and its relationship to immunological function. We had amazing interviews with Alice Bast and Dr. Doherty. That was a wonderful introduction to this important topic, but there are many questions still unanswered and we are going to pick up the topic again in this month's issue.

I don't want to overpromise and under-deliver; there will still be unanswered questions at the end of this issue. This is an evolving story that is opening up at a very dramatically rapid rate and it would be presumptuous of me to suggest that we'll be able to bring all the ends together and close this into a tidy, fully understood conclusion by the end of this issue. But I think we will make some extraordinary advances forward in our understanding of this issue, particularly because of the remarkable research that you are going to hear much more about from our researcher of the month, Dr. Kristina Harris, who works in the group of Alessio Fasano at the University of Maryland School of Medicine in the Department of Pathology. I think you are going to be absolutely amazed at what you are going to hear from Dr. Harris and her recent published work.

What you will be hearing first is Dr. Harris' interview about her work and the implications it may have beyond just celiac sprue. Then I will continue the discussion after the interview, and I will look at some of the other clinical implications, particularly things like neurological-related issues, such as autistic spectrum disorder. Without further ado, let's get right to the heart of the matter with Dr. Harris.

INTERVIEW TRANSCRIPT

Clinician/Researcher of the Month
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This is the place in Functional Medicine Update where our energy rises because we have a chance to hear from a leading clinician or researcher of the month. We are going to be doing two-part series on a topic that is extraordinarily important. This interview will be one of the components in this two-part series on gluten/alpha-gliadin and its relationship to autoimmunity and celiac disease. The reason this topic really

requires two parts is because this field is rapidly exploding with new information, both clinical and at the basic research level. To help guide this discussion, we are very fortunate to have with us Dr. Kristina Michelle Harris, post-doctoral fellow at the University of Maryland School of Medicine.

I think Dr. Harris has an extraordinarily interesting background. She did undergraduate work at Southern Illinois University Hospital where she did a lot of work in histology, which gives you good eyes to the world because you are looking at how cells perform and trying to understand, from the morphology of cells and their architecture, their function. From there she went to the University of Maryland School of Medicine in pathology and recently finished her PhD there. I call her a young investigator because with each passing year it is amazing how many people now are young relative to my age (so I guess it's a compliment to Dr. Harris). She has done some extraordinary work. I think the title of her thesis tells you why this is such an important topic for us to be discussing. The title of her thesis was "Investigation of IL-23 Response to Wheat Gliadin, the Primary Etiological Agent in Celiac Disease."1 Many of you may already be tapped in to the connection, but if you do not understand the Interleukin-23 component, you are going to learn much more about it today.

Dr. Harris has recently authored a number of important contributions to the literature. One of these articles appeared in the Journal of Immunology in 2008 and was titled "Cutting Edge: IL-1 Controls the IL-23 Response Induced by Gliadin, the Etiologic Agent in Celiac Disease."2

Dr. Harris, we welcome you to Functional Medicine Update. How did your journey in life go from your work in histology into the work that you are doing now?

KH: Well, that's a very interesting question. As with most things, they just kind of tend to happen. I was doing histology at SIU because that was one of the only laboratories that I was able to get into immediately after finishing my Bachelor studies and I wanted laboratory experience, period. We had a great histology director there. I was doing that work for the spring semester. To get a full-time job I had applied to various positions out here on the east coast. Dr. Mann brought me in and he had a really interesting project dealing with vaccine adjuvants for cancer, which is work described in one of the first papers that I published.3 So I actually came to Maryland looking at cancer immunotherapy and Dr. Mann brought me in as a lab tech to do that kind of project. I did that for about a year.

Another investigator here at the university is big in celiac disease research, and that is Dr. Fasano. He was looking for somebody to collaborate with in the field of immunology. At the time I was thinking about going back to school to do my doctoral studies, so we thought it would be a great collaboration to get together and let me kind of take over the immunology aspect of the celiac studies. I was particularly interested in the innate immune response because of the discovery of IL-23 and the IL-17 pathway in tissue-specific autoimmunity.

JB: That's a wonderful introduction. I like to think of myself as a student of science and a reader of the journals. Occasionally when we are reading science we hit on an article that just lights us up and it is an "a-ha" experience. I really want to compliment you because I think this cutting edge article in the Journal of Immunology that I described earlier (co-authored with Dr. Fasano and Dr. Mann) is really one of those "stand up in the crowd"-type articles. To me there are so many things not too far below the surface in this article that are extraordinarily important. It's very rich and really good work. I think you are to be complimented on the quality of this work. Let's get into it.

KH: Thank you so much.

Reviewing the Major Players in the Immune System

JB: Thank you. For those who are not as familiar with the whole immunological cascade as you are, let's kind of review the players in the immune system that relate to environmental responses in the gastrointestinal-associated lymphoid tissue (or the GALT). Maybe you could tell us a little about the dendritic cells and the peripheral blood mononuclear cells and how they get activated to produce pro-inflammatory cytokines, and the CD16 cells, and the Th-17. Maybe you could take us through the players so we understand the cast of characters.

KH: With the gastrointestinal immune system, the epithelial layer really provides the first barrier to the environment. This is really a unique lymphoid organ because it is exposed to a vast array of exogenous antigens from food and commensals that line the intestinal tract. It is really important that the epithelial cells and these intestinal dendritic cells (which also can kind of extend their dendrites out into the lumen) have cross-talk and kind of maintain a nice homeostatic level of inflammation and a healthy gut. Upon insult by either an invading pathogen or tissue damage, these dendritic cells, or monocytes that come in from the peripheral blood, actually can sense various pattern molecules. When they detect these danger signals (as we like to call them), they will then become activated and secrete numerous types of chemicals that we like to call cytokines and chemokines, that then direct the downstream events (i.e. the adaptive immune response).

JB: How does that relate to things we have often heard about: the thymus-dependent 1 and the thymus-dependent 2 (or Th1 and Th2) type of responses? We recognize that that is kind of a simplified view; there is really much more orchestration.

KH: Depending on the combination of cytokines that these dendritic cells (or other types of antigen-presenting cells) secrete, that will ultimately direct which type of T-cell response you get (if it is Th1, Th2, or now the Th17). We also have regulatory T cells that are involved in all of this. It is really a complicated process with a lot of different entities interacting/controlling these responses.

The Role of Toll-Like Receptors in the Signaling Cascade

JB: Sitting on the surface of all of these immune cells are different receptors that pick up these messages (these exogenous messages), one of which is the family we have heard a lot about recently: the TLRs, or the toll-like receptors. Can you tell us a little bit how the toll-like receptors fit into this signaling cascade?

KH: The toll-like receptors are considered a pattern-recognition receptor. They will recognize certain sugar structures/ lipid structures that are conserved on bacteria or fungus. Upon sensing them, they induce a cascade of activating signals within the dendritic cell that then leads to transcription of the pro-inflammatory mediators that I was describing.

JB: Good. Now we are getting to the next level of complexity. Could you tell us a little bit about Th17? What is its personality relative to these thymus-dependent lymphocytes?

KH: Sure. For many, many years autoimmune disease was ascribed to the IL-12/Th1 paradigm. I'm not necessarily saying that should be completely discarded because I think that's still a really important part of the process. But now we have this other player involved-the Th17 cell-and it's primarily thought to be a

pathogenic memory-type CD4 T cell. In response to IL-23, IL-1beta, and perhaps IL-6 and TGFbeta, these cells will secrete pro-inflammatory cytokines that then recruit neutrophils and other types of cells that directly destroy the surrounding tissue.

JB: Good. Now you have introduced IL-23, a new cytokine that has to do, somehow, with the antigen-presenting cell physiology. Where does that come from?

Interleukin-23 (IL-23): A New Cytokine and Its Role in Cell Physiology
KH: IL-23 is primarily produced by antigen-presenting cells, so dendritic cells, macrophages, monocytes. No one has ever provided any evidence of that any lymphocyte population can make this potent pro-inflammatory cytokine.

JB: Is there a strong connection, then, between secretion of IL-23 and various autoimmune diseases, and if so does it cut across multiple autoimmune diseases or is it specific for a certain diagnosis?

KH: Yes. There is a very tight correlation with increased levels of IL-23 in various different types of tissue-specific autoimmune diseases, so rheumatoid arthritis, psoriasis, multiple sclerosis, Crohn's disease- all of these autoimmune diseases have been associated with increased IL-23 production at the lesion.

JB: I don't want to lead you into saying something you don't feel, but let me just tell you quickly about the functional medicine concept and see what you think of it. The functional medicine concept is less focused on differential diagnosis and more focused on what we call patient-centered assessment, which relates to antecedents, triggers, and mediators producing signs and symptoms of different duration, frequency, and intensity in the patient. So rather than what we call it, it's where it came from that is the focus in the functional medicine model. To look at the antecedents, which in the model could be things like HLA-DQ2, for instance, and then we'd say a trigger could be gluten, and then a mediator could be IL-23, and it would span out into a variety of different clinical presentations depending upon the patient's own individual characteristics. It is less what we call it; it is more the mechanism by how it got there. Does this, at all, ring into this model that you are describing with some resonance?

A Focus on the Production of IL-23 Rather than Downstream Effects
KH: Yes, exactly. That's actually why I was so excited about this project and why I think it is so important, because upon discovering IL-23 and the Th17 pathway, it seemed like everybody was focused on the downstream effects of IL-23 (the specific effects within each different type of disease). But nobody was really paying attention or focusing on what was initiating the production of IL-23 and that's why we decided to go that angle, because it seemed like if you block that then you could potentially have therapy for multiple chronic inflammatory diseases versus just one.

JB: I'm having fun. I hope you are...

KH: I am. Absolutely.

JB: You are teaching me a lot. Now let's move from that to celiac disease. In my past nomenclature, using the kind of differential diagnosis etiology model, celiac disease is considered a Th1-dominant disease. But now it would suggest, from what you are saying in your work, which you are going to go into, that this IL-23/Th17 pathway may open up different ways of thinking about celiac disease beyond just a

Th1-dominant disease with no other confounders. Is that reasonable to say?

KH: Sure. Yes. Absolutely.

JB: Tell us a little bit about how you came to that understanding.

Research Opens Up Different Ways of Thinking about Celiac Disease
KH: We wanted to compare the innate immune response, looking at IL-23 and IL-1beta and the other innate-type cytokines in people that have celiac disease, and compare those responses to individuals that have the HLA type but are perfectly healthy and never developed the disease. We thought this might point us in the right direction of what is different between these individuals. What is so confusing about celiac disease is that about 30 –
40{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} of the normal healthy population share the DR3-DQ2 haplotype and are exposed to these dietary glutens regularly but they never develop the disease. We think that might be because they have different genetic polymorphisms in the genes that encode the innate-type proteins. We took peripheral blood cells from either celiac patients or the HLA-DQ2-positive healthy individuals, and we just exposed them, in vitro, to the gluten-derived antigen, gliadin. And then we harvested the cell culture supernatants and measured the levels of IL-23 and IL-1 and the other cytokines. What we found was that the peripheral blood cells from the celiac patients produced significantly higher levels of IL-23 and the related pro-inflammatory mediators in response to gliadin than did the healthy donors, indicating the IL-23 pathway may be part of the pathogenesis of celiac disease.

JB: I don't want to interrupt, but there is a question that was burning in my mind as I read your work and I may have missed this...I'll ask the question and see if I missed it. In your healthy population, did that include any of the genotyped HLA-DQ2 or DR3 people that didn't have symptoms?

KH: Yes. In the paper that we published in JI, all of those healthy donors were DR3 and DQ2 positive and did not have disease.

Study Focuses on People with the Genotype Who Do Not Express the Inflammatory Condition
JB: I want to make sure our listeners understand that because I think this is a very critically important thing. There is 95{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} penetrance, if I'm not mistaken, in celiac disease, with HLA-DQ2, and DR3 positives, and so I think clinicians might think that that is a one-to-one correlation. But what we are saying here is that there are people with those genotypes who do not express the inflammatory condition, so it is only an antecedent, and it is not an expression pattern yet.

KH: Right. I would say the majority of individuals that are DR3 and DQ2 do not develop celiac disease. Only like 1{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36}.

JB: For the other 99{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} of people with those HLA haplotypes, do they go on to get other autoimmune diseases or can we say that they are just like the run-of-the-mill people? They don't have any increased prevalence of any autoimmune disease?

KH: I think we can say that about 35{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} of healthy individuals have that haplotype and remain healthy. But I would say-and I think it's important for people, if you are looking for a possible diagnosis of celiac disease-if they have relatives that have other class II-associated autoimmune diseases, like type 1 diabetes or rheumatoid arthritis, etc., that those individuals are about 8 to 15 times more likely to develop celiac disease than someone that doesn't have autoimmune diseases in the family.

JB: I think that is such a clinically important observation you just made because in our functional medicine model (to restate what I've already said), what we call it is not as important as the soil in which it was embedded. If there is a family susceptibility to immunological disturbance, then it may present in one as MS, another in type 1 diabetes, and a third...

KH: Right.

JB: Exactly. Rather than the diagnosis being a kind monozygotic penetrance into the disease, basically.

KH: Right. It seems like depending on the combination of what you inherit you may or may not develop one of these diseases.

Gluten-Derived Gliadin Peptides and Innate Response

JB: Let's move to the next part of your incredible work. Tell us a little bit about what you think is unique about gluten-derived gliadin peptides and the response (the so-called PTGs-the pepsin-trypsin digest of gliadin), which I always find interesting because the pepsin-trypsin digest produce proteoids, or these peptides, that may have different immunogenicity or haptenic effects than that of the intact protein itself. Tell us a little bit about that.

KH: When we do the pepsin-trypsin digestion we get a very heterogeneous product, which consists of basically at least over 50 different proteins. This makes it very difficult to narrow down what exactly is inducing the innate response (for me, anyway-I've actually been trying to do this for about the last four months now and I still haven't been able to pinpoint exactly which fractions, or which epitopes, are inducing this inflammatory response in my dendritic cells). But we do know for the T-cell response, that the pepsin-trypsin gliadin contains epitopes that are particularly well-suited for the DQ2 and DQ8 binding pockets, so this has helped explain the DQ association and the adaptive immune response. But as far as the properties of gliadin that are inducing the innate immune response, I think it has to do with glycosylation, and I think that certain fractions of the wheat gliadin are probably involved because only certain fractions are thought to by glycosylated, whereas the others are not. But I have not been able to pin this down, so I can't say that with any definition.

JB: Again, I think where we are going here-where you are taking us, not where we are going, you are taking us-is extraordinarily important new territory. Even without having the answers, the questions themselves are very clinically important. For the sake of the clinicians who are not necessarily immunologists, let me make sure that they are following along.

Dr. Bland Summarizes the Research

You have observed that these pepsin-trypsin digests of gluten, which have this composite of different weight molecular peptides, some of which are glycosylated and some of which may not be as

glycosylated, can trigger monocyte release of IL-23, and there then could be immune-dominant epitopes present within that mixture-you're just not yet sure which they are when you do a fractionation-but some of them do have preferential binding for the pockets of HLA-DQ2 and DQ8 or DR3. Am I summarizing what you just said correctly?

KH: Yes. Excellent.

JB: Now the question that we might have is: Given that this pepsin-trypsin digest that we do in a test tube is at least similar to what goes on in the human...?

KH: Right. We do this because this is supposed to be most representative of what happens in the digestive tract.

JB: So now we have different digestive functions among different people.

KH: Exactly.

JB: So could that account for differing populations of these immuno-dominant epitopes from gluten?

KH: Perhaps.

JB: Part of your work, I think, will be taking us through something related to looking at how things need to be broken down maybe to lower their immunological memory.

KH: Exactly.

Different Results with Synthetic Peptides
JB: So now let's ask a question that I know you have studied because I've seen it in the paper. What happens if you make synthetic derivatives of the alpha gliadin-type peptide sequence? I know you are studying incubated p31-43 alpha-gliadin peptides, or 25 overlapping synthetic peptides spanning the entire sequence of alpha-gliadin. What did you learn from that, with these synthetic peptides?

KH: These synthetic peptides did absolutely nothing as far as inducing the pro-inflammatory response that I get with the whole pepsin-trypsin digest. They are missing something. They are not the proper structure, and they don't have any of the post-translational modifications. It is just peptide. I really do think that ultimately it is going to come down to some carbohydrate structures on there that are important for the innate immune response.

Beta Glucan Also Showed Positive Response
JB: That ties to something that for a lot of the clinicians listening may be kind of an "a-ha" for them (it was an "a-ha" for me). I think I knew it, but you really drove it home with your article much more strongly for me. Tell us a little about beta glucan, because beta glucan also showed positive response in IL-23 as I read your paper, but we don't think of beta glucan as being a gluten-related molecule.

KH: Right. We used beta glucan because other immunologists had used that. Mainly they'll pick beta glucan from yeast or something to induce the IL-23 immune response. We opted to go for beta glucan

from barley because barley is one of the triggering agents in celiac disease. With beta glucan you just have glucose molecules; I think it is over a thousand glucose molecules that make up beta glucan. What we found is that if we just add beta glucan alone, that we could induce IL-23 from the monocytes and the DC subsets. What was interesting is that it took way higher concentrations of the beta glucan compared to the pepsin-trypsin digest of gliadin. On the one hand it looks like we are getting a similar response, but I don't know that they are initiating it the same way. It seems more likely that the pepsin-trypsin gliadin might be using a 2-receptor signal because there is protein and carbohydrate and multiple epitopes in there, whereas the beta glucan is just a bunch of glucoses.

JB: I think it is important, again, for clinicians who are listening, to make sure they understand this concept of structure/function. Yes, beta glucan is just a polymer of glucose, but it has a specific branched configuration of the way glucoses are put together into the polymer that is different than starch, amylose, or dextrins. I believe this construct that shape of molecules triggers immunological response is a very important part of the story for people to understand because I think there is a simplistic view in the minds of many that somehow gluten is just a foreign protein no matter what we call it, when really gluten is just a descriptor for a whole complex array of molecules that are both in the gluten fraction and can get further broken down into other peptic digests that then have different personalities, so we have to be conscious about what we call things, I believe.

KH: Right. Absolutely. In fact, I really don't like that gliadin is called gliadin because it seems singular. Whenever anyone ask me, "What is it?" they expect me to say, "It's an octopeptide," or something, but it is a huge array of proteins. You are absolutely right.

JB: In your paper you made an interesting comment. I'd have to say it is pushing at my edge of understanding, so maybe you can help us. You said (as I recall) that the response from this work with the synthetic peptides and the p31-43 alpha-gliadin peptides suggests that the response had to be derived from beta, gamma, or omega gliadin, not necessarily alpha. Could you help me understand what that means?

Innate Immune Response May Be Tied to Omega Gliadins: More Research is Needed
KH: Sure. Within gluten there are the two main protein fractions: gliadin and glutinen, which are also thought to be antigenic in celiac disease. But then gliadin can be broken down further into four other fractions: omega-5, omega-1,2, alpha beta, and then gamma gliadins, depending on their amino acid structure, their glutamine content, and their molecular weight. The synthetic peptides that we used were based off the alpha-gliadin structure. We didn't look at any of the other (the omegas or the gamma gliadins). Actually the omega gliadins are the ones that have been shown to be glycosylated. I really think that these are going to be the ones that are important for inducing the innate immune response, but I haven't proven that yet.

Could Different Cultivars Be Related to the Increasing Frequency of Gluten-Related Problems?
JB: I've had this question asked of me: "Doesn't it seem, Jeff, interesting to you that this frequency of gluten-related problems seems to be increasing at a fairly dramatic rate in our population?" If you look at the literature it went from 1 in several thousand to 1 in (depending on what literature you want to look at) 300 or something like that. Could this be because we have different cultivars of wheat now, or grains, that have differing epitopic presentations, or is it that our immune systems (as humans) are all confused? Could it be the fact that what we think is gluten isn't the same gluten that we ate when we ate the original

cultivars in Italy, or something?

KH: I think the different cultivars could be part of it. I also think a lot of that increase in frequency has to do with detection methods, because a lot of cases went undetected for awhile. As far as immune response, there is still the issue of molecular mimicry. If you have more virus or vaccines that might be triggering immune response it might, accidentally, cross react with gluten peptides. All of these things could contribute to the increased incidence.

Could Gluten Sensitivity and Autistic Spectrum Disorder Share Common Factors Related to Immunological Alteration?
JB: That's very interesting because one of the questions that I've had asked of me is, "Do you think there is a correlation between the rising frequency of what we call autistic spectrum disorders, which is immunologically related dysfunction, and that of gluten sensitivity?" It doesn't necessarily mean one causes the other, but maybe they share common factors related to immunological alteration. It's an interesting hypothesis.

KH: It's very interesting. This has caught my attention on several occasions. Recently it seems like there is a lot of autism awareness out there. And rotavirus-that one really perks my ear because there are antibodies in celiac patients that cross react with rotavirus. There does seem to be a connection, here. It is definitely something we need to think about.

JB: Again, I'm trying to be cautious not to lead you into things that you feel uncomfortable saying, but it seems to me in reading your papers that the HLA-DQ2 genotype in symptom-positive people may not have a severe presentation of symptoms unless there are other underlying factors like the things that stimulate IL-1beta production, because it seemed like IL-1beta aggravated the production of IL-23 and maybe made the condition more amplified. Am I on the right track, here?

KH: You are, yes. That was one of the surprising things we found, that IL-1beta, alone, could induce secretion of IL-23. So if you have a situation where you have overproduction of IL-1beta, which is a pro-inflammatory cytokine, versus its natural inhibitor, the IL-1 receptor antagonist, then ultimately you would be driving an IL-23 response. This is an imbalance that has been noted in multiple different types of chronic inflammation. It goes back to that functional medicine [concept].

JB: The basic concept of kind of a network biology approach to looking at the immune system...

KH: Right.

JB: So if you had an underlying inflammation that was associated with higher levels of IL-1beta and lower levels of IL-1 receptor antagonist and then you laid on top that a DQ2 and a gluten diet, now you might be loading the dice, is what we are saying.

KH: Right.

JB: You can see-I'm tracing with you my thinking as I walk through your paper-why it was so profound for me. It then suggests that there are other inflamed tissues beyond the mucosa of the small intestine that could be influenced because it might be produced locally but act globally, and so we start talking about

IL-1beta inducing IL-23 p19 mRNA in human synoviocytes and myofibroblasts, which suggests to me that maybe things happen systemically from what we thought of as a regional GI problem. Is there something to that?

KH: Well I definitely think that the gut is connected to just about everything else. I don't know if you are aware of the skin condition of celiac disease (the dermatitis herpetiformis), where people actually develop skin lesions and if they go on a gluten-free diet then it is completely remedied. And then there is also gluten ataxia, which affects the nervous system. So I think this immune response that may initially start in the gut can definitely affect extra-intestinal sites.

JB: That would talk, clinically, to things like the reports talking about MS-like symptoms in patients with neuritic plaques that, when put on a gluten-free diet, the plaques remain but the symptoms go into remission.

KH: Yes.

JB: There may be something about neuritic inflammation, here, beyond just a plaque formation.

KH: Yes, or the autistic children that go on a gluten-free casein diet and seem to do much better with that.

JB: This leads us to ask how we take all of this extraordinarily interesting and complex information and take it from the research lab to the clinic. Are there certain tests that we should be doing? What about genetic testing for celiac predisposition? What kinds of things lead us to the right questions, as a clinician?

KH: I think the first thing is heredity. Does the family have a history of other autoimmune-type diseases? And then the test for detecting celiac disease is relatively easy-you just do the antibody for tissue transglutaminase from the peripheral blood (at least for the screening). So that would be an easy way, I think, to screen for celiac disease.

JB: And if a person was found on a genotypic test to be DRQ2-positive, meaning at-risk to gluten-related sensitivity, but they didn't present with clinical symptoms, would they go on a gluten-free diet, do you think, or do you think they would look for the antibodies, or they would look for mucosal biopsy?

KH: Do the mucosal biopsy. I don't want to complicate matters, but there is also gluten sensitivity that isn't celiac disease. And only half of those people have the DR3-DQ2 haplotype. This is yet another facet of what dietary gluten can do.

JB: How can that be? Tell us a little bit about that. How can you have this immunological thing going on without having digestive....

Multiple Factors May Be Involved in Innate Immune Response

KH: Well they don't develop the autoimmunity, so it appears that it gets initiated. With what I am doing perhaps maybe the gluten is inducing the innate immune response, but then at some point it gets regulated. But these people are uncomfortable; they don't feel well, but their intestinal epithelium is still intact. But they do have an infiltration of lymphocytes within the epithelium, and if they go on a gluten-

free diet then it remedies. This is new information to me and I'm still trying to process it. It's very interesting that only half of them are the DQ2. I think that perhaps the combination of genes that may be involved in the innate immune response may be the same in these individuals and those with celiac disease, but yet there are other genes or environmental insults that are then adding the push toward to the autoimmune disease and celiac disease, but that doesn't occur in these individuals.

JB: Wow.

KH: And it is not an IgE allergy, either. We're not exactly sure what it is yet, but I'm just putting it out there.

Could Probiotics Favorably Influence the Antigenicity of Gluten in People with Gluten Sensitivity?
JB: That's fascinating. I could continue this discussion ad infinitum, but I'll restrain myself and ask just one last question. I have seen some reports recently (preliminary reports) suggesting that there are enteric bacteria, or what might even be called probiotics, that could favorably influence the antigenicity or the epitopic sensitization of gluten in people that have gluten sensitivity.4,5 Have you seen any of these reports and if so is there any plausible mechanism that you can think of from your work that would explain that?

KH: I haven't read those reports yet, but as soon as I get off the phone with you I'm going to because I had actually thought of this, too, with regards to the IL-1 receptor antagonists and the IL-1beta story. I had thought that if you could introduce a probiotic or something that would upregulate the anti-inflammatory mediators, such as IL-1RA, that that might be protective for patients with celiac disease. That would be a possible mechanism-that these probiotics are inducing immunoregulatory mediators that are keeping the immune system at bay.

JB: I can't tell you how much I appreciate this. You should see my face-I've got a big smile. This conversation really lights up every neuron of cognitive interest that I've got because I think you are really bridging many different disciplines. This is truly integrative, translational research. I think it is opening up a whole new door. I think it is courageous research because it is not so siloed; it has many different implications. I applaud what you are doing, and if you stay on this track we are going to be hearing your name all sorts of places because this is very important work. I hope you are getting support from your associates and from the funding agencies to continue this work.

KH: Thank you so much for having me. This is really quite an honor to get to do this. I really appreciate it.

JB: I just want to say in close that this is, as I said, is the first time in 27 years that we have had a new investigator who is in the first phase of her clinical and basic science research talk to the community. I say this sets a very good precedent. Thank you very much for charting new territory for us.

KH: Thank you.

I hope that you were as excited and stimulated by the discussion that we just had with Dr. Harris as I was. This is one of the landmark, threshold-types of discoveries, I think, in terms of helping us to understand and pull together information about gluten and its effect on the immune system. Why are we seeing more

prevalence today? Is there a difference in the composition of gluten? What about our grains and our agricultural methods and our seeds? And how about the body's immune function? Are there other covariables that work together to trigger immunological dysfunction and make a person's reaction to gluten more prevalent? All of these are very, very interesting questions. And then what does the gut have to do with this? Is the gut ecology of some importance as it relates to gluten sensitivity? That question will be further advanced as we move into the August 2009 issue. We are going to be discussing with Dr. Delzenne and Dr. Cani (from Université catholique de Louvain in Belgium) some extraordinary work they are doing on the gut microecology/the microbiome and its relationship to immunological function.

Dr. Bland's Interview Takeaways

Let me go back and pick up a couple of the very important points laid out by Dr. Harris in her interview (to review a couple of the clinical takeaways). First of all, we recognize celiac disease is categorized as a member of the autoimmune disease family related to auto-antibodies of the small intestine that then lead to localized and regional inflammatory response and ultimately can produce histopathology of the small intestine. We recognize that there are certain genetic lineages or linkages to this condition through the HLA-DQ2, and to some smaller extent the HLA-DQ8, polymorphisms, which then code for increasing susceptibility to adverse or immunological response to gluten. This appears to have some relationship to the composition and structure of individual members within the gluten family.

Gluten is a Term that Applies to a Family of Different Proteins
Recall, if you would, that gluten is a term that applies to a family of different proteins that have similar electrophoretic mobility and similar kinds of personalities as it relates to their primary amino acid sequence. But there may be differences in specific composition within the members of the family as it pertains to post-translational glycosylation reactions, and so they may have different degrees of glycosylated residues that change their epitopic personalities slightly, one member to another. So we might say "gluten" as the general generic family, but we really should be talking about the specific members within the gluten family that are the antigenic determinants that really create these autoimmune-type responses in genetically susceptible individuals.

I believe Dr. Harris said that the HLA association only accounts for about 40{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} of the genetic requirement for Crohn's disease, celiac disease, and its relationship to gluten. It's not just kind of a hard-wired genetic effect; there are many people that are carriers of the HLA-DQ2 polymorphism that never experience celiac disease, even when consuming gluten in their diet. Why? Why are some individuals experiencing the condition and others not? I think that is the environmental functional variable that is the interesting component of Dr. Harris' presentation.

The Relevance of Dr. Harris' Research
What did the Harris and Fasano group really help us to understand? They helped us understand that although many individuals that carry the DQ2 and the DQ8 polymorphisms who consume gluten never experience the disease, there are other variables that relate to the specific personality of these antigenic determinants and autoimmune disease in these individuals that modify the expression of these characteristics. Their group recently provided the first evidence that this IL-23 immune response pathway may be involved in the cascade of events that manifest themselves ultimately as celiac disease. This new connection to IL-23 and its connection back upstream to the release of IL-1 and TNFalpha by the gut-

associated immune system may then help us to understand covariables that set the stage for the DQ2 and the DQ8 polymorphisms to be more susceptible to expressing themselves as celiac disease. So we are talking about covariables, or modifiers (other factors within the web that set the stage for the person, then, to express these characteristics more overtly as celiac disease). So this IL-23 immune response pathway is involved in the cascade of events that manifest as celiac disease.

IL-23, as Dr. Harris pointed out, is a potent cytokine that is related to innate immunity. It has been implicated in the pathogenesis of other tissue-specific autoimmune diseases, particularly those that influence the endocrine system, like thyroiditis. When we go into this discussion a little bit more deeply, we're talking about systemic autoimmune susceptibilities, as well as regional effects on the gastrointestinal system. I think this is where the topic gets much more broadly implicated for looking at immune-related dysfunctions of a wide variety of different diagnostic codes, including, possibly, even those of the nervous system (having to do an anti-myelin antibodies/anti-phospholipid antibodies) with cardiovascular disease), and spreading this into other areas where we have this immunological activation that occurs systemically through these pathways.

The downstream effects of IL-23 have been the primary focus of autoimmune research recently. The agents that initiate the production in the context of autoimmunity are still being developed or understood. By looking at IL-23 response induced by gliadin, Dr. Harris' and Dr. Fasano's group was able to actually demonstrate that this (for the first time) looks like a primary etiologic agent in the expression of celiac disease. And what they went on to show, just to remind you, was that the CD-16 monocytes were identified as the primary source of IL-1beta, and ultimately trigger the production and expression of IL-23 as related to the ingestion of gluten. It was found to be significantly overexpressed in the peripheral blood mononuclear cells from Crohn's disease patients, suggesting a role for this activated pathway in the pathophysiology of Crohn's disease. Neither monocyte-derived immature dendritic cells, nor monocytes that were colony-stimulating-factor-derived macrophages recapitulated the response, however (as was discussed by Dr. Harris), incubation with interferon gamma generated a population of these dendritic cells that ultimately secreted IL-23, IL-6, and tumor necrosis factor alpha upon exposure to gliadin. There has to be the priming of the cells by some kind of low-grade inflammation, it appears, before these cells become reactive to gluten and then release IL-23 and start this cascade of events that seems to be associated with the pathophysiology of Crohn's disease.

These are obviously novel findings. They are pretty remarkable new findings that suggest a functional role for a number of candidate genes other than just HLA-DQ2 and HLA-DQ8 that are associated with altered innate immune response and activation of certain pathways associated with inflammatory conditions, and even suggests that there has to be a presaging, low-inflammatory potential going on at the gut to amplify the relative expression of cells that secrete IL-23 and start the cascade of events that we ultimately diagnose as Crohn's disease. As you heard from Dr. Harris, one of the other interesting features of this work was the recognition that you could initiate some of the same response by agents other than gluten that are found in specific foods. Admittedly, these agents had to be at higher levels in order to initiate this reaction, but it didn't appear as if it was necessarily gluten-specific; there were other reactive molecules that were glycosylated glycoprotein-type molecules that could initiate these IL-23 responses as well.

One of the most remarkable features of this discussion with Dr. Harris-and I'm sure you took this away-was that the patterns of glycosylation of specific gluten proteins appeared to be directly related to the

effects they would have on triggering (through a specific cell type) the release of IL-23 and initiation of this condition. That would suggest that if you had gluten without certain glycosylation patterns of those proteins it might still be gluten in terms of its definition by electrophoretic profiling on a protein map, but it may not be an antigenic determinant/epitopic determinant for this immune-type response that produces inflammation. This may explain-I want to speculate on this for a moment-why people have been able to travel in certain places in the world that have other cultivars of grains and consume wheat-like products, and have more tolerance of those wheat-like products than they have had by eating grains in the United States. Could our glycosylation patterns of gluten proteins have been modified through genetic hybridization in such a way that we actually are starting to witness altered epitopic determinants that then trigger this cascade of events that leads to IL-23 release and so forth? That's a question that I don't think has been fully resolved nor answered, but I think it is a very interesting operational question that deserves more explanation and exploration. This may also help us to understand why there have been reports that have demonstrated that various types of enzyme preparations, when orally consumed, may help patients who were previously gluten sensitive tolerate gluten-containing grains. The increased digestion resulting from breaking down glycosyl residues on gluten proteins and reducing antigenic polypeptides down to leuko-antigenic digestive products may then result in lowering the memory of antigenicity and making them more neutral to the immune system. We are starting to witness examples of possible therapeutic agents that can be orally administered to try to make these grains more tolerant to people that have some degree of sensitivity (immunological sensitivity) by reducing their immunological recognition to the inflammatory cascade, presumably by their influence on gut-immune function and ability (possibly) to secrete enzymes that help break down glycosyl residues and alter the antigenic determinants within these gluten-containing molecules. What we are starting to see is the gastrointestinal milieu or the gut microbiome may play roles also in increasing or decreasing the relative sensitivity to gluten. This opens some very interesting and important potential therapeutic doors. For most patients, when you tell them they have to be on a total gluten-restricted diet (gluten-elimination diet), it's a very, very complicated thing to live in the "normal" world because gluten, in very small amounts, is found in so many different products and even at trace amounts may contribute to this sensitivity. If one could make it more easy for a patient to actually modify their gluten intake by still being able to live in the normal world, this would improve compliance, adherence, and certainly improve clinical outcome. It may be that new digestive preparations-enzyme preparations and different strains of probiotic organisms--can help to improve gut ecology and gut function in such a way as to lower the immune reaction to certain levels of gluten-containing materials.

I want to emphasize there are those people who are exquisitely sensitive to these molecules. It is like a peanut allergy: sometimes peanut oils being cooked at a restaurant can produce enough antigens in the vapor to trigger anaphylactic response in a person who has a peanut allergy. I don't want to underestimate the seriousness of gluten reaction and immunological sensitivity. I think it is important to demonstrate or to at least understand the range of reactions that people can have (from very acute immune response to more mild response). I'm speaking about modifying those that are more on the mild end of gluten response, and making their regime and diet a little bit more easily complied with. For those people that have the extreme gluten response, I think elimination (rigorous elimination) is the sine qua non for their management.

There is a clinical art in this; it can't strictly be determined by antibody testing. I think the proof of the pudding is the clinical outcome in the patient with elimination/provocation. People that can't even stand 20 parts per million of gluten in their food are those individuals who are obviously on the side of very

high sensitivity and have a large immunological reaction. But, as has been pointed out, one of the variables that can lower sensitivity to gluten (based upon the work of Dr. Harris and Dr. Fasano) is to lower the general immune response and inflammation vigilance of the gut-associated lymphoid tissue by resetting the gut function. There are many people that have chronically activated gut-immune system inflammatory response just by the nature of eating a high fat/high sugar diet; they put the immune system of their gut on notice continuously. Do you know the old Pasteur saying "Chance favors the prepared mind"? If the immune system of the gut is already activated then it is more readily sensitized to other triggers, like these antigenic determinants/epitotic determinants in gluten, which can put the straw on the camel's back and push this over into an acute inflammatory response.

I think gut ecology becomes a very important part of the therapeutic approach towards patients with gluten sensitivity. Even for those patients (as Alice Bast pointed out in the June issue) that have been put on a rigorous gluten-free diet, often years of activation of their gut function results in inflammatory, low-grade, chronic problems of their gut-immune function, which needs to be normalized to restore really good health and to get them feeling good again and to absorb nutrients more effectively and restore their gut mucosal absorptive surface. I think the 4R Program, which we've discussed so many times over the years in*Functional Medicine Update* becomes, again, such an important clinical tool: Remove, Replace, Reinoculate, Repair.

I think this is almost like a mantra if you have listened to *Functional Medicine Update* over the years. "Remove" means to get rid of the organisms that may be parasitic or those organisms that could produce toxic byproducts. Get rid of the food allergens, get rid of the environmental chemicals that might activate immune-gut dysfunction. That is the Remove "R."

The next one is "Replace." That is to assist by improving digestion and bile acid secretion. If necessary, this can include exocrine pancreatic replacement therapy using digestive aids to acidify the chyme in individuals that have aplastic anemia type-B with parietal cell loss and low stomach acid secretion and have an under acid chyme that then produces a lowered stimulation of bile release and pancreatic enzyme release. Again, there is a clinical art in this to balance the patient properly, to improve their digestive function through the second "R," the Replace phase.

The third "R" is Reinoculate. This is where we add back the friendly probiotic organisms, the symbiotic bacteria, and has to do with the use of both prebiotics and probiotics in combination. We're going to talk about this in much more detail with our clinicians/researchers of the month in the August 2009 issue, Professor Delzenne and Dr. Cani.

The last "R" is the Repair phase of the 4R program, which is adding back those nutrients in adequate quantities that help to restore proper gut mucosal activity, knowing that the gut mucosa turns over every week or so and it is replaced. The gut is a constantly regenerating cell line and so we want to give it back the nutrients necessary (L-glutamine, L-arginine, pantothenic acid, zinc in a non-irritating form, vitamin E), a variety of agents that help to restore proper gut-immune function. It may even have to do with the addition of certain phytochemicals that help to balance the gut-immune-inflammatory response, like curcumin from turmeric, or ECGC from green tea, or iso-alpha acids from hops, all of which have been demonstrated to have favorable effects on gut-immune-inflammatory response.

These are, I think, very interesting approaches that can be employed clinically. People have often asked

me over the years, "In functional medicine, if you were to develop one tool from the tool kit of the functional medicine process, what would you go to first? What would be the most important singular tool to develop expertise with?" I would have to say it is the 4R program. Once a clinician becomes comfortable and skilled in the art of applying the 4R program, they will be amazed at how many patients with a wide variety of complaints--from dermatological to neurological, gastrointestinal to cardiovascular, insulin to even things like metabolic syndrome--improve. This gut-immune connection to so many functions of the body is one of those very important cornerstones in the functional medicine matrix. I know I have said this probably ad nauseum, but the 4R program is really more than just an acronym to recite on demand. It really becomes a series of very important clinical steps in designing a program personalized to that patient's need to use their gut-immune system as a friend rather than a foe and to allow them to be more tolerant to this complex environment (nutritional, internal, and outside environment) that we are exposed to.

Going back again to Dr. Harris' comments, I think it is very important for us all to recognize that the personalized response an individual has (notwithstanding their HLA determinants that give them some relative risk--the HLA-DQ2 and HLA-DQ8) to gluten is going to depend upon things that relate to the gastrointestinal milieu and the underlying sensitivity that the GI immune system (the gastrointestinal-associated lymphoid tissue and the mucosal-associated lymphoid tissue, or the MALT) have to the local environment. Balancing the immune system of the gut, using appropriate prebiotics and digestive aids, and of course using either a rotation or an elimination-type diet become the sine qua non for better clinical approaches towards this problem.

This also relates, obviously, to other kinds of inflammation modulating nutrients. In last month's *Functional Medicine Update*, we talked about omega-3 fatty acids and the favorable role they have on immune modulation. We have also talked about such things as stabilizing insulin using a low glycemic load type of diet because high insulin levels can be contributing to proinflammation and activation in the gut of various types of cell proliferative processes and inflammatory immune response. Again, we use a systemic, network-thinking-type of approach to this problem. It is not just solely elimination of gluten in the absence of looking at all these other covariables that may be modulators of both the susceptibility and the severity of response to the triggering molecules (the glycosylated versions of gluten).

Exploring a Connection Between Gluten Sensitivity and Central Nervous System Disorders, Including Autistic Spectrum Disorders (ASD)

With that in mind, then, let's finish up this discussion by taking one more step forward by talking about the connection between gluten and central nervous system-type problems. The one that has been in the news most significantly of late is autistic spectrum disorders, or ASD. I know this is a very controversial topic and I don't want to make a very complex topic overly simplistic and be misleading, but I do believe that there is increasing evidence to support the fact that ASD, as it expresses, is not just a sole response to a monozygotic genetic linkage. It is a lot like this gluten connection to autoimmune diseases. There may be genetic linkages that increase the relative susceptibility, but it is not just a gene that produces this condition called ASD in absentia to other covariables.

There are many covariables that can modulate the immune system in such a way as to result in activation of brain neuronal pathways that ultimately present themselves as this definition of autism or autistic

spectrum disorders. I am reminded of this from my experiences at the Institute for the Achievement of Human Potential in Philadelphia, PA, where they have worked with what they call mid-brain-injured children, who are really those that are often diagnosed as autistic. By utilizing their approach, which is a multi-phasic and multi-parameter approach using physical training and nutrition and stimulation, patterning, many of these children (who have very significant difficulties) have achieved significant improvement over the course of therapy, with their parents providing the therapeutic approach at home. The approach involves dietary modification, including taking antigens out of their diet and encouraging improved nutrient quality. This requires getting these children on higher protein diets and lower sugars and simple carbohydrates-the things that most of us would think are important for overall health. Getting these children into proper programs of structured physical and neurological training-all of this is part of the approach. I think it is very interesting that they have recognized the importance of gut ecology in these children as well-I guess you would call it gut hygiene. Getting these children to have regular bowel movements and applying the 4R Program in ways that can conveniently be done at home by the use of probiotic and prebiotic organisms and so forth.

We recognize that this complex etiology that we call autism has many different variables, but one of the interesting variables appears to be oxidative stress (what we call oxidative neuronal stress). Where does oxidative neuronal stress emerge? What is the triggering agent? I don't think it is "the" triggering agent, I think there are many triggering agents for neuronal oxidative stress in specific regions of the brain. Certainly one of these can be hypoxia.

Overview of the Biochemistry of Oxidative Stress
We recognize that one of the most paradoxical situations is that oxidative stress occurs in the states of low oxygen tension within tissues, which seems almost counterintuitive. How could a low oxygen tension produce a high oxidative potential within tissues? The reason for it, simply (not to go into the very complex biochemistry), is that lowering oxygen tension in an oxygen-requiring environment alters mitochondrial redox potential (the reduction/oxidation potential), and shifts that redox into a state where you get incomplete oxidative chemistry and you get more intermediate free radicals being formed (these are both oxygen and nitrogen free radicals) that can be involved with what we call oxidative stress or free radical pathology. Ischemic events are associated with a whole array of different neurodegenerative conditions, some of which are related to autism itself.

Study on the Effects of Hyperbaric Oxygen Treatment in Children with Autism

There is this very nice clinical trial that has recently been published in *BMC Pediatrics* in 2009.[6] This is a collaborative group of investigators working with autistic children and their families across multiple centers that have been looking at the effects of hyperbaric oxygen treatment with children with autism. This is a multi-centered, randomized, double-blind, controlled trial that includes the International Child Development Resource Center, the Center for Autism Research and Education, the True Health Medical Center in Naperville, IL, the Princess Anne Medical Associates in Virginia Beach, Therapeutic Pathways in East Troy, WI, Biognosys in Nanuet, NY, and the Rimland Center in Lynchburg, VA. These centers looked at 62 children from ages 2 to 7 with a mean age of about 5 years, randomly assigned to 40 hourly treatments of either hyperbaric treatment at 1.3 atmospheres and 24{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} oxygen, or slightly pressurize room air, and looking at the Clinical Global Impression outcome, the Aberrant Behavior Checklist, and the Autism Treatment Evaluation Checklist response.

They found very significant improvement in the treatment group that got the hyperbaric treatment versus those children that did not. How does it work and why does it work? Why do these kids' brains not seem to be getting enough oxygen? Is it a respiratory problem? Is it a transport problem? Does it have anything to do with anemia? Does it have something to do with mitochondrial oxidative phosphorylation (you have to force the furnace of the cells to work harder)? There are many, many variables, obviously, that regulate oxygen delivery to the brain.

I find this a very interesting study because it was 50 some years ago that the Institutes for Human Potential (the Domans being the principals there) started talking about childhood neurological problems being associated with a deficiency of the most important nutrient in the brain, oxygen. They really framed their whole program around ways of helping that child to deliver better oxygenation to the brain through physical training, mental training, and nutritional intervention. There is a convergence, I think, between this work of hyperbaric treatment for children and ASD and the work being done for 50 some years to improve oxygenation. At The Institutes for the Achievement for Human Potential, they have even developed a therapy called inhalation therapy, using rebreathing (bagging the children) to improve the CO2 and get the bore effect to drive more oxygen into their cells, and showing significant improvement in many of these kids (the technique is also called masking).

There certainly seems to be something about oxygenation of the brain and these children with these "mid-brain" or autistic-related changes. How does that relates to things like immune function, perfusion, and endothelial function? Think back, if you would, about delivering oxygen. It has to do with vascularization. It has to do transport of oxygen. It is not just necessarily passively diffusing; it has to be transported and delivered to the site where it is used. The reducing agent and the oxidizing agents that are present in the mitochondria control the energy efficiency of the body, so you need to have an oxidizing agent and you need to have a reducing agent. The reducing agent comes from nutrients and the oxidizing agent comes from oxygen, so you get this ability to have the oxidizing agent reduced into water (molecular oxygen, or diatomic oxygen).

I think we are starting to witness kind of a theme emerging that takes an observation (hyperbaric oxygen treatment improving clinical outcomes in autistic children), and ties it back to a complex mechanism of understanding of why oxygen could be a limiting nutrient in those children and what factors we can use other than hyperbarics to improve oxygenation, oxidative chemistry, redox potential, mitochondrial oxidative phosphorylation, and neuronal energy production. This ties into the interview we had with Jill James on autism a year ago in which she talked about the work she is doing in glutathione as a centrally important substance and as an intercellular redox agent (cellular antioxidants that couple between glutathione and oxidized glutathione disulfide). How does the glutathione connection have anything to do with the hyperbaric connection that has anything to do with the gluten connection that has anything to do with what we know as nodular ileal hyperplasia and its relationship to immunization and that of autism? All of these things are interrelated, one to the other through a process of distortion of the web of the immune system and function and its influence on vascular function and inflammation.

I think that an emerging theme-a conceptual framework: to look at autism with a broader lens from a functional approach. There may be genes that ultimately determine relative susceptibility in certain children, just as there are genes that determine susceptibility to gluten-induced celiac disease (as we have mentioned, the HLA-DQ2 and DQ8). But those genes in and of themselves are not the sine qua non determinants of the expression into the disease. It is other environmental modifiers that regulate promoter

regions of genes and may have epigenetic influence on how genes can ultimately be expressed that then become the modifiable factors that relate to the expression of that condition.

Delivering oxygen through hyperbarics may treat an oxygen deprivation condition in the region of the brain. This begs the question: why did that person have that oxygen deprivation? What led to the poor relative perfusion of certain regions of the brain that power up mitochondria? Could it have been an immunological problem that leads to a vascular constriction, or endothelial dysfunction, or to toxic burden on mitochondrial that then downregulates the activity of the electron transport chain and requires more oxygen to drive this process through? All of these are questions that I think are very important as we start to look at the environmental links to this rising tide of autistic spectrum disorder that is seen in our society.

I believe that what Dr. Harris has shared with us as it relates to her (or their) emerging understanding of the mechanism, has a spreading effect into a variety of companion relationships that connect the gut to the immune system to the nervous system to bioenergetics to redox potential, and ultimately even into these observations of the beneficial effects of hyperbaric oxygen in treating children with autistic spectrum disorder. This, to me, is the language and logic that underlies the functional medicine model, versus that which is singular in its drive to a diagnosis and then once a diagnosis has been determined, finding the drug that modifies the endpoint that correlates with that diagnosis.

Obviously the functional medicine model, as I am describing it, has some degree of confusion. It is not as comfortable because it doesn't have the clear edges that the diagnostic model has in which you have a comfort zone, and can say, "I now have a diagnosis and I now have a drug or a series of drugs that I can use for the treatment of that condition that matches that diagnosis." In the case of what we have been describing over the last two issues of*Functional Medicine Update* as it relates to this gluten story, we can see that there are many variables that intersect to give rise to increasing relative susceptibility and severity of response to a trigger, which in the case of gluten, could be celiac disease as the endpoint.

What we have said is that you not only need to deal with the immediate obvious trigger, but you need to also be considerate of the environment in which that trigger is operating, because only 40{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} of the people with the genetic susceptibility ultimately have the trigger lead to the mediators that are associated with celiac disease. We also then said this relationship of an environmental agent (in this case, the dietary agent gluten), in the form in which it has epitopic relationship with the immune system (so specific glycosylated forms of the gluten molecules) then triggers, in effect, not only regionally but systemically, and so that correlates with things like autoimmune thyroiditis. It correlates with things like anti-phospholipid syndrome that now is associated with vascular disease risk. It correlates with dementia and neurodegenerative diseases. You can only understand those "comorbidities" if you understand the potential mechanisms by which this immunological shift can occur in the patient and the variables that augment or modify its expression. That is, I think, the discomfort or insecurity of the functional medicine model, because it doesn't have those clean edges that the diagnostic model has. With functional medicine, there are other questions. Why are these conditions being seen in this patient? What are the other variables that relate to the triggering (their antecedents, their genetic past, their medical past, their environmental past, their ecology) the outcome that we see as that dysfunction? Withthis model of gluten and celiac disease there is a very tight correlation of an environmental factor to an autoimmune disease then gets confused (to some degree) when focused through the functional medicine lens, because now we

say, "Hold it. Not everybody with those genes that are susceptible end up with a disease when they are exposed to that environmental agent, gluten. And not every gluten produces the same response." So there are many modifiers and variables that need to be evaluated if we are really going to improve patient outcome and not just use the one-size-fits-all-type mentality and then lose people on the edges.

The functional medicine model allows us to ask (and actually even demands of us to ask) the right questions. I think the work of Dr. Harris and Dr. Fasano that was described in this issue helps us to understand the evolving science that supports asking these questions, because there is more below the waterline than meets the eye in every one of these stories. As we start to explore it you see that we start to broaden our understanding of how other covariables influence the expression of those conditions into the disease that ultimately results.

I'm very emboldened and encouraged by the last two issues (the June and July issues) of *Functional Medicine Update*, which have really helped us to pinpoint the importance of gluten as it relates to a triggering factor for a variety of systemic and regional inflammatory conditions. This may be an operational model to support the difference between a drive toward a sine qua non of diagnosis versus a functional medicine model, which is to drive towards a sine qua non of mechanism and understanding the variables that influence, in that patient, the expression of those signs and symptoms. I hope I have given you some tools to use and through the wonderful experience and knowledge of our clinicians and researchers of the month that you have taken some big steps forward in better understanding this gluten story. We're going to explore the next step in August when we talk with Professor Delzenne and Dr. Cani. Thanks for being with us.

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