November 2006 Issue | John J. Bright, PhD Senior Investigator and Director of Neuroscience Research Laboratory

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Welcome to *Functional Medicine Update* for November 2006. I am frequently asked questions about some of the topics I discuss and how these topics may relate clinically. One question that has come up recently from a number of listeners has to do with the connection among vitamin D-calcium-magnesium, gut function, and risk to colorectal cancer: How does this work? Is this clinically relevant? And how much calcium, magnesium, and/or vitamin D might be useful?

To introduce my response, I'd like to go to an article that appeared in 2005 in the *Journal of Steroid Biochemistry and Molecular Biology* titled (and this sounds like a functional medicine title), "The Vitamin D Endocrine System of the Gut-It's Possible Role in Colorectal Cancer Prevention."[1] I think we all know that the gut represents the seat of the immune system. Over 50 percent of the immune system is clustered around the gut (the gut-associated lymphoid tissue), and 70 percent of antibodies are produced by the gut-associated lymphoid tissue (GALT). It is a very rich site of immune function. A lot of receptors are on the surface of the gut mucosal cells, and these receptors pick up messages that they then translate to the rest of the body through cell signaling processes-such as with cytokines and chemokines and other regulatory molecules.

The Role of Vitamin D in Gut Signaling
We know vitamin D plays a role in signaling at the gut level because there are receptors for vitamin D, and because of the ability of the colon cells (the mucosal cells) themselves to convert vitamin D into 1,25-dihydroxy D3 (the active hormonal metabolite of vitamin D). If you take colonocytes in the laboratory and grow them, you can show they possess vitamin D-synthesizing CYP27B1, which produces 1,25-dihydroxy D3, and also they have the activity of CYP24 hydroxylases, which are the catabolic enzymes that break down 1,25-dihydroxy D3. It is a dynamic equilibrium between production in the gut and breakdown in the gut.

A low calcium diet upregulates the expression in the gut mucosa of the CYP24 hydroxylases, which are the catabolic enzymes that break down 1,25-dihydroxy D3. This tends to lower the activity of the hormonal form. The hormonal form of vitamin D is a very important agonist for a number of cell signaling processes that relate to the regulation of insulin signaling, the regulation of inflammation signaling, and the regulation of cell proliferation and cell cycling. If you increase the breakdown of 1,25-dihydroxy D3 signaling, it increases cellular proliferation, decreases differentiation, and decreases some of these cell regulatory processes that occur under proper immune balance. That occurs when your diet is shifted toward low vitamin D and low calcium. As people take away dietary calcium and/or

vitamin D (through either the absence of sunlight exposure or vitamin D in their foods), it tends to shift these cell signaling processes toward altered gut-immune function and increased cellular proliferation, both locally and systemically.

This raises a question, doesn't it? What about the oriental diet? It is not a dairy-product consuming diet and historically very low in calcium. How is it that there is such a low incidence of colorectal cancer in Asian communities, where they don't eat a lot of calcium in their diet and they don't take a lot of vitamin D? It is a northern latitude population, so you might not expect that they are getting a lot of sunlight exposure. Recently, it has been reported in a number of interesting papers that the soy isoflavones are activators of the hydroxylases (the CYP27B1), and therefore promote (in the gut mucosa) the formation of 1,25-dihydroxy D3. You can get-through the combination of calcium and isoflavones-a very beneficial effect on this synthesis and the prevention of accelerated break down of the hormonal form of vitamin D locally/regionally in the gut mucosa.

Now, we start looking at epidemiological cultural variations in the diet and the influence that has on cell signaling. Is this an example of co-evolution? Did we evolve, by natural selection over millions of years, the ability to accommodate and use different signaling molecules to produce salutary beneficial effects on regulating the immune system? Obviously I don't have a crystal ball, but it sounds like a reasonable explanation.

What we do know (epidemiologically), is that diets that are higher in isoflavones and lower in calcium seem to have a similar beneficial effect on regulating epithelial differentiation in the colonocyte, as does a diet that is higher in calcium and vitamin D. This might account for differences in dairy-product-consuming individuals with higher calcium intake versus low-dairy-product-consuming individuals who have higher isoflavone intake. If you take soy out of the diet (or other isoflavone-rich foods) and you take calcium out of the diet, now you have a double-barrel problem because you are not upregulating the synthesis of 1,25 at the colonic level, and you are increasing the breakdown, so you can shift this toward proliferation, dedifferentiation, and poor regulation. That is the story that is starting to emerge from these observations.

The Relationship between Nutrients and Cell Signaling

There is a very interesting take-away concerning the relationship of nutrients (bioactive compounds in our food) and cell signaling, and how this regulates gene expression patterns. We are not talking about changing the genes. We are talking about changing the expression of those genes (polymorphic genes) that then regulates the phenotype of cells. A person is not born hard-wired to get colon cancer, but there may be people who are more susceptible based upon their polymorphisms. An environmental modulator can be seen as a variable that penetrates through all the genotypes and is a modifier with different levels of sensitivity.

An interesting paper has been published recently in *Nutrition Cancer* titled "Vitamin D Receptor Gene Polymorphisms, Dietary Promotion of Insulin Resistance, and Colon and Rectal Cancer."[2] The authors of this article investigated an interrelationship between insulin resistance, downregulation of the signaling of vitamin D active hormonal metabolite formation, and the dedifferentiation of colonocyte cells (increasing oncogenic risk). You are going to hear more about this as we move into the discussion in this month's *Functional Medicine Update*.

We should not leave other conditions out of this. This is a complex web-insulin resistance plays a role, genetic polymorphisms play a role, dietary calcium plays a role, and sunlight exposure plays a role. There are many different variables, including exposure to environmental *in situ* carcinogens (produced either by gut fermentation or direct intake). It is an example of the complexity of gene-environment interaction.

Vitamin D Hydroxylase Expression and Tumor Prevention and Therapy
Nutritional regulation of vitamin D hydroxylase expression has potential applications in both tumor prevention and therapy. There was a very interesting paper published in *Future Oncology* in 2005 that discusses this whole mechanism that I have been describing to you.[3] This is an emerging story about calcium and vitamin D, and we should add magnesium to that story because magnesium is a critically important agonist for the calcium dynamics of the cellular membrane, and may have some of the effects on the enzyme activity of these hydroxylase enzymes forming 1,25-dihydroxy D3. I encourage you to reflect back on our October issue, when I discussed the role of magnesium in these processes.

Calcium, magnesium, vitamin D-do we need supplementation? The answer really comes from a diet evaluation. Is the patient consuming adequate levels of these nutrients, or exposed to sunlight in an adequate way (for vitamin D)? If not, then we might start talking about vitamin D supplementation (1000 IU) to support of function. We might talk about soy isoflavones or a soy-enhanced diet (1 or 2 portion sizes a day of soy with isoflavones; 20 to 30 mg a day soy isoflavones). We might talk about calcium in the range of 1000-1200 mg. Lastly, 400 to 500 mg elemental magnesium in a non-GI-irritant form. That is the range of therapeutic intervention that you might consider if a patient really has evidence of compromised status (relative to those nutrients that help regulate gut-immune function) and has evidence of GI imbalance in terms of immunological regulation through these cell signaling pathways (the cytokines, chemokines, and intercellular communication agents).

Because we are on the subject of the gut, let me take a little sidebar related to one of the most significant gut-inflammatory conditions that we are seeing with high prevalence in our population. In fact, it is one of the most common afflictions in western countries; I am talking about diverticulosis. Diverticulosis is so common that its presence is more normal than its absence among older Americans. Is diverticulosis normal? I don't think we consider it optimal, but it is the way people are as a consequence, primarily, of what is acknowledged as a low-fiber diet.

Fiber, by the way, has more than just a physio-mechanical effect upon the gut mucosa (like a scouring pad that wipes the villi clean). It is able to be fermented by various types of microbiota that live in the colon, which produce secondary trophic factors that can have favorable influence on the physiology of the colon. One of these factors is butyrate (a colonocyte fuel). We should think of fiber (or the appropriate types of fiber) as a precursor to support proper formation and function of gut anatomy and physiology.

Many gastrointestinal symptoms (such as hiatal hernia) are attributed (rightly or wrongly) to diverticulosis. We know there are two very real complications of diverticulosis that can carry serious consequences-diverticulitis and diverticular hemorrhage. Diverticular diseases (diverticulitis, in particular) are problematic because they can exist as abscesses of the colon and can create chronic sites of focal infection. Obviously, this can lead to perforation and very serious problems of sepsis. Even under the best of care I think there is about 7 to 11 percent mortality in people who have abscess and perforation as a consequence of diverticulitis. This is not to be taken lightly; these are serious considerations.

Generally, the medical treatment for diverticulitis is bowel rest and total parenteral nutrition (TPN), followed by probiotics and prebiotics to improve gut flora. Specifically, patients are put on a high antibiotic regime, which is a combination of antifungal, antibiotic, and antibacterial agents (broad spectrum). They are on bowel rest (TPN) for 2 to 3 weeks, or sometimes as much as 2 months (depending upon the degree of inflammation of the gut). Following rest and the recovery, the GI specialist and/or surgeon will evaluate whether the patient is a candidate for surgery. Hopefully, there is a resolution of the problem without the need for surgery, because if surgery occurs it is generally a two-step surgery: first a colostomy (to allow cleaning up of the environment and "cooling off") and then a reconnection of the GI tract afterwards. It is a pretty invasive process. The attempt, conservatively, is to first allow the bowel to heal by intervention with bowel rest and antibiotics, and then a diet that hopefully will renourish the colon with high fiber and friendly bacteria.

Use of Probiotics in Patients with Diverticular Disease
You might ask about using probiotics when you've got potential abscess (maybe even a perforating abscess) of the colon-is it really a good idea? What happens if you add bacteria to the gut-aren't you running the risk of potential sepsis and infection? This question was recently addressed in a review article titled, "Probiotic Use in Clinical Practice: What are the Risks?"[4] This appeared in the *American Journal of Clinical Nutrition* in 2006. I would encourage those of you who are probiotic users to take a look at this article because I think it does a very nice job of reviewing the literature; I'll give you a quick appraisal of it.

The authors of the article review papers that have been published about different strains of *Lactobacillus acidophilus*, *Bifidobacterium* supplementation, *Lactobacillus reuteri*, and even *Saccaromyces boulardii* (all of these various types of probiotic organisms). They look at cases of bacterial sepsis temporally related to probiotic use in humans. They cite 6 different studies that have been published that go back over the last 10 years that are case reports of potential septic disorders that were associated with the administration of probiotics. This includes liver abscess, endocarditis, and bacteremia (most common). These were using various kinds of LGG and *Bacillus subtilis*. The most common one is LGG (associated with 4 of these 6 reports).

I need to emphasize (as the authors emphasize in the review) that these problems are not proven to be necessarily a consequence of the probiotics. These were sick patients who had perforated bowels and they had all sorts of other things going on, but because they were also supplemented with bacteria and they were able to culture their blood and find these probiotic organisms in their blood, there is some suggestion that it was a contributor. The authors' take away is that if a person has serious gut inflammation with perforation, be somewhat cautious about the use of probiotics.

The authors then go on to look at cases of fungal sepsis temporally related to probiotic use. The number of case histories is longer and principally associated with *Saccharomyses boulardii*. There are over 20 case reports with *S. boulardii* being associated with fungemia and fungemic shock. Again, the authors point out that we consider these safe organisms used for reinoculation of the bowel, but in cases where the individual is immune compromised (many of these case reports were people with perforation of the bowel in combination with HIV infection, so these were immune-compromised patients or very much older patients-patients that might have might have been in the intensive care unit) there may be sensitivity. These are extreme examples, maybe, of immune compromise and sensitivity to any kind of burden of foreign organisms that are not normal. Without raising too big of a red flag I want to put this

out, just as food for thought.

We recognize that probiotics often have a very beneficial effect on gut micorflora and they may (as has been suggest by a number of studies) lower gut inflammation when used appropriately. There are many more studies published in the literature showing favorable effects of probiotic supplementation with chronic gut inflammation than there are that describe an association with sepsis (or bacteremia or fungemia). I think the concept here is that nothing is completely safe. When we get up in the morning, we don't have assurance that the day will be 100{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} safe; we just get up and move around our world. There is always some risk just to living, to breathing, to drinking.

In a relative risk-benefit, it appears as if probiotics (certainly in the earlier stages of nonperforating diverticulitis) is beneficial in the remission of the disease. There are many good reports about this, including a recent paper that appeared in the *World Journal of Gastroenterology* in 2006 titled "Management of Diverticular Disease is Changing."[5] The authors of this article advocate the use of probiotics after bowel rest and antibiotics as part of the therapy for the management of diverticulitis. Everything in balance, I guess, is always the watchword. We should keep in the back of our minds the issue of what the state of the dysfunction or the pathology is of the patient when we use probiotic supplements.

What we are really speaking to here is an interesting connection to inflammatory disorders, and that is going to be the theme throughout the rest of this month's *Functional Medicine Update*, with specific focus on modulation of autoimmune disease. I am very intrigued with the story that you will hear because part of the clinical concern we have relates to the biomarkers that we use to identify whether a patient has early insipient inflammatory disorders (prior to joint disfigurement, renal failure, carditis, and some of the more acute symptomatologies that develop with autoimmune tissue-specific reactions and inflammation). It doesn't take a very high level of diagnostic acumen to recognize an arthritide when it is deforming. But, well before that occurs, there is a sequence of events at the cellular level that occur from imbalances of the immune system. These events can be occurring over, maybe, decades before resulting in deformation and the serious pathophysiology associated with autoimmunity.

Biomarker Development for the Early-stage Recognition of Inflammatory Disorders
What are the earlier-stage markers that we might clinically evaluate? That is a question that I wish I could give you a definitive answer for because it is a question in flux. What we are really talking about is biomarker development for early-stage recognition of inflammatory disorders. Paul Ridker certainly helped us to understand some of this in greater detail when he and his colleagues at Harvard got us to think about high sensitivity C-reactive protein. That is an example of a biomarker that is used for earlier warning recognition of potential insipient (or smoldering) inflammation. As we learn more about inflammation, we recognize that it is much more than just a marker called CRP. There are literally hundreds of differing signaling molecules that give rise to an alteration of immune function, which may express as an inflammatory disorder. Downstream from that, ultimately, is the production of C-reactive protein by the liver.

Let me speak briefly about biomarkers because this is a term that we are going to be talking much more about in the years to come. The development of good, accurate, and respected biomarkers will really be the lynchpin upon which the future of functional medicine rests. In the absence of good biomarkers, we

can speak philosophically all we want about early stages of disorders ,and antecedents and triggers and mediators, but the clinician doesn't really know what to do. It just sounds like an interesting philosophical exercise: What do I look for in my patient? Exactly how to I know if they have an early precursor marker? How do I know if their genotype is expressing itself as an inflammatory phenotype that later will be a disease if I don't have something that I can measure and evaluate and track? Biomarkers are very important. The sensitivity, precision, and accuracy of a biomarker with clinical specificity to a condition provides reliability in the way to assess, intervene, and then track the success of therapy.

Serum Cholesterol as a Biomarker

Serum cholesterol would be considered a biomarker. It is really not a measurement of pathology for any disease. If you are asked what disease is diagnosed with elevated cholesterol, the answer is really none. Elevated cholesterol is a prognostic marker-a physiological function marking a trajectory toward a disease (cerebrovascular disease, cardiovascular disease, and so forth). We have started down the biomarker road by including cholesterol in the standard SMAC test (the multiphasic screening laboratory analysis), but clearly cholesterol represents a pretty broadbrush-type of biomarker and there must be other individualized biomarkers in the sea of new genomic markers that are being discovered that would give better specificity and ability to identify personalized needs of the patient. Biomarkers are really part and parcel part of the development of personalized medicine.

Recently, in the journal *Genome Technology,* there was a review paper about biomarkers on the horizon. The title of this article is "Betting on Biomarkers: Researchers Aim for the Clinic."[6] This is where clinical laboratories are starting to focus-to endorse and incorporate some of these new biomarkers. Biomarkers are central to personalized medicine, but they are only slowly trickling into clinical trials and there is some concern that although research is discovering potential new biomarkers, they are not being put to clinical tests.

Although it may not appear that they are getting out into clinical evaluation adequately to help spur the development of personalized medicine, there is a great impetus to develop biomarkers. Pharmaceutical companies need better biomarkers for tracking some of their new individualized drugs, such as inhibitors for cancer treatment (which are based upon the individual tumor type of the patient).

Categories of Biomarkers

We can really break down biomarkers into a few categories. First would be the diagnostic biomarkers, which differentiate healthy from a specific disease state. Second, which for functional medicine devotees is probably the most important family of biomarkers, are what we call prognostic biomarkers. Prognostic biomarkers are those that predict the likely course of disease; here we look at expression analysis, epidemiological studies, and mechanism of disease, which is really what functional medicine has tried to focus on. How do genes get expressed? What environments alter gene expression? How does that track against epidemiological studies, where we can see some real effects in human populations? And, what are the underlying mechanisms that may cut across the ICD-9 codes? This really speaks to the origin of the disease. The biomarkers that understand the disease prognosis, help with the trajectory toward that disease? From a functional medicine perspective, this is the area that is most directed at clinical outcome. For example, C-reactive protein (CRP) is one of the trajectory type of markers, which by its early-warning recognition of smoldering inflammation, can help us to understand the course of later disease.

In the course of our discussions in *Functional Medicine Update* over the next year, you are going to hear

me speak much more about where biomarker development is going and how it relates to the field of functional medicine. What are some of the candidates-the players-that would help to identify personalized needs? Of the potential biomarkers that are emerging for inflammation, the inflammatory cytokines (including things like Interleukin-1, Interleukin-2, Interleukin-6, and tumor necrosis factor-α) are very interesting. We have all heard these names many times. These are proteins that are produced by components of the immune system or inflamed arteries, which then liberate these bioactive molecules that trigger cells at a distance to put the guards on the gate and generate a phenotype of inflammation.

Is TNFα a Central Player in Inflammatory Processes?
I have wondered if tumor necrosis factor-α (TNFα) is a central player in inflammatory processes. If it is a central player, why don't we develop an analysis for TNFα, and why don't we then develop a broad-scale therapy to block TNFα? That might be the easiest way of approaching the management-or the recognition and then later management-of many inflammatory conditions. But, if you explore that question a little more, you might ask whether TNFα signaling is the same in every cell. In other words, is there only one pattern of expression of TNFα? We often try to make these things a little simpler than they really are in human physiology.

You have probably heard of some of the pharmaceuticals that have been approved for the treatment of autoimmune disease (rheumatoid arthritis, for instance) that are TNFα-blocking agents (or that modulate TNFα production). We see good clinical outcome from these blocking agents, but we also see some potential adverse side effects that occur from the generalized activity of these blocking agents. So what is the story around TNFα as either a biomarker or a target for therapeutic intervention? The best way I can get to that is to cite what (probably for most of you) would be a very esoteric article that you wouldn't really necessarily read, but I hope I can demythologize this. This article appeared in the *Journal of Biological Chemistry* last year and is titled "Bruton's Tyrosine Kinase is Involved in p65-mediated Transactivation and Phosphorylation of p65 on Serine 536 during NFκB Activation by Lipopolysaccharide."[7] Now this is probably not the article you are going to gravitate to for light reading. What is it all about? Let me summarize what it really says.

It has been shown that NFκB sits in the cytoplasm of cells in a dormant state, bound up with an inhibitor called inhibitor-κB (or IκB), and this IκB-NFκB complex is quiet in a non-activated cell. When an alarm message arrives at the surface of a cell (coming from an outside message or something that would trigger an arousal of the inflammatory process), it is picked up at a membrane receptor on the outside of the cell, and it translated through a very complex network of pathways that involves kinases. That message then weaves its way through the membrane into the cytoplasm of the cell through kind of intracellular kinase relay race. The kinases involved in this relay are very specific for that message that came to the cell. For instance, bacterial lipopolysaccharides might trigger a different relay than do allergic responses, or toxic metals and xenobiotics. The messages from these relays ultimately arrive at the NFκB-IκB complex.

Then, through a very sophisticated series of steps, again mediated by specific kinases, the IκB is phosphorylated and it falls off the complex, liberating the NFκB, which then travels to the nucleus as free NFκB. The NFκB is now in the nucleus and its role is to sit down on specific DNA sites to activate the genes that are associated with inflammation. So far, everything is good; I hope you are following. Now the question is: does that NFκB (when liberated) always affect the same genes in every cell so you get the same exact mechanism? Here is where the story gets interesting; the answer is no. Throughout all of this

process I have described-the translation of the initial message into the cytoplasm of the cell, the liberation of IκB from NFκB, the translocation of NFκB to the nucleus, and lastly, the sitting down of NFκB onto the genome at specific loci that would up upregulate the expression of the genes associated with inflammation-is a very site-specific and very environment-specific function.

A generalized NFκB inhibitor may produce good and bad effects, because there are some cells in which NFκB is playing a very important role in regulating function of the immune system. There are other cells in a person with autoimmune disease where NFκB is excessively activated and its modulation would be beneficial to the outcome of the patient. But if you generally block all activity of NFκB, you get no differentiation of the good from the bad. There are different sites on the NFκB molecules that can be phosphorylated by different kinases under different environmental circumstances that then cause it to identify and to associate itself with different reporter genes, so you would get a different outcome in those cells, which leads to specificity of the response.

What am I trying to say is that this process is much more exquisitely complex than we have dumbed it down to be. The reason for that-in a teleological argument, if I can be so bold as to suggest an argument-is that if we had only one path to NFκB, and if NFκB is the only path to all inflammation, then every time we associate with a proinflammatory stimulus, every cell in the body that has NFκB in it (which are all cells) would become inflamed. Clinically, that is not what is observed, right? You know that a patient does not necessarily have every cell of their body inflamed when they come into contact with a proinflammatory agent; they generally have cell-specific types of inflammation. But if we use a general NFκB inhibitor, it does not matter if NFκB is working in a favorable way to modulate immune function or in a dysfunctional way to enhance inflammation-all of those processes would be blocked. This is a little bit like the argument of the COX 1 and COX 2 with the selective COX 2 inhibitors that we learned later produced adverse effects on the favorable effects of COX 2 (on the vascular endothelium). How does the natural system work? It works by very tight regulation of these cell signaling mechanisms through kinases that regulate only certain cells that need to alter their NFκB activation of genes to produce, then, specific types of cytokines.

If you could imagine this from a therapeutic approach, what you would really like to have are biomarkers that identify what specific pathways within the NFκB cascade are associated with what dysfunction that will later lead to a disease (not just general NFκB dysfunction). So you would not necessarily just measure TNFα, which comes from the activation of gene expression by NFκB. You would want to know what cells are activated by specific types of cell signaling from NFκB that produce, then, TNFα, and you would want to regulate your therapy, specific to the biomarker that is associated with that process of activation of TNFα.

Where I am going with this is to say TNFα is kind of generic. We do not know what cell it came from; all we know is that certain cell types were activated into inflammation through NFκB to produce TNFα, and that that came through upregulation of a specific kinase relay that is unique to that cell line. What we would like to know is what-specifically-needs to be modulated to target that cell line or that tissue type without just a general suppression of all forms of NFκB activation of immune function. That is a new pharmacology, isn't it? That really leads to personalized medicine.

Individual Tissue Regulation of Inflammation and Immunity: Identifying Kinase Pathways
What kind of biomarkers might emerge from this concept or this objective? Biomarkers that would

identify the individual kinase pathways associated with the individual tissue regulation of inflammation and immunity. In oncology, which is a good example of how this is being harnessed, things are happening with specific inhibitors in which (in the laboratory) tissues are being taken on biopsy and they are analyzed in that patient for that specific kinase. Using a general kinase inhibitor would not work because kinases are very important for the regulation of all sorts of important immune functions. But if you target on specific kinases that are associated with altered cell function in an individual patient, you have a possibility for a cancer therapy that is personalized. That particular strategy is actually being investigated for a number of the new oncology drugs.

To me, that strategy is similar to where functional medicine is going around a whole series of other disorders. Those disorders are associated with a variety of complex chronic diseases. We could start by identifying those specific regulators of tissue-specific activation and then the molecules that would be useful for normalizing their function. I think you will learn more about this when you hear our researcher of the month speak because you will see how the environment modulates function. It may be that we have been modulating our function from time immemorial through all the things we are exposed to-by subtle alterations of these cell-specific kinase pathways that regulate our function in appropriate or inappropriate ways (e.g., from toxic foods, toxic environments, toxic thoughts). All these things then create dysfunctions in kinase signaling that are specific to certain cell types that give rise to different types of diseases.

Let me close this argument with a less esoteric and a more specific clinical example. Those of you who have been following our discussion of autoimmune disease know that I have proposed a model. It is actually not my model-it comes from the literature, so I can't claim this was invented here, just that it is communicated here. This is the idea that autoimmune disease is a consequence of building up responses to foreign agents in our body that were really part of the normal immune function of the body. What happens if our host-friendly cells become foreign cells and then the body's immune system does what it is supposed to do-it forms an antibody or a protective factor or a destructive factor against that foreign substance? How can our body go from being a friend to being a foe-what would do that? Damage to proteins or damage to DNA by radiation or chemicals or various cell physiological mechanisms could take a host-friendly cell and make it into a foreign molecule (like a foreign protein that had a different amino acid-an oxidized or a glycated amino acid or a phosphorylated amino acid) that looks not like the host anymore. A very adept immune system is like a seek-and-destroy mechanism; that is called an autoantibody.

Is there evidence for this model? Of course there is-I've cited numerous studies from the literature showing that DNA autoantibodies are often not to host DNA, they are to altered DNA. We call them antinuclear antibodies, but they are actually antinuclear-altered DNA antibodies.

Estrogen and Autoimmune Disease

What about the connection of estrogen to autoimmune disease? We know oral contraceptives affect things like SLE, so it sounds like estrogen is involved. We know that women who are pregnant and have autoimmune diseases often go into remission during pregnancy. And, we also know that women who are postmenopausal and get autoimmune disease often have some dysfunction of estrogen metabolism, or menstruating women often have a flare during their time of the menstrual period when they have autoimmune disease. This doesn't all seem to fit; it seems inconsistent, clinically.

Are we sure these conditions associated with estrogen are associated with 17β estradiol and not altered estrogen (the estrogen metabolites-the "funny"molecules-that can cause clastogenic or mutagenic influences on DNA and other molecules)-the twisted estrogen molecules like the 4-hydroxy estrogen and possibly the 16-hydroxy estrogen? Maybe we are looking in the wrong place. Maybe it is the altered estrogen metabolites that are associated with exacerbation of autoimmune disease.

I reported on a clinical study that was published in 2001 in the journal *Lupus* that indicated that supplementation with indole-3-carbinol in women with SLE actually led to improvement of their symptoms and increased their 2-to-16-hydroxy estrogen ratios.[8] I would like to throw out one last concept to you. There is a paper in the *Journal of Nutrition* titled "Lifespan is Prolonged in Autoimmune-Prone (NZB/NZW) F1 Mice Fed a Diet Supplemented with Indole-3-Carbinol."[9] (This is the animal model for SLE.) These mice were fed a diet supplemented with indole-3-carbinol, a phytonutrient that increases the 2-hydroxylation at the expense of lowering the 4- and 16-hydroxylation (so it is improving estrogen metabolism). What did they find? I won't go through the whole study, but they found that the estrogen urinary metabolite ratio of 2-to-16 was increased in the indole-3-carbinol-fed mice, and these same mice had a remission of symptoms, and they did not die (death did occur in 70{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} of the animals that were not I3C supplemented).

The point I am trying to lead you to is that we are looking for biomarkers that are sometimes far away from what you might think of as the origin of the disease. You might not think of the 2-to-16-hydroxy estrogen ratio as a biomarker for autoimmune disease, but it may one for women who have estrogen exacerbation of their symptoms. For these women, phytonutrient supplementation with 200-400 mg of indole-3-carbinol a day, and following their 2-to-16-hydroxy estrogen ratio, may be very beneficial in ameliorating the course of their disease. Indole-3-carbinol comes from cruciferous vegetables-the broccoli, cauliflower, cabbage, Brussels sprouts family. Does this indicate there are certain foods that might be beneficial for the amelioration of autoimmune disease? A big question that we are going to discuss with our researcher of the month, Dr. John Bright.

INTERVIEW TRANSCRIPT

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Once again we are at that portion of Functional Medicine Update that I think we all are very excited to hear. This month we have Dr. John J. Bright as our guest, a senior investigator and director of the neuroscience research lab at Methodist Research Institute of Clarian Health in Indianapolis, Indiana. Before joining as director of neuroscience research at the Methodist Research Institute, Dr. Bright was at the department of neurology and pharmacology at Vanderbilt University School of Medicine in Nashville, Tennessee.

While going through some abstracts related to autoimmune disease, I found that Dr. Bright's studies in neuroscience and pharmacology to be very pioneering. As I was following a track of his research, I determined that he was also looking at the role that various nutraceuticals and plant-based materials have on the immunological system, pertaining specifically to autoimmune disease. It was a fascinating kind of circular connection that I came to while reading his work. I think his studies are very exacting, very precise, and very interesting. I would like to introduce Dr. Bright to you, the audience of Functional Medicine Update. Dr. Bright, thank you being with us today and sharing your very interesting work.

Experimental Allergy Encephalomyelitis (EAE) and Multiple Sclerosis Research
JJB: Thank you, Dr. Bland. I'm glad that I can share some of the studies that we have done on autoimmune disease that may benefit many millions of people who are suffering.

Autoimmunity is a process in our body. Usually the immune system takes care of our body and is at war to fight against infection. At some point (and due to unknown reasons), immune response damages our own tissues and this is called autoimmune disease. This autoimmune response (attacking individual specific organs like the heart, brain, or pancreas) leads to specific diseases, including multiple sclerosis, rheumatoid arthritis, type 1 diabetes, and myocarditis. There are many autoimmune diseases that effect human health.

There is no one hypothesis or theory that explains how these diseases happen, but one thing we know is that there is immune response against our tissues, our cells. We use an animal model for multiple sclerosis called EAE (Experimental Allergy Encephalomyelitis) to learn the pathogenesis mechanisms in this disease process and also to test the different molecules and drugs that may be useful in treating multiple sclerosis or other autoimmune diseases.

EAE is a mouse model that we use. You can induce MS symptoms in different animal models, but our experiments mostly are in mouse model. We induce this disease by injecting neural antigens (or proteins from the brain), purified or synthesized in the lab. We usually inject two times (we call this "vaccination"). Two vaccinations lead to the development of a disease like multiple sclerosis.

We score the animals by looking at clinical symptoms of paralysis. Usually the paralysis in these mice starts from the tail, and we call paralysis in the tail "score 1;" from there the paralysis progresses toward the anterior. If we see paralysis in the tail and the hind limb, this will be "score 2." Paralysis in both hind limbs is "score 3." Paralysis in the tail, both hind limbs, and the forelimbs will be "score 4." If the animals cannot move or the animals die, this is "score 5."

By using different mouse clans we can induce different types of disease. There are two main types of multiple sclerosis we see in human beings: lapsing/limiting type and chronic progressive. We use SJL-type mice to induce lapsing/limiting disease; they get better after 25-30 days. The whole disease process of our experimental course is something like 0-30 days. When we induce the disease we usually score these animals clinically, and we also take immune cells from the animals and put these cells in culture, trying to learn the mechanisms of how drugs regulate autoimmune cells.

We look at the specific signaling pathways that are blocked or regulated by these drugs so we can define the mechanism of the drug effect in these animals. We initially started looking at one cytokine called interleukin-12 (IL-12), usually produced by macrophages, microglia, or dendritic cells in the body.

Microglia are macrophage-like cells present only in the brain. We found that if you induce the disease in this EAE model, the levels of IL-12 are higher during the disease and then come back down when the disease goes away (by treatment or otherwise).

Interleukin-12 (IL-12) and the JAK-STAT Pathway
We thought IL-12 was a good candidate to follow and treated the animals with anti-IL-12 antibodies (neutralizing antibody). There is a p40 subunit that is very important in this disease process, and if you neutralize IL-12, you can inhibit the disease. That was our early finding-we realized that IL-12 was a major player in this disease process. IL-12 gene expression and IL-12-induced function are the two aspects we wanted to study. IL-12 function means IL-12 acts on T cells to make them cells that can induce the disease. We call these encephalitogenic T cells, and they are mostly CD4-positive T cells. IL-12 induces Th1 (or T-helper-1-type) encephalitogenic T cells by signaling through a pathway called the JAK-STAT pathway ("JAK" for Janus kinases, "STAT" for signal transducers and activators of transcription). That is the signaling pathway that IL-12 uses to make Th1-type encephalitogenic T cells in EAE.

What we did was use a compound that has been shown to inhibit inflammation; it is a compound that binds PPAR-g (peroxisome proliferator-activated receptor gamma). PPAR-g is a member of the family of nuclear receptors. These proteins sit in the nuclear membrane; if you cross link them with the ligand, it gets activated and inhibits inflammation (that was our understanding of this molecule). Synthetic ligand for PPAR-g has been used as a treatment for diabetes; there is a prescription (FDA-approved) drug for diabetes now.

There are natural ligands for PPAR-g and there are synthetic ligands for PPAR-g. In our earlier experiments, we took those natural and synthetic compounds of the PPAR-g ligand family and treated these animals after inducing the disease EAE. We found that both natural and synthetic compounds can inhibit EAE in a dose-dependent manner. We went on to look at the mechanistic action and we found that it inhibits IL-12 production, as well as IL-12 signaling through the JAK-STAT pathway. That was our finding at that time and it was not just a drug effect.

We used knockout animals. The homozygous knockout for PPAR-g protein is embryonically lethal, so we used a wild-type, a heterozygous animal for PPAR-g. If you knockout part of the PPAR-g function, the disease in EAE is severe. If the PPAR-g activity is low, the severity of the disease is higher, showing that it has a physiological function in the demyelization process. We believe this may be true in human illness as well (that PPAR-g is playing a role in regulating disease process). We have followed up on this with our interest in nuclear receptor family of proteins.

Then we started looking at a different molecule-vitamin D. Vitamin D has been shown to be involved in regulating immune responses. We believe this (that sunlight correlates to disease) because inflammatory or demyelinating diseases are more common in the colder regions where vitamin D has more limits. We tested an actual metabolite of vitamin D-the 1,25-dihydroxyvitamin D3. We treated animals with vitamin D after inducing EAE demyelinating disease and it seems to protect animals from getting EAE. In this experiment, we also looked at the mechanisms of action that involved inhibition of IL-12 production, as well as IL-12-induced function in immune cells.

Studying Molecules with Anti-inflammatory Properties: Quercetin and Curcumin

With the knowledge that many natural compounds (nutraceuticals or phytochemicals) have anti-inflammatory properties, we selected some molecules to study-the most favored for us are curcumin and quercetin. Quercetin is a phytoestrogen and it comes from these kinds of compounds. Soybeans are rich in flavonoid compounds, as are many fruits and vegetables. In our experiment we used a very purified preparation of the compound quercetin and we injected it into mouse models of MS.

The second compound we used was curcumin. Curcumin is a powder or extracted compound from a plant called turmeric. It is an underground stem (a rhizome). You can purify it. Usually it is a yellow-colored powder. People use it in Asian countries as a spice, to color the food products, and (in very low quantity) in their daily meals/food. In ancient days, in Asian countries, curcumin was used for wound healing and for treating inflammatory-type of diseases. We took curcumin and injected it into the EAE animals (doses of 100 and 200 micrograms, or 50 and 100 micrograms-different doses) every other day-IV injections in our animals. We found that curcumin inhibits (dose-dependently) the EAE disease in mouse models, suggesting that it may be useful for treating multiple sclerosis.

We found there are different mechanisms involved in this process. At least one mechanism for curcumin inhibition of EAE is by inhibiting IL-12 production, as well as IL-12 signaling through the JAK-STAT pathway. There have been a few reports that curcumin can bind on PPAR-g, so there are connections between these compounds, but otherwise, we don't know how curcumin inhibits the JAK-STAT pathway. At least we know that if curcumin binds on PPAR-g, PPAR-g may be able to interact with the JAK-STAT pathway and downregulate the signaling pathway leading to Th1 differentiation or encephalitogenic T cell development in the EAE model.

There may be so many other compounds out there that may be useful, but we need to study more about their use and their side effects. Generally we believe natural compounds have very few side effects, and hopefully these kinds of compounds will reach patients for their benefit. We need to study more and find support for conducting this kind of research to further understand its mechanistic action, as well as safety and efficacy in human beings. We believe these natural compounds are very important to study and will reach human patients for their benefit.

JB: Dr. Bright, I want to congratulate you. That was one of the most remarkable, eloquent, and fluent descriptions of a very complex topic, connecting together a tremendous amount of information in a way that was really quite incredible. Obviously, you've practiced that before-that is a very thoughtful way that you articulated that to our listeners. I'd like to go back, if I could, and pick up some of the high spots because many of our listeners are not as familiar with biology or some of these signal transduction mechanisms as a specialist such as you (a neuroscientist), so if you wouldn't mind I want to emphasize a few of the points by just taking us through some of the things that you've said.

Let's start, if we could, by addressing the question I think a lot of people have about animal models. How respected is the EAE animal model to correlate with human disease in the autoimmune area-the MS area? Is it a generally accepted model that has a pretty strong human clinical correlation with its pathophysiology?

The EAE Animal Model

JJB: I believe this is the best model available so far for studying MS pathogenesis or therapeutic aspects. There are controversies; there are differences. Even among MS patients, the disease is different between

patients, so there is no uniform pattern of disease, but overall there are two classifications: lapsing/limiting and chronic progressive. If you see lesions, there is no clear pattern; each individual looks differently.

There have been-I think-two or three review articles/opinion pieces recently published about use of EAE as a model. There is confusion about it. If you read the recent review articles on EAE, I think most of the comments state this is the best model available. Until there is a better model, we need to continue to use this one because EAE is what we have used to test and find a useful drug for MS. Any FDA-approved drug now approved for the treatment of MS are drugs tested and approved based on results using EAE.

JB: That really answers that beautifully. In past editions of Functional Medicine Update, we have interviewed two investigators that have some overlap with your areas of emphasis: Dr. Colleen Hayes at the University of Wisconsin and Dr. Michael Holick at Boston University Medical School. You probably know Dr. Hayes' work on vitamin D metabolites and MS, and also Mike Holick who was originally at Wisconsin and was one of the discoverers of the 1,25-dihydroxy-D3 before he moved on to dermatology and immunology at BU. I think their work very closely affiliates itself-and certainly is consistent-with your observations. Have you had communication with either Dr. Hayes or Dr. Holick over your years?

JJB: Not really. I know their work and I strongly believe their observations are real and have been confirmed in our studies. By inhibiting the JAK-STAT pathway, we have proven that pathway is important in disease pathogenesis, so we have added at least one step further in the understanding of its mechanism of action.

JB: Yes, I think that is what I was going to reinforce. There is a question that has been raised at meetings that I have attended where either Dr. Hayes or Dr. Holick have presented. Very compelling evidence exists, but no mechanism as to how it works. Your work has started to piece together the mechanism through these kinase signaling pathways. I think it is a big step forward in recognizing the importance these agonists (or JAK-STAT) have in how it influences IL-12 and downregulates Th1 activity. It is a very big step forward.

When we look at autoimmune disease, people often say there is no clinical autoimmune disease in the absence of high autoantibody titers. From your evaluation of both the literature and your experience, are autoantibodies the sine non quo for the autoimmune process and how do they derive out of the signaling activation that you have described?

The Role of Autoantibodies in Autoimmune Disease

JJB: In the really early days, people were working mostly on T cells as the target for inhibiting diseases (mostly autoimmune diseases). We now know definitely antibodies are playing a role and autoantibodies will be playing a role in MS, RA (rheumatoid arthritis), type 1 diabetes, myocarditis-T cell-mediated disease, mostly Th1 types of mediated disease. But, during the course of analysis, we and other people have found that antibodies specific to neural antigens are present in humans as well as in the animal models. My point is that even to produce autoantibodies by B cells requires Th1 cells. Th1 means T helper cells. Helper cells help B cells also to make antibodies. Even though B cells are involved, T cells are the prime target and if you target Th1 cells, it will inhibit Th1-mediated tissue damage, as well as help B cells. It will be the target, I think, to go after.

JB: That makes very good sense. It is interesting that there is more literature coming out now saying that autoantibodies always have some level of importance. They are part of the body's defense process; they are not all bad. A zero titer of autoantibodies is not associated with good health and it relates to the distribution of the type of immunoglobulins. If you shift a pattern from an IgG-dominant over to an IgM-dominant, that suggests that you are getting a shift in the personality of your immune system (moving toward autoimmunity). I find it fascinating that we have changed our view about autoantibodies by saying they are not all bad, they are actually part of the natural defense process.

JJB: That is what I also believe-that is it part of the natural defense process as well. It is not basically blocking out or inhibiting antibody or Th1 response. We need to regulate-regulate at a threshold where it won't cause any harm to our tissues, but it will maintain and regulate whatever regular physiological function needs to be taken care of.

Is there a Pre-clinical Stage of Autoimmune Disease?

JB: Thank you. The next question is, I know, a very controversial question. If autoantibody titers start increasing well before the diagnosis of disease (and I think there is ample evidence for that published in some fairly good journals), then it suggests there is a pre-clinical stage of autoimmune disease that may exist prior to the onset of a frank diagnosis. Does your work (through looking at the signaling mechanism) suggest that early on (if we had proper biomarkers) we could identify the trajectory toward autoimmunity if we could ask the right questions?

JJB: We believe in that kind pattern. In our animal experiment (which is a 30-day course), usually the disease peak is around 12-15 days. We did a course of PCR analysis after IL-12 gene expression. In the brain we found that you can detect high level of IL-12 around 6 to 7 days (5 to 8 days before the peak it is coming higher); this is what we call pre-disease. We believe that this pattern may be seen in humans as well, but we don't get to do a similar kind of analysis (it is hard to identify such a patient before they come to the clinic). If such a study is done, we believe we may be able to identify pre-disease increase in pathogenic antibodies as well as the cytokine level.

JB: I think that is a very important message for our clinicians to keep in mind because it has been suggested that there are approximately five fold as many people that have increasing titers of autoantibody prior to the onset of diagnosis than there are people being treated for frank autoimmune disease. If you consider that there are 3 to 4 million people with various autoimmune diseases and you multiply that by five, it is a substantial portion of the population that has a chronic state of immune imbalance that we might call pre-clinical autoimmunity for which the drugs that are used to treat autoimmune disease are probably too harsh. What can be done with these patients to improve their function? Some of your investigations and discoveries related to some of these natural products may come into play. At an earlier stage, they may be the best compounds for modulating these functions.

JJB: We believe that natural compounds may play a very important role. Because of a low risk of side effects, we believe that you can start taking low quantities of these natural compounds even if you don't have a disease. If there is a pre-disease state, you may be able to subvert substantially the possibility of getting clinical disease. One thing to keep in mind is that most of our studies used high doses of these molecules. We used these molecules to inhibit the disease after the onset of disease or during the course of the induction of the disease. People usually eat low quantities of nutraceuticals in their regular diets. If you keep doing that routinely (like people do in some of the Asian countries), you may be able to prevent the onset of these kinds of autoimmune diseases.

Correlation between Insulin Resistance, Hyperinsulinemia, and Autoimmune Disease
JB: We know in the United States and elsewhere in the developed world there is a rising tide of insulin resistance, hyperinsulinemia, and type 2 diabetes. Your work on recognizing that there is a connection between PPAR-g agonists and the downregulation through the JAK-STAT pathway of IL-12 expression suggests (and I don't want to put words in your mouth, but I guess this may be a question) that there may be a correlation between insulin resistance, hyperinsulinemia, alterations in the PPAR-g pathway, and exacerbation of inflammatory types of autoimmunity. Does that kind of story that I just developed hold any water or is that making too many leaps?

JJB: That is something that we want to study as well. Since all of our experiments with PPAR-g ligands are in an animal model, and since these drugs are taken for type 2 diabetes as a prescription, we are trying to see (in the record) if we can find some MS patients who are type 2 diabetic and are taking PPAR-g ligands by prescription. If we can find some number of patients like that and determine whether they do better or they get worse, or what the relationship is between taking PPAR-g ligands and MS and diabetes health, we can connect it. At this point, we know that PPAR-g ligands definitely inhibit JAK-STAT pathways. Some of those pathways are required for fighting infection because IL-12 is required for Th1 response and Th1 response is required for fighting many of the pathogens. We cannot shut down the whole pathway of Th1 response because then the patient would need to live in a sterile environment. Our objective is to regulate these pathways to achieve a threshold level of autoimmune response that won't harm the tissue, but will be an immune response to infection. The JAK-STAT pathway is the target; we think it is one of many of these pathways that these kinds of molecules are targeting. We need to remember that these pathways are required for normal physiological function as well.

JB: You just said something that I think is very important for clinicians. The drugs-the breakthrough new biologicals-that we have available to the rheumatologists to manage autoimmune disease are these biotech products that are basically anti-TNF-a blocking agents, or they are mimics that bind to receptors. Because of the physiological activity of these compounds, they are capable of really suppressing the immune system. The black box label warnings for these products often warn about tuberculosis or malignancy. What I am hearing from you is that we have to be cautious when we use these drugs because they may have an effect on suppressing immune systems, and if we can intervene early we may get some clinical advantage.

JJB: That is what we stress. When we write manuscripts, I put a warning like that in the conclusion. We need to watch the dose we use when we take these drugs because all the pathways are important for normal physiology and fighting against infection or cancer. The timing is very important. I think if we start taking low levels (or a normal dietary level) of these compounds before we get a disease, this may be beneficial in the long run.

Published Research by Dr. Bright
JB: With that I am going to give our listeners the titles of four of your papers that I think bear on the next question I want to ask so they will have them in their thoughts. One is your more recent paper in the Journal of Neuroscience Research in 2006 titled "1,25 Dihydroxyvitamin-D3 Modulates JAK-STAT Pathway in IL-12/IFNg Axis Leading to Th1 Response in Experimental Allergic Encephalomyelitis." 10 The next is from the Journal of Clinical Immunology in 2004 and is titled "Quercetin, a Flavonoid Phytoestrogen, Ameliorates Experimental Allergic Encephalomyelitis by Blocking IL-12 Signaling Through JAK-STAT Pathway in T Lymphocyte." 11 And the next is a curcumin and autoimmune disease

study, "Curcumin Inhibits Experimental Allergic Encephalomyelitis by Blocking IL-12 Signaling Through Janus Kinase-STAT Pathway in T Lymphocytes;" this appeared in the Journal of Immunology in 2002. 12 And then your review paper, "Targeting Autoimmune Diseases Through Nutraceuticals" appeared in the journal Nutrition in 2004. 13

With that as a background (just so our listeners know you are speaking as an expert) I would like to ask kind of a philosophical question. Within the cellular signaling pathways, antennae that stick out to receive messages are like receptors that are sensing the outside world and translating those messages through these kinases, these complex networks of signaling, to the gene to create different expression patterns that change the personality of that cell (to go from a cell at rest, say, to a cell that is in an inflammation state). Why it is that specific nutrients that we have either consumed or been exposed to (like 1,25 dihydroxy D3 or curcuminoids or quercetin) would influence those pathways? What does this mean about what we eat, the environments we live in, the regulation of our gene expression patterns, and the changing environment that then maps against the diseases that we have seen in prevalence in developed western society? Do you philosophically feel that there is some interesting connection here?

JJB: I think so. First of all, I appreciate you introducing some of our published manuscripts with the titles and volumes. We are glad we could take some lead on publishing articles about the use of nutraceuticals in treating autoimmune diseases. Curcumin is a favorite for us. When we presented at a conference in New Orleans several years back, someone wrote an article saying that curry spice can cure multiple sclerosis. There was a lot of attention, and many patients started calling me-"How many teaspoons?"-and then the multiple sclerosis society called me and asked me to send a PDF file to put on their website because many patients were calling and asking about it.

At that time, I cautioned many of the patients that this is an animal study and this is a compound we usually use in very low quantities in our daily food and it regulates normal physiology. These are conditions we have to learn more about through clinical trials with human MS before we can say how many teaspoons we should eat. No human being can survive without food. Everybody eats food and everybody gets some kind of nutraceuticals (biologically active functional molecules) through food everyday of our lives. For example, many people eat curcumin from childhood to adulthood.

Through the studies we have done, we came to understand one mechanism by which some nutraceuticals regulate body functions. We have shown the JAK-STAT pathway can be regulated by natural products or nutraceuticals or environmental factors. I think there is an interplay between environment and autoimmune disease. When we talk about autoimmune disease, we don't know the etiology yet, and we believe that environment is one factor which modulates genetics and our behavior. Nutraceuticals can be called behavioral-you choose what you eat, and your choice of food can change the final outcome of your health (through autoimmune diseases or otherwise). We believe nutraceuticals are natural products. Environment can influence molecular mechanisms or physiology, which can lead to the pathogenesis of many diseases, including autoimmune disease.

JB: That is a really eloquent answer. I have one last question. It seems what is emerging from your work and work of others is that these environmental factors that signal certain cellular functions through these kinase pathways operate through very complex web-like signaling pathways. That suggests there is more than one mechanism for controlling a disease (a disease is not just a breakdown of one mechanism), and that there is the possibility of synergy among these different compounds that are consumed in a daily diet

or that we are exposed to in our environment, and it is not just one molecule at a time, but rather it is multiple pathways responding to multiple molecules that gives rise to the orchestration that we call our function. This is a very different model than the traditional pharmacological model, which is one molecule for one function for one disease. Do you feel that as this emerges-this concept of regulators of intercellular communication from the environment-that we will start looking more at complex arrays of molecules affecting multiple pathways?

JJB: I believe so. There is not just one pathway; JAK-STAT is one pathway, but we know there are many other pathways involved in the whole process of autoimmune response, autoimmune disease, and inflammation. These pathways include the MAP kinase pathway, the NFkB pathway, the AKT pathway, and JAK-STAT. I think there is a network among these pathways that leads to the final outcome of the disease. We believe that if we block one of these pathways it will block the whole network.

We are trying to learn one pathway at a time. We have recently started using a computer infomatics program to map all the pathways in autoimmune disease (using inflammation and EAE and MS as a model) and to look at novel targets in various different pathways, how they interact, what the directionality of the interaction is, and the strength of the interaction so we can identify novel molecular targets at the junctions of these pathways. There are many approaches like that I think people are taking approaches like this and hopefully not single tract therapy.

We believe nutraceuticals have multiple biologically active functional compounds and that they may all work together in concert to regulate these signaling pathways. Curcumin has low bioavailability; that is one complaint we have. People have published that curcumin bioavailability can be increased by other compounds. What we realize from these kinds of experiments is that even one natural or phytochemical and nutraceutical compound can increase the bioavailability of another compound or its action on one pathway. The biomolecules of nutraceuticals help each other to enhance activity on the molecular targets as well as the molecular signaling pathways. These nutraceuticals may help regulate all these networks together, and these molecules together may regulate the network of signaling pathways in a beneficial way for human health.

JB: Dr. Bright, I want to compliment you. This has been one of the most information-dense and concentrated-but-eloquent presentations we have been fortunate enough to have and it opens up a whole vision of where our future might be taking us in looking at the role that diet and lifestyle and environment play in both the prevention and management of complex chronic disease. Keep up the tremendous work. You are really a pioneer and we have so appreciated you sharing this information with us.

JJB: Thanks so much, Dr. Bland, for the opportunity to share our work and I hope we will have more and more discussion and some of this message will be useful and beneficial for the patients who are suffering with autoimmune diseases. Thank you.

JB: Thank you.

I am guilty for waxing very philosophical at times and getting very emotive and excited about these interviews but I think my excitement is justified at the conclusion of Dr. Bright's eloquent presentation. The implications of what he has discussed may be quite dramatic, clinically.

When Dr. Colleen Hayes of the University of Wisconson talked about her work and the animal model (the EAE model for MS), she showed that vitamin D metabolite (1,25-dihydroxyvitamin D3), when vitamin D metabolite (1,25-dihydroxyvitamin D3) was instilled directly into the brains of totally paralyzed animals, the animals were walking and then running around the cages in a matter of less than an hour. The dramatic influence of these agonists that may have nutritional origin could be quite significant, clinically. The chapter still remains to be fully written, but the story sounds very fascinating from a clinical perspective. Thanks for being with us. We look forward to being with you next month.

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