October 2009 Issue | Trevor Marshall, PhD Adjunct Professor

http://jeffreybland.com/knowledgebase/october-2009-issue-trevor-marshall-phd-adjunct-professor/

DOWNLOAD AUDIO |DOWNLOAD SUMMARY NOTES |

Welcome to *Functional Medicine Update* for October 2009. If you have been listening and following along with our intellectual content over the last several months, you'll recognize that we have been developing this concept of signaling to intercellular communication that occurs by outside environmental agents, like gluten, allergens, toxins, or inflammatory proactivating agents, and how that signaling ultimately influences the phenotype of cells or the expression of what we call later the clinical presentation of the patient. This follows very nicely within the context of the functional medicine assessment concept: looking at antecedents, followed by triggers that trigger the release of various mediators from specific cell types. These mediators go on to regulate function downstream in multiple cell lines to ultimately produce what we see in the clinic as signs and symptoms with different duration, frequency, and intensity.

This is a very different model than the traditional differential diagnosis model. Rather, what we are doing in this particular process is understanding the origin of the disorder and how it spreads out into multiple different presentations, knowing that we have comorbidities, which refers to multiple organ systems being influenced in different ways by shared processes or shared mechanisms. That is the functional concept that underlies the functional medicine model as we have been describing it for many years.

We have really had a wonderful journey with a number of clinicians and investigators over this 2009 year, who have been helping us to understand genomic uniqueness and the influence of expression signaling from outside agents, including things like gluten (which we spent quite a bit of time on) and more recently gut enteric bacteria (how they influence signaling systemically). I hope these interviews have opened up a richer and more robust view of the origin of chronic disease and new opportunities for both its prevention and management based upon this strategy

Just as we are developing this theme, what do you think appears in *Scientific American*magazine in their August 2009 issue? It is an incredibly rich article-beautifully written-and (as always with *Scientific American*) wonderfully illustrated, titled "Surprises From Celiac Disease," authored by none other than Dr. Alessio Fasano, now at the University of Maryland, and the person who was the principal investigator in discovering many of the mechanisms at which gluten can initiate, at the brush border cell and at the mucosal barrier, alteration in gut mucosal integrity that he has termed (and we have used this term for many years) "leaky gut."[1] As a gastroenterologist, Dr. Fasano has started to put this term "leaky gut" and gut permeability on the map related to localized gut inflammatory response, as it pertains to the gut as an immune organ.

I think you will find this a very interesting article. I urge you to go to the *Scientific American*website and

look at this mechanistic discussion of gluten and gut permeability. Included is a beautiful diagram/illustration that looks like we a functional medicine teaching diagram. The brush border cells have proper intercellular junctions that get disturbed by various proinflammatory signaling processes from outside molecules (like gluten) in genetically susceptible individuals, leading to breakdown of gut permeability and opening of the portals of entry to larger molecular weight molecules that can initiate a generalized immune response. I think this article is a very nice confirmation that this field is growing in greater recognition and visibility.

We are really talking about the changing of metabolism as a space in time that relates to altering the web of physiology. As you know, one of the fundamental tenets of functional medicine is this web-like interaction, and that concept is really gaining traction now in the field of general science and certainly in systems biology. A recent paper that was published that helps us to understand this better appeared in the *Journal ofProteome Research* in the October issue of 2009, in which they talked about individual human phenotypes actually reflecting the influence of the environment .[2] If you look at identical twins, which they did in this particular study, and do pattern recognition of their metabolites by complex dendritic analysis (this is very complex pattern analysis-it is kind of almost artificial intelligence of the data set [multiple analytes]), they found that a pair of identical twins in different environments, particularly different nutritional environments, showed a difference in how their metabolites clustered. There is a genetic underpinning (your basic map) that is common between the two identical twins, but then there is this environmental factor laid on top that modifies or distorts their web, making them less the same than they were prior to being in different environments, and diet is a major modifier of that . The gut connection to enteric bacteria and its spreading effect through the whole systemic circulation through information signaling molecules is a whole new paradigm that is opening up in medicine, and that is the topic we will be discussing and focusing on in this issue of *Functional MedicineUpdate*

INTERVIEW TRANSCRIPT

Researcher of the Month
Trevor Marshall, PhD
Adjunct Professor
School of Biological Sciences and Biotechnology
Murdoch University
Western Australia

Director
Autoimmunity Research Foundation
Thousand Oaks, CA

www.trevormarshall.com
www.autoimmunityresearch.org
www.marshallprotocol.com

Here we are once again at that portion of Functional Medicine Update that I know you, like I, look forward to each month with anticipation and that is our Functional Medicine Update Clinician/Researcher of the Month area, where you hear the "news-to-use" from the people who are really making the new medicine happen. You are not going to be disappointed this month, I can assure you, because we have

one of the clearest, fresh, and innovative thinkers that I think I have had the privilege to interview, Dr. Trevor Marshall.

Let me tell you a little bit about Dr. Marshall. If we want to talk about a Renaissance man, this gentleman has diverse interests, ranging from biotechnology and medicine, into things like digital information sciences and digital media and the way that one assembles complex information into systems and cross-disciplinary thinking. You'll see it woven beautifully into the work that he is going to share with us today, which has to do with the burden of autoimmune disease, which cuts across many different diagnostic categories and affects every subspecialty of medicine when we talk about dysregulation or altered regulation of the immune system that often gets put under the rubric of autoimmune disease. Dr. Marshall is an adjunct professor at the School of Biological Sciences and Biotechnology at Murdoch University in Western Australia. He is also the past Chair of the Engineering in Medicine and Biology Society, and he is currently Director of the Autoimmunity Research Foundation in California, which you will be learning more about as we go through this interview.

Dr. Marshall, it is really a great privilege and pleasure to have you here to share your diverse background and talents and discoveries with us. Given that I've already presaged that this interview is going to involve discussions about the immune system (the immune system kind of gone into overdrive), could you help us review the concept of innate immune response and its relationship to bacteria, and viruses, and things like antimicrobial peptides? Your work is founded upon this whole emerging concept of the innate immune system. Can help us understand a little bit about the background?

The Difference Between the Adaptive Immune System and the Innate Immune System
TM: It's great to be here. The focus of research, particularly on the autoimmune diseases for the last four or five decades, has been on what we call the adaptive immune system. That is where the antibodies are generated and are recognized by the lymphocyte clones and then become memory cells. Should a pathogen attack again at a later point, the body has the ability to recognize them with antibodies generated in the past. This is what is called the adaptive immune system.

The innate immune system is like the final line of defense that the body has. Once the pathogens manage to get within the cells of the immune system itself (within the cells that generate those antibodies and generally protect the body), the innate immune system kicks in to try and protect the cells from the pathogens. There are a number of obligate cytoplasmic pathogens (that means pathogens that get within the actual phagocytic cells-the cells of the innate immune system that are supposed to gobble up these bacteria; the known ones include mycobacterium, of course, and some others as well). Typically, the methods by which those pathogens invade the innate immune system vary. What we have found is that there is a persistence-what I call a metagenomic microbiota. Metagenomic means there are many genomes involved, many more than one species accumulate during the lifetime. And microbiota is a community of microbes; you might think of it as a biofilm-protected community because there is usually a biofilm central area of these inclusions and then a cytoskeleton (an exoskeleton) around the outside. Think of it as a vacuole in a phagocyte...a vacuole which is full of living and persisting bugs rather than being filled with dead pieces of bugs. That is really what we are dealing with these chronic diseases.

JB: To me, this sounds like an extraordinary step forward in understanding this connection between...what would you call it?...I guess the genome that is not a eukaryotic genome...It would be this diverse microbiome genome and that of our own cells in the immune system. It sounds to me like what

we are saying is that we are starting to get information from a non-relative genomic that then has an effect both internally in our innate immune system and systemically.

Genomes of Bacteria Have Now Been Sequenced

TM: Clinical medicine has retained the concept that the human body is a sterile compartment. Basically, that there are no pathogens within the human body except for the normal, acute-phase pathogens that cause sickness. And those acute-phase pathogens, by and large, are capable of being cultivated in the laboratory and observed with the tools that medicine has had available to it prior to the explosion of the genome in this 21st century (in the last decade). There are now over 2400 bacteria whose genomes have been fully sequenced (essentially fully sequenced), in addition to the human genome. Typically the bacterial genomes are smaller, but they are still producing a large number of proteins, enzymes, lipids, etc., and we can actually study these bacteria in some detail now by looking at their genomes and comparing the genomes with that of Homo sapiens itself, looking for things like molecular mimicry, and also with other known pathogens, looking for things like the way that the bacteria evade the immune system, for example.

JB: How does the body regulate this burden? It obviously must have built in systems that can get to these biofilms or this microbiota burden. What are the mechanisms by which the immune system has some protection against this?

TM: The body has this innate immune system I spoke about, which consists of a number of factors. The key defense are the so-called antimicrobial peptides, which are relatively small molecule proteins (there are larger molecules-actually the molecules transcribe from the genome as larger molecules and then they break down to the smaller peptides), which target specific known pathogens, against which Homo sapiens has managed to survive in the past. There is one very important antimicrobial, which is called cathelicidin. Cathelicidin is the main antimicrobial that allows the phagocytes to protect themselves from intraphagocytic invaders (or invaders that actually get through the outer layer of the phagocyte and try and persist in the cytoplasm). But there are also others; there are the beta defensins and alpha defensins, which are pretty active in the gut and in other areas of the body as well. Totally, there are about 24 families of antimicrobial peptides that have been identified at this point in time (or antimicrobial genes--let me put it that way) that translate into peptides and proteins that have been identified at this particular point in time. There are a number of ways that these transcribe from the genome, but many of them are transcribed by what we know as the type 1 nuclear receptors).

JB: That leads us into a really interesting cross fertilization in this field. Our field of science often tends to be compartmentalized and different disciplines don't talk to one another, but I think you have done a very good job of helping us to recognize that the body doesn't subscribe to any specific professional society; it works as a community. We are going to transition now to one of those nuclear receptors, which you have really brought to the forefront of our attention. That is the vitamin D receptor and how that plays a role in this whole process. Can you help us understand that?

Defining the Vitamin D Nuclear Receptor (VDR)

TM: The vitamin D receptor is one of the nuclear receptors. Let me call it a sister receptor because it is part of a complementary team with the progesterone receptor, with the androgen receptor, with the alpha and beta thyroid receptors, with the glucocorticoid receptors, with the mineralocorticoid receptor, and the PPAR alpha and gamma. They are the main ones. They form a set, which we call the type 1 nuclear

receptors, and they work together as a set, transcribing genes or expressing genes from the genome into the proteins and later the enzymes and other metabolites that the body needs in order to function correctly.

There is one nuclear receptor called the VDR (the vitamin D receptor), which is responsible for transcribing or expressing about 3{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} of all the genes in the human body. It is fairly important. There is quite a lot of redundancy in the human genome; it is remarkable resilient and able to deal with challenge. So there is quite a bit of redundancy, but the VDR is responsible for transcribing over 900 genes that have been confirmed at this point, which is about 3{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} of the total human genome. This particular receptor transcribes genes ranging all the way from the metastasis suppressor type 1 all the way through to many, many genes associated with the development of the human fetus. It is a very, very important transcription factor. A transcription factor is something that will transcribe the genes that are on the genome into proteins that the body needs in order to work.

JB: You are helping us take an important step, and that is to start off with understanding a little bit about the innate immune system and the complex relationship it has with microorganisms and the complex microbiome. We have said that part of the regulation of the innate system and its expression of genes (over 900 genes) is regulated by the VDR (the vitamin D receptor), which plays a very important role in concert with other orphan nuclear receptors in regulating genomic transcription. Going upstream from there, the question is: What are the various types of activators or suppressors or modulators of the vitamin D receptor? One of them must be vitamin D. Let's talk a little bit about that?

Confusion about Vitamin D Dates Back to Early Research

TM: Right. The vitamin D receptor became known as the vitamin D receptor because of the substance (or a metabolite of the substance) that was called vitamin D back in about 1903 through about the 1920s when the vitamin was being studied. A substance was identified back then-a secosteroid-and that means a steroid which is a little bit more flexible than the other steroids. This secosteroid was called vitamin D. In its activated form, it is the substance in a healthy human body that allows the VDR to transcribe all of these genes. The whole confusion about vitamin D and the VDR and whether it helps people fight off cancer or whether it prevents cancer or whether it makes cancers worse...all of the dissension at the moment in the clinical world around what vitamin D actually does is based on the mistaken notion that it is a nutrient. It is not a nutrient. The body produces all of the vitamin D it needs. It can get excess vitamin D from, for example, exposure of the skin to sunshine, and from the diet (for example, eating large quantities of fish will increase the level of vitamin D in the bloodstream), but the vitamin D that is needed in order to transcribe the genome is produced inside each of the cells where the VDR is present. All the exogenous, or externally supplied, vitamin D can do is to try and disturb the homeostasis that is set up within the cell itself.

JB: I want to make sure that our listeners are following because this is a fairly complex area. The secosteroid, which would be that which converted in the body from 7-dehydrocholesterol into cholecalciferol then gets further converted, as you say, into a vitamin D receptor modulator, which is 1,25-dihydroxyvitamin D3 (what you now are really talking about as a hormone modulator), so that goes through several control points...

TM: Yes, it's a nuclear hormone, though. It's not an endocrine hormone. It is not a signaling hormone. It leaks from the cell into the bloodstream and it can be measured in very low quantities in the bloodstream, but it doesn't have a signaling mechanism an endocrine hormone); it's a nuclear hormone.

Assessment of Vitamin D Status

JB: I know in our field the clinicians are measuring, as a status evaluator for (or biomarker for) vitamin D status, the 25-hydroxy precursor to the 1,25. It would sound, from what you are saying, that we ought to have some information about 1,25 levels as well as 25. Can you tell us a little bit about assessment?

TM: These pathogens, including mycobacteria, Borrelia, and Epstein-Barr virus are known to downregulate the VDR. They actually stop the VDR from doing its job properly. Whether you look at those or you look at the pathogens (the metagenomic microbiota) that accumulate to ultimately cause chronic disease, they do this by knocking out the VDR. As we said earlier, the VDR is responsible for a significant proportion of the body's own antimicrobials. If a pathogen is going to survive and persist, it clearly has to knock that receptor out, and it does, in fact knock that receptor out. When the receptor is knocked out, you usually detect a lowered level in the bloodstream of this metabolite called 25-hydroxyvitamin D, which is the normal one that is measured to assess vitamin D status. The 1,25-dihydroxy (the active hormone), which is much, much harder to measure usually rises when the VDR becomes dysfunctional in chronic disease, but the one that is easier to measure (the one that we measure for vitamin D status) drops. I'm sure you would have seen all of the studies over the last decade that show that just about every chronic disease is associated with a drop in the blood level of 25-D. The reason for that is the body's own homeostasis (the control systems that control the generation of metabolites within the cells); it is the body's own homeostasis trying to force the 25-D to a level where the VDR can do its job properly. It fails to do its job when pathogens manage to overcome the innate immune defense system. My colleagues just published a paper in Autoimmunity Reviews pointing out that although the low levels of vitamin D are associated with the chronic diseases, you cannot correct or reverse the chronic diseases by increasing the level of vitamin D.3 Vitamin D is not a nutrient, it is a marker.

JB: I think this is a very important point for clinicians because we have been led to believe that the assessment of 25-hydroxy D is measuring a vitamin deficiency and that by repleting the vitamin you manage the deficiency, which then treats the downstream deficiency signs and symptoms. This model that you are presenting, based upon this background, is certainly a different model.

Vitamin D Supplementation is More Complex than Just Treating a Deficiency

TM: Yes. Our paper documents a number of studies that have showed conflicting results. In fact, just last week the Institute of Medicine of the National Academies held a hearing where two of my colleagues spoke.4 They have done a complete report of all of the literature (a summary of all the literature) on vitamin D (whether it is beneficial or not beneficial). Their official study came back with the conclusion that there is just too much variance. There is no distinct thread that can be pulled out of all the studies that have gone on in the past.

The reason for this is actually fairly simple. I said that the VDR is responsible for transcribing about 3{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} of the total human genome. When you construct an experiment to change the way that the body transcribes that 3{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} of the total human

genome, a lot of things change (at least 900 metabolites, plus all of the downstream affects that those have). A clinical study-especially the clinical studies that have been based on the concept that vitamin D is a nutrient-are not capable of measuring that many variables. If you have a typical cohort of a few hundred patients, for example, you would be lucky to be able to deal with 4 or 5 variables, and typically you try and constrain yourself to look at just one endpoint. The problem with the VDR and its ligand, activated vitamin D, is that when you change the homeostasis around the VDR, you are changing about 3{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} of the total genome, and lots and lots of things change. It's just not possible to measure all of the changes that occur and understand how they fit into the scheme of things unless you study it at the level of the biology, and not just at the level of the evidence base.

JB: That's a really important point. Let me parrot back something to make sure that I'm following correctly. What I have heard you say (and I'm going to sound bite this to hopefully make it simple) is that the effect of the burden of the microbiome on our innate immune system then has implications on the secosteroid metabolism through the VDR effects so that the 1,25-dihydroxy and the 25-hydroxy levels reflect more as an assessment of the burden of the microbiota on our immune system than a pure vitamin D-deficiency relationship. Therefore, ipso facto, if you just give more vitamin D to treat what you thought was an apparent vitamin D deficiency (based upon a 25-hydroxy serum level), you may be actually going the wrong way.

TM: Right. You are trying to force the body to increase the metabolite that the body itself has decided needs to be lower in order for the cells to continue to function correctly. 25-D is regulated down by the cells in the chronic disease processes (those processes which are based on microbiota which overcomes the VDR in order to survive). You'll recall the reason that overcoming the VDR is an important survival mechanism is because many of the antimicrobials are produced by the VDR.

There is one step we took that I didn't explain in any real detail. We started talking about microbiome. Since we've been able to identify the genomes of the bacteria that coexist with Homo sapiens, we have found, for example, that the saliva contains over 100 species of bacteria, and that ranges from Yercinia, Neisseria, obviously strep and staph, and all the way through; about 100 species are in normal saliva from healthy individuals. In hip joints you will find a whole range of bacteria, many of which have never been seen as being human pathogens or actually capable of being internalized in the human body (Methylobacter, Lysobacter), and even the Eubacteria, which were previously only found in hydrothermal vents at the bottom of the ocean (hydrothermal vent Eubacteria). These can now be found because their genomes can be found and identified with DNA sequencing.

JB: I think you've actually said it in your publications that more than 90{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} of the genomes in the human are not eukaryotic.

TM: Yes. That's a number that came from the National Institutes of Health (NIH) Microbiome Project, which seeks to more closely define how many pathogens and species we are dealing with and where they are located in the human body.5 That's an ongoing project. Some of the early results are in. I mentioned the salivary microbiome, for example. There will be a lot more studies coming in over the next few years that will enable us to really understand the way in which Homo sapiens lives in harmony with the bacteria flora that (on this planet, at least) are far more numerous than Homo sapiens, itself.

JB: That begs one interesting question related to polymorphisms or genetic variations that could have influence on the regulatory components of the innate immune system, for instance, polymorphisms of VDR. Have certain SNPs been identified that are more sensitive to dysregulation or altered regulation in this process?

TM: This is a very difficult and technical area. One can make the argument that the concept of a polymorphism and a SNP is based on the concept of a single genome for Homo sapiens and not on a mix of genomes in the samples being measured and analyzed. When the bacteria living in symbiosis with Homo sapiens interfere with (or affect) the transcription and repair mechanisms of Homo sapiens itself, then there is clearly the opportunity for genetic variation to occur as a result of the pathogens. But honestly, there just is too little known at this point about precisely how the human genome protects itself and precisely how the pathogens integrate their DNA with the human DNA. Once again, over the next few years that will become a lot clearer. I'm not an expert in that area because I frankly think at this point it's a waste of time to be looking at genetic variations in one genome when there actually are hundreds of genomes present in the body. You get a measurement error potential, and you certainly get interpretation error potential.

JB: Thank you. Let me just go back to one point that we talked about earlier. If we can, I want to see if we can get clarity for the clinicians. Based on what you have said, is it or is it not, in your opinion, important to measure (if you are doing an assessment of this whole immune interrelationship with vitamin D) both 1,25-hydroxy and 25-hydroxy or is that still not going to give you the information you are looking for?

Vitamin D Status as a Marker for Chronic Disease
TM: Well it actually is a fairly good marker of chronic disease. One of my colleagues, Dr. Greg Blaney, from British Columbia, just published in Frontiers of Autoimmunity, which is put out by the Annals of theNew York Academy of Sciences, a paper that explains (with his particular patients) how the best indicator of chronic disease has been an elevated 1,25-dihydroxyvitamin D, frequently associated with a depressed vitamin D status (or 25-hydroxyvitamin D).6 That is a paper, showing that it is one of the best markers of chronic disease, in fact. By chronic disease, I mean the chronic disease where the pathogens have overcome the VDR in order to persist in the human body.

JB: That's very helpful. Thank you. The big next step in the journey that we are taking with you is to talk about how this understanding of the emerging view of the innate immune system and its regulation by endogenous and exogenous factors could influence the clinical approach to various autoimmune diseases. Principally, the way we have been treating them recently is just to knock down the immune system and try to treat the symptoms rather than the cause. Can you tell us a little bit about how this model is helping us maybe moving toward clinical approaches?7
TM: Yes. You are correct. It is typical that in all of the rheumatic, autoimmune, and many other chronic diseases, including asthma, for example, that corticosteroids are used in order to suppress the inflammation based upon the concept that it is the inflammation that is causing the problem and the disease symptoms. But, when you are looking at inflammation, that is driven by bacteria (by pathogens), then the inflammation is actually beneficial because it is the body's own response trying to knock down the pathogenic load and generating inflammation from the cytokine storm that it produces as part of doing that. And, indeed, applying corticosteroids to reduce the inflammation and reduce the body's ability to deal with the pathogens, is in the long run going to bring relapse. That has been documented in some of

the more serious chronic diseases. For example, if you take sarcoidosis, which is a really end-stage chronic disease because in sarcoidosis the phagocytes, monocytes, and macrophages actually exist in clumps or granuloma, which don't consist of any other tissue-related cells specifically, but where more than 90{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} of the cells in the granuloma are these innate immune primary defense cells (the monocytes and the macrophages). In that particular end-stage disease,the vitamin D dysfunction has actually been known for some time. And with that disease, it becomes clear to see that when you use corticosteroids to make the patient feel better and suppress the symptoms in the short run, that there is almost invariable relapse. I can remember a paper which came out in the 90s (quite a decent study by Gottlieb, et al) which showed that something like a 78{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} relapse rate following withdrawal of corticosteroid in sarcoidosis. We would expect the same pattern in the other chronic diseases and autoimmune diseases as well.

JB: I know that in your papers you've suggested (maybe at a lower level of risk, I'm not sure, than corticosteroids) that excessive use of vitamin D in these cases might lead to kind of temporary immune suppression but later-stage rebound effect like you are describing. That is kind of a highlight that you have cautioned us about, I believe.

TM: The body is designed to be able to deal with vitamin D from external sources (vitamin D from sunshine, for example, on the skin). When vitamin D falls on the skin, the skin reacts to protect itself from, for example, carcinogens that are produced as a result of UV light falling on the skin. The skin acts to protect itself, and in doing so, in healthy people, it produces some 25-D, which accumulates in the bloodstream and then in the tissues of the patients. Also when patients have food which contains a significant amount of vitamin D, such as, for example, fish or mushrooms (especially irradiated mushrooms), then that also accumulates in the bloodstream. This doesn't do any harm until it gets to a level at which it starts to interfere with the processes within the cell. There is an attenuation between the bloodstream and inside the cell, which is about 20 to 1 (10–20 to 1), so at concentrations around 20 nanograms per mil (around 20–25 nanograms per mil) 25-D, the vitamin D in the bloodstream starts to become immunosuppressive; it starts to interfere with the way that the VDR is activated within the cells.

JB: This very complex regulatory process that you have been describing takes us beyond the simple concept of measuring a number and then giving an agent to treat the number. That is, I think, what we are taking away from your discussion.

TM: Right. We've known for a long time that if you give vitamin D to a group of people, that every one of them will behave differently, that every one of them will end up with a different value for the blood metabolite. It is not a linear "vitamin D in, goodness out"-type of paradigm, which is what you would expect from a nutrient, incidentally (toxicity issues aside). A nutrient you would expect to be a first-order mass action-type of system, but what you actually find with the vitamin D metabolites is a very complex control system, more of an eighth-order, involving two other transcription factors (PXR and CBP), which produce the enzymes. It is quite a complex diagram. I published it in Figure 1 of my bioessay in 2008 if anybody is inclined to actually go into all of the various things that affect the operation of the innate immune system, affect the operation of the VDR itself.8

JB: Let's move into an example of an autoimmune disease that certain receives a tremendous amount of

attention and one that you have helped us understand more about. That is fibromyalgia, which is an interesting kind of "wastepaper basket" diagnosis for many people because it doesn't seem like it has a clear etiology, and therefore, often, the treatments are kind of shotgun. We have a new drug approved in the states, Lyrica, which is a gabapentin analog that basically treats (maybe) the pain without treating the cause; there are a lot of questions about that. It seems that there hasn't been a treatment that is focused on the cause because no one knew the cause. People say, "Well, it is a disorder of the HPA system (the hypothalamus/pituitary/adrenal axis)." What does that mean? Where is did the origin of that come from? Your model-and your concept-really lends itself to a better explanation of an etiology. Can you tell us a little bit about that?

TM: Fibromyalgia was one of the pleasant surprises that we got once we started to focus on the disease sarcoidosis. We found that fibromyalgia and another disease (another diagnosis), chronic fatigue syndrome, both responded well to the VDR agonist. We use a VDR agonist, which is a small molecule. In this case, it is a sartan that was developed primarily to target the angiotensin 2 receptor, but it also targets the VDR quite well when the dosing is changed. When we used the VDR agonist to switch the VDR back on again, there are a number of reasons why vitamin D, itself, can't switch the VDR back on again, but to understand them I've really got to go in to the structure of the proteins and things and that's pretty heavy stuff. When somebody gets sick, then vitamin D can no longer activate the VDR; it has to be activated with a different drug. We were fortunate enough to be able to find a drug that would do that, which is in the US formulary and it is regarded as a very safe drug; that drug is called Olmesartan medoxomil. When that is dosed in the correct way, then the VDR is reactivated again, and the innate immune system is incrementally activated and it allows the body to start to recognize the pathogens and also make sure that it keeps transcribing the 913 genes, and particularly the anti-cancer genes that are amongst that set. Once we started to do that, we found that there were a whole range of diagnoses that were responding that we hadn't really expected to respond. As I said, chronic fatigue syndrome, fibromyalgia, and some of the other neurological conditions as well: obsessive compulsive disorder and bipolar were some things that were fairly common in our cohorts. The symptoms tended to resolve at the same time as the underlying inflammatory diagnosis resolved.

JB: Did you find that this was a class effect in the angiotensin receptor blocker drugs or was it specific?

TM: No. It is unique to Olmesartan medoxomil. Only Olmesartan medoxomil is the correct shape to get into the VDR and activate it.

JB: And the doses that are required are generally...?

TM: Just a little bit above the levels that are used for hypertension. But it has to be taken fairly frequently, and it has to be the slightly higher concentration because the affinity of the drug for the VDR is less than the affinity of the drug for the angiotensin type 2 receptor.

VDR Research Links to Obesity and the Gut

JB: You know, it would sound to me, as you are unweaving this story for us, and then talking about what has been called the Marshall Protocol, that you are really discovering an interesting generalized mechanism that relates to the etiology of many chronic-related illnesses. I know you have also done some collaborative work on the obesity epidemic, which might seem far away from what we are talking about, but by this mechanism, it actually interconnects. Can you tell us how those all kind of fit together?

TM: Well, when I wrote the bioessay in 2008, I can remember one of the peer reviewers came back and objected to me referring to obesity as a disease. We got into a discussion. I pointed out that there had been a number of studies on closed communities. There were studies on native Indian communities, for example, in the United States, where the entire way of life of school children was changed in these small tight communities in order to try and curb the incidence of obesity, and it totally failed. The normal connection that we accept--that obesity is a result of lifestyle--has never been able to be confirmed in trials. In fact, quite the opposite has been shown-that obesity appears to be a disease-related process. And since we find it surging in the American population at the same rate as we see other conditions surging (the other conditions that we know are VDR-related in our population), obesity is likely to end up considered a disease of the same nature, where the pathogens are overcoming the VDR in order to survive and persist.

JB: It's very interesting. We just had the privilege of interviewing Professor Delzenne and Dr. Cani at Louvain University (Catholic University) in Belgium, who have been doing quite a bit of work on the gut enteric microflora and its relationship to obesity in which they have been able to show (at least in the animal model) that by altering gut flora they can alter the energy economy of the animal and actually treat obesity. It would seem that what you are describing has some correlation with their work. Would that seem reasonable?

TM: Oh, yes. There is also work being done at Washington University in St. Louis (I'm not sure if that was what you were citing as well as the Belgian work), but there is quite a lot of research being done on trying to understand gut flora. Much of the flora in the body is in the gut, of course, because it accumulates there. It comes in through food and water and things that we ingest, and it accumulates there. Many of these pathogens have learned how to persist. For example, Helicobacter pylori persists for year after year after year in some patients; it is very, very difficult to eliminate. I'm sure your listeners would understand that many bacteria species are competitive, for example, strep always tries to kill off staph that are in the vicinity, and staph does the favor in return, trying to kill off any strep in the vicinity. In fact, some of the antibiotics that are most effective against staph were isolated from strep bacteria forms in the past. So there is a competitive environment set up, and as we fully understand that competitive environment we will probably be able to figure out interventions that will allow us to more effectively deal with diseases like obesity.

The Autoimmunity Research Foundation

JB: This all interestingly connects with your activity as a Director of the Autoimmunity Research Foundation, which is centered in California. Can you tell us a little bit about that? It sounds like a very interesting organization.

TM: The Autoimmunity Research Foundation is a 501(c)3 nonprofit organization. All of our staff are volunteers at this particular point, although we are transitioning right now. It was set up back in 2004 primarily to handle the clinical study (the phase II clinical study) that was running from 2002 to early 2008. It was a fairly loose organization with myself and some colleagues, just to give us a focus for coordinating the corporate structure and the things that one needs in a corporate structure. Now we are starting to collaborate with others. For example, we have signed a deal with West China Hospital, which is in Szechuan Province in China. West China Hospital is the largest clinical center in the world, with 4600 beds and 2.5 million outpatients a year. We just signed an agreement with them to commence a number of studies (collaborative studies) to implement this new science in a Chinese environment (make

the fruits of these discoveries available in China), and also at the same time, of course, produce good evidence-based double-blinded studies that can help persuade people in the west that science is changing medicine.

JB: Tell us a little bit, if you would, about this 2002–2008 open-label clinical study. That sounds very interesting.

Dr. Marshall's Background in Engineering

TM: I'm not an MD. In fact, I have a PhD, and my PhD was on mathematical modeling of insulin and glucose homeostasis in diabetic and healthy individuals and dated back to the late 1970s/early 1980s. My primary focus, throughout my career as an academic, has been on teaching science subjects, but not medical science subjects. I started to transition to an interest in biomedicine back then in the 80s. But what happened towards the end of the 20th century, with the push towards the genome and sequencing the genome and then understanding the genome, is that translational people (people who could not only understand the biology involved, but could also understand the computer systems involved and the computer programs which are needed to analyze the genome and analyze how proteins move and exist in the environment) were starting to become available. I started to get back into biomedicine with a vengeance back around the turn of the century (around 1999/2000), and used the experience that I built up over my lifetime both from the computing side of things with the biology. We had done some work in the 90s. For example, I designed the big array computer, which we used in the early PET scans (Positron Emission Tomography scanners) from Hamamatsu Photonics in Japan. Basically, what really opened up the fields of discovery was the availability of these tools (these computing tools) that could enable us to analyze exactly how the human body worked to a much greater degree of accuracy and detail than we had been able to do to that date.

JB: And with all of this extraordinary visionary science (really translational science), it seems very interesting that you have made this connection between the genomes of various species, regulatory signaling, ultimate changes of phenotypes, and how that translates or maps against the appearance of what are fairly new disease prevalences in the chronic disease area in our society. It begs a question: Are we just burdened now by more "funny bugs," or is it because we are living longer that we are seeing more of this, or is has it always been there and we just didn't see the elephant in the corner?

New Studies to Be Done in China

TM: It's all of the above. First, we are burdened by more bugs. For example, I was talking to one of my colleagues in China who went to London for her postdoc, and about 3 months into her postdoc she came down with really bad rheumatoid arthritis, which she has been obviously unable to shake and which will probably significantly impair her as she goes through life. That's an MD/PhD person who I'm sure was being very careful and not doing anything silly. These pathogens that are present in the various environments, in the various countries, in the various food chains, in the various populations differ. There is quite a lot of work (research) being done at the Imperial College in London on what is called the metabolome. What they are doing is they are measuring (in urine) proteins which cannot be produced from the human genome, but can only be produced from various bacterial species. And they are using this to gain some insight into the diversity of how the human body operates in symbiosis with different species in the different areas. You get totally different sets of proteins from Japanese residents, for example, than you get from Chinese residents, and that you get from USA residents. They are carrying a totally different flora, and we are talking about urine, so we are not talking just about gut flora, here, but

rather we are talking about overall flora in the body. But the fascinating thing is that when Japanese move to the USA, suddenly the metabolites they start to produce become more assimilated with those that are in the USA. In other words, they are picking up the species that are found in the USA and vice versa, and you can see that in some of the data. My final slide, when I gave the keynote at the World Gene Congress last December, in fact is a slide of that data.9 As travel has allowed us to move around more freely, we have all become more exposed to these things, plus there is the global food chain. Our food now is sourced from all over the world, and the food is most definitely not sterile (not when we are talking about these intracellular pathogens). So food is a factor. Travel is a factor. The use of inappropriate antibiotics is a factor. The antibiotics that we use most commonly (penicillins and cyclosporins, the beta lactams) in fact get rid of the acute infection, but they make a latent-phase infection even worse. There are a number of factors. And then, of course, there is the overriding factor that sometime during the 20th century we decided that vitamin D was a vitamin and that sunbathing was good for you. The population is going to take some time to recover from the combined effects of all of those problems.

JB: Thank you. This is extraordinarily interesting. Just one last question. If I didn't ask it I know it would be in the minds of some of our listeners. This hangs together so wonderfully-the way that you have developed your concepts, your published studies over the years with your collaborators-so it begs a question about why this concept has not been generally accepted. What do you think is necessary to get it generally accepted?

TM: Time. Because of my background in computing, I was able to very quickly ask the right questions of the molecular programs (the molecular simulations that I am doing of the genomes I am looking at) and make the discoveries ahead of mainstream. My colleagues gave presentations last week at the Institute of Medicine (National Academies), and one of the members of the IOM committee came up to a colleague after the presentation and said, "Look, you can't do what you guys are trying to do with proteins. You just can't do this. I am a chemist. I know. You can't do this. You can't use molecular forces and interatomic forces to analyze how these proteins interact." It is going to take awhile for acceptance and understanding of all of these new tools. I just happen to have been able to do it quickly and efficiently because of my background, that's all.

JB: That's very, very exciting. Just another note: I found very interesting, Dr. Marshall, as I had a chance to learn more about you and your background, this kind of Renaissance thinking that you have has cut all the way across into the entertainment industry and you did some work on electronic music synthesizers as well and some of the media that has come out of that technology.
TM: Oh, goodness. I was running a company while I was putting myself through engineering school, providing sound equipment for rock music groups and some of the people I worked with (for example, Bon Scott, who went on to head AC/DC, and some of the others...Doc Neeson also) became fairly famous in the intervening years. It was interesting, but it was really a job. As soon as I graduated I took an academic position in Papua New Guinea and left the music behind. But I did enjoy that period of my career; it was fun.

JB: You are a very interesting man and we will keep in touch because clearly you have got your finger on the pulse of some possible explanations of things that have been enigmas for a long time. We thank you so much for spending this time with us and wish you the best in your continued work.

TM: Thank you very much, indeed.

JB: It has been our pleasure.

We really thank Dr. Marshall for being a provocateur par excellence for our Functional Medicine Update series. This all derives out of his group and his own recent publications: "Autoimmune Disease in the Era of the Metagenome" and "Vitamin D, An Alternate Hypothesis.", These are very nice publications that have come from his research and collaboration with colleagues at Georgetown University and Weill Cornell Medical College (Amy Proal and Paul Albert), that appeared in Autoimmunity Reviews in 2009. We are starting to see some very interesting provocation of new concepts that tie together some observations from epidemiological animal studies, some human observational trials, historical information, and the emerging science of molecular genetics and systems biology, all of which are pointing us toward new thoughts, new questions, and new hypotheses that may help us to resolve some of the complexity of managing a patient with complex chronic disease.

A press release came out recently that particularly focused on the vitamin D and autoimmune disease question. This was a press release that was generating a lot of visibility because the title was "Vitamin D May Exacerbate Autoimmune Disease."10 This was a press release that followed the Autoimmunity Reviews paper and was released by Paul Albert, who is one of the collaborators working with Dr. Marshall. Paul Albert is at the Weill Medical College associated with Cornell University. In this press release, which appeared in the April 2009 media, he states, "Deficiency in vitamin D has been widely regarded as contributing to autoimmune disease, but a review appearing in Autoimmunity Reviews explains that low levels of vitamin D in patients with autoimmune disease may be a result rather than a cause of disease and that supplementing with vitamin D may actually exacerbate autoimmune disease." The press release goes on to talk about the paper that you heard Dr. Marshall review in his presentation, looking at the insights on molecular biology, showing that 25-hydroxy D inactivates (rather than activates) its native receptor. As you heard Dr. Marshall talk about, his team explains that by deactivating the vitamin D receptor, and subsequently the immune response, 25-hydroxy D lowers the inflammation caused by many of the bacteria that are insulting the immune system and allows them to spread more easily in the long run if we do short-term kind anti-inflammatory modulation but we don't arrest the growth of the bacteria. It is kind of a good story on the front end and maybe a bad story on the longer term outcome; this is the position that is being taken.

Other Researchers Are Also Studying the VDR

There is a very nice paper that appeared recently in the *Annals of the New York Academy of Sciences* titled "Dysregulation of the Vitamin D Nuclear Receptor Contributing to the Higher Prevalence of Some Autoimmune Diseases in Women" that describes more of this relationship that Dr. Marshall is talking about.[11] There are many groups around the world that are actively involved in this whole area, so I don't want to give the impression that it is just Dr. Marshall and his colleagues. There is also a very nice review that appeared in *Trends in Molecular Medicine* in 2008 titled "Therapeutic Implications of the Toll-like Receptor and Vitamin D Receptor Partnership," showing that the allergens (or proinflammatory agents) that activate the toll-like receptor that triggers inflammation couples itself together with cross communication with the vitamin D receptor.[12] This article describes (just as Dr. Marshall was leading us to understand) how the innate immune system provides the host with an immediate and rapid defense against invading microbes, and how detection of foreign invaders is mediated by this class of receptors that are known as the pattern recognition receptors, which are a family of toll-like receptors (TLRs). There are ten functional toll-like receptors that have been identified, and they respond to pathogen-

associated molecular patterns that are associated with bacteria, mycoplasma, fungi, and even viruses. The activation of these toll-like receptors leads to direct antimicrobial activity, even against things like biofilms (discussed by Dr. Marshall). This activity induces an antiviral gene program. It was reported in this paper that the toll-like receptor 2 activation, for instance, leads to the use of vitamin D as a mechanism to combat mycobacterium tuberculosis. Now investigators are focusing on findings that can relate the toll-like receptor-induced antimicrobial mechanisms in humans and the therapeutic implications of these findings and the interconnection between this vitamin D receptor communication pathway, gene expression, and activation of TLRs. Toll-like receptors are attractive therapeutic agents and they interface, in their activity, with vitamin D metabolism of 25-hydroxy D (the secosteroid, the pro hormone) into its 1,25-dihydroxy form.

This research is emerging from many different avenues. Another paper that was very interesting appeared in the *Journal of Clinical Investigation*. This article was from 2007, and investigators looked at induction of toll-like receptor-2 activation and the release of antimicrobial peptides by immune cells through a vitamin D-dependent mechanism.[13] This is work out of the University of California, San Diego, and the department of dermatology at the David Geffen UCLA School of Medicine. It is really interesting that many investigators are using systems biology thinking to pull together observations from different fields to look at how antimicrobial influences are expressed by genes in specific cell lines, activated through certain receptor site pathways, and how they relate to these class I nuclear orphan receptor activations, of which the vitamin D receptor is a partner.

Vitamin D is more than just the sunshine vitamin. I think that is what we are taking away from this. There is a lot more to the story that relates to activation, repression, and regulation of over 900 genes that are tied together with this vitamin D receptor. In fact, we also know that altered vitamin D receptor coactivator interactions can lead to over-activated receptor as well as under-activated. A little is good, but a whole lot more might not be better. This concept was described the *Journal of Steroid Biochemistry and Molecular Biology* some years ago.[14]

Dr. Marshall related to us that there are ligands that can modulate the vitamin D receptor and this process of signaling. One of those is the class of drugs called angiotensin-receptor blockers that can have effects on the immune system versus the VDR and class I nuclear orphan receptors. In fact, Dr. Marshall and his colleagues were involved with theoretical modeling of the relationship of the combination of these molecules, specifically with the active site of the VDR. This was some beautiful work published in *Biomed Central* in 2006 that really shows how these two things fit together and might influence the activity of the vitamin D receptor by modulating its function.[15] This research is one of the reasons Dr. Marshall was talking about The Marshall Protocol for the management of fibromyalgia and use of higher levels of intervention with a specific member of the angiotensin receptor blocker family of drugs. Th is particular angiotensin receptor blocker seems to fit in best in that active site and downregulate the overly active VDR signaling and therefore allow proper antimicrobial activity of the body with the antimicrobial peptides to be produced so that the body has its own natural antibiotics, basically, being produced and not being suppressed.

Lastly, of course, is the topic of the enteric bacteria in the gut. We have a kilogram and a half (in most human GI tracts) of living critters (many different species). Do they influence, through their signaling processes, aspects that have to do with activity of cellular metabolism and things like insulin sensitivity

and energy storage or energy utilization in mitochondrial oxidative phosphoylation? The answer is apparently yes. We heard this from Professor Delzenne and Dr. Cani recently. We also alluded to it in this interview with Dr. Marshall. And there are more and more papers coming out from other groups, one of which is a recent paper titled "The Microbiome and Obesity: Is Obesity Linked to Our Gut Flora?"[16] This was from Frank Tsai and Walter Coyle at the Department of Gastroenterology and Hepatology at Scripps. They have been looking at the human gut as a lush microbial ecosystem with about one hundred trillion microorganisms, whose collective genome (called the "microbiome") contains a hundred fold more genes than the entire human genome. The symbiosis of our extended genome plays a role in host homeostasis and energy extraction from the diet, and so there are now many studies that have advanced our understanding of how the microbiome has effects on metabolism, obesity, and health. The so-called "metagenomic" studies that Dr. Marshall was alluding to demonstrate that certain mixes of gut bacteria may protect or predispose the host to obesity and may have signaling processes that regulate activities of the toll-like receptors and then signal through the VDR and have influence on gene expression across multiple cell lines.

This is a new biology, isn't it? This is a new era that we are moving into in understanding, at a much deeper level, both the complexity, and the beauty, and the opportunity for modulating chronic disease in a different way, rather than just treating the effects. It is about actually getting down to the root causes of how specific uniqueness in a person may be translated into their own disease process by exposure to specific agents. I want to be very cautious here that we don't throw the baby out with the bathwater and we recognize that vitamin D is still a very important vitamin, and there are people that are insufficient relative to the precursor, which is the cholecalciferol molecule, and therefore this doesn't mean [we should] stop concerning ourselves with vitamin D, or that nobody should be supplementing it. This discussion just puts in balance, once again, this premise that we have learned time and time over the years: if a little is good, a whole lot more is not necessarily better. We want to find the right dose for the right person to produce the right outcome; that is the basic watchword.

Bibliography

1 Fasano A. Surprises from celiac disease. Sci Am. 2009;301(2):54-61.

2 Bernini P, Bertini I, Luchinat C, Nepi S, Saccenti E, et al. Individual human phenotypes in metabolic space and time. J Proteome Res. 2009;8(9):4264-4271.
3 Albert PJ, Proal AD, Marshall TG. Vitamin D: the alternative hypothesis. Autoimmun Rev. 2009;8(8):639-644.

4 http://www.iom.edu/CMS/3788/61170/68400.aspx

5 Proal A, Albert PJ, Marshall TG. Autoimmune disease in the era of the metagenome. Autoimmun Rev. 2009;8(8):677-681.

6 Blaney GP, Albert PJ, Proal AD. Vitamin D metabolites as clinical markers in autoimmune and chronic disease. AnnN Y Acad Sci. 2009;1173:384-390.

7 Gottlieb JE, Israel HL, Steiner RM, Triolo J, Patrick H. Outcome in sarcoidosis. The relationship of relapse to corticosteroid therapy. Chest. 1997;111(3):623-631.

8 Marshall TG. Vitamin D discovery outpaces FDA decision-making. Bioessays. 2008;30(2):173-182.

9 http://www.vimeo.com/2585394

10 http://www.bio-medicine.org/biology-news-1/Vitamin-D-may-exacerbate-autoimmune-disease-7924-1/

11 Proal AD, Albert PJ, Marshall TG. Dysregulation of the vitamin D nuclear receptor may contribute to the higher prevalence of some autoimmune diseases in women. Ann N Y Acad Sci. 2009;1173:252-259.

12 Liu PT, Krutzik SR, Modlin RL. Therapeutic implications of the TLR and VDR partnership. Trends Mol Med. 2007;13(3):117-124.

13 Schauber J, Dorschner RA, Coda AB, Büchau AS, Liu PT, et al. Injury enhances TLR2 function and antimicrobial peptide expression through a vitamin D-dependent mechanism. J Clin Invest 2007;117(3):803-811.

14 Eelen G, Verlinden L, Van Camp M, Claessens F, De Clerq P, et al. Altered vitamin D receptor-coactivator interactions reflect superagonism of vitamin D analogs. J Steroid Biochem Mol Biol. 2005;97(1-2):65-68.

15 Marshall TG, Lee RE, Marshall FE. Common angiotensin receptor blockers may directly modulate the immune system via VDR, PPAR and CCR2b. Theor Biol Med Model. 2006;3:1.

16 Tsai F, Coyle WJ. The microbiome and obesity: is obesity linked to our gut flora? Curr Gastroenterol Rep. 2009;11(4):307-313.

17 Cannell JJ, Vieth R, Umhau JC, Holick MF, Grant WB, et al. Epidemic influenza and vitamin D. Epidemiol Infect. 2006;134(6):1129-1140.

18 Cannell JJ, Zasloff M, Garland CF, Scragg R, Giovannucci E. On the epidemiology of influenza. Virol J. 2008;5:29. Review.p>