VDR Receptor Competence Induces Recovery from Chronic Autoimmune Disease

PRESENTED BY PROF TREVOR MARSHALL
Director, Autoimmunity Research Foundation

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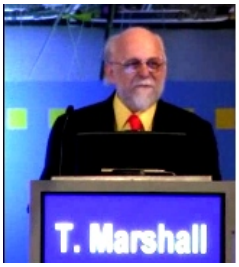
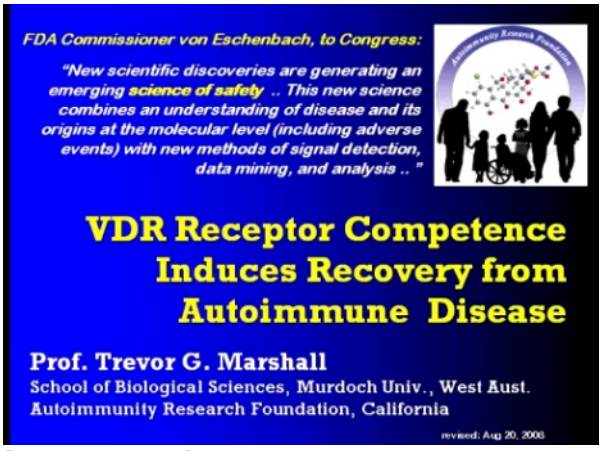


Session: Vitamin D Receptor (VDR) and Vitamin D in Autoimmune Disease

Chairs: T. Marshall (USA) and H. Amital (Israel)

Presentation: VDR Receptor Competence Induces Recovery from Chronic Autoimmune Disease

Author: Trevor Marshall; Autoimmunity Research Foundation and Murdoch University.



[00:01:28 SLIDE 1]

[00:01:59 SLIDE 2]

Howard Amital: Good afternoon everyone, my name is Howard Amital of the Meir Medical Center in Israel.

First of all we appreciate all the attendees at this time of the day, and we hope we all enjoy a fascinating afternoon dealing with vitamin D.

It is a great pleasure for me to introduce Dr. Marshall who is bi-continental researcher, working both in the US and in Australia and who is currently the director of the California-based Autoimmunity Research Foundation and an adjunct professor of the Murdoch University in Western Australia.

Dr. Marshall will discuss "VDR Receptor Competence Induces Recovery from Autoimmune Diseases."

(00:01:11)

Trevor Marshall: Thank you. I am going to talk about the VDR receptor. It is one of the family of nuclear receptors, which includes the glucocorticoid receptor, the thyroid receptors and a number of other very important receptors.

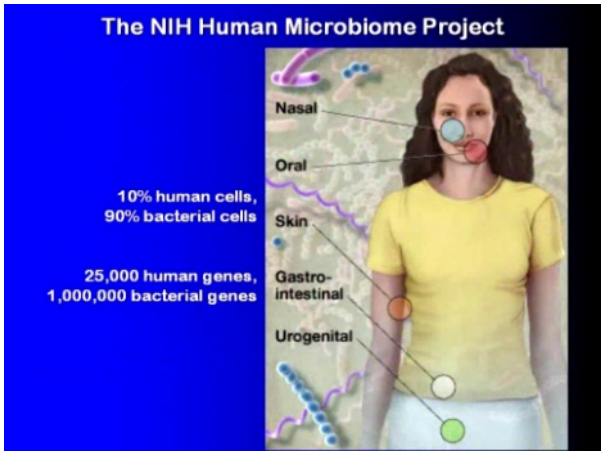
I just want to point out this statement from FDA commissioner Von Eschenbach to Congress two years ago, pointing out that "New scientific discoveries are generating an emergent science of safety, where the new science combines an understanding of disease and its origins at the molecular level." That is what I am going to talk about in this presentation: Understanding Autoimmune Disease Pathogenesis at the molecular level.

in-vivo, in-vitro, in-silico (00:01:59)

There are three types of biology in common use today, in-vivo, in-vitro and the newer in-silico.

The first time I came across in-silico biology was back in 1981. This is a photograph of myself and my colleagues at the Hospital for Sick Kids back in 1981, which was when IBM showed us the in-silico techniques that they had used for the synthesis of the first human insulin, the Humulin, the first human insulin. That was the first time I came across in-silico and realized the power of being able to emulate the operation of the human body at the level of individual atoms.

Since then the in-silico work I think that everybody is most familiar with is the decoding of the human genome and probably more important now, but less well known, is the decoding of around 740 microbial genomes that have been fully decoded to this point in time.



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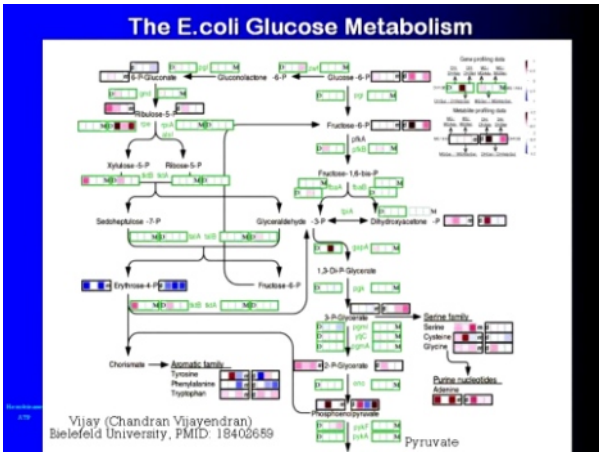

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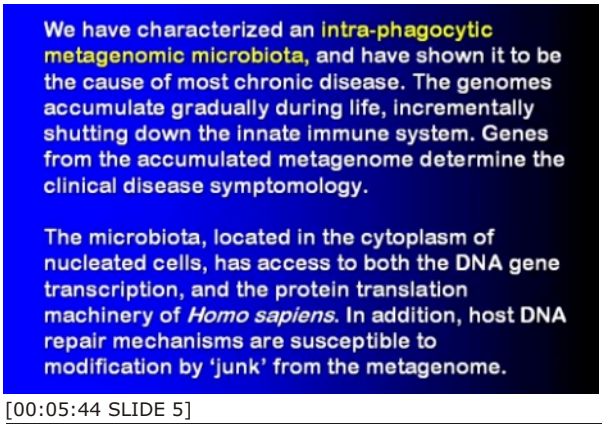


[00:05:44 SLIDE 5]

The NIH Human Microbiome Project (00:03:05)

And in fact, NIH has just started a big Human Micobiome Project, with the idea that they wanted to characterize all the DNA that is available from human sources, all the DNA in the human body. The normal infectious areas that we are aware of: the nasal, oral, skin, GI and urogenital cavities, but also within the cells of the body itself. Because NIH has estimated that around 10 percent of the total cells in the human body are human cells and as many as 90 percent of the cells could be bacterial cells.

You will be hearing a lot about the Microbiome project over the next decade.

The E.coli Glucose Metabolism (00:03:46)

Why is that important? Well it is important because the bacterial cells, in many cases, perform functions that are very similar to those of the *Homo sapiens* itself, of the host.

And what I have got here is a slide showing the E.coli Glucose Metabolism. I know very few of you are going to be able to follow every subtlety of it. Don't worry. I just wanted to show you that this chart exists. I got this one from Vijay at the Bielefeld University in Germany. But it shows the way that the bacteria E.coli gets from Gluco-6-P substrate down to Pyruvate and produces the Serine, Cystein, Glycene Amino acids, the Purine Nucleotides Adenine, the Tyrosine, Phenylalanine, Tryptophan.

All of these are produced by bacterial genes — actually by proteins that are transcribed from bacterial genes — but the bacterium itself, the organism itself, is capable of working on exactly the same metabolites as used in the human body for the production of energy and it produces very similar intermediates.

You have got Fructose-6-Phosphate here. You have got Glycenaldehyde, Glycerate, many of these intermediate metabolites are very common to people who study, familiar to people who study the human genome.

So if you have got the bacterial genomes working in the same environment, within the same cells — in fact, infected cells — as the human genome in the human body, you can imagine the amount of interference between the operation of the two of them.

We have characterized... (00:05:44)

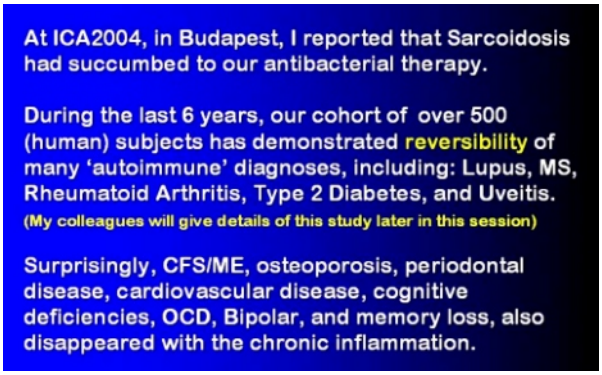
And that is exactly what we have found. We have characterized that there is an intra-phagocytic metagenomic microbiota (metagenomic: many genomes, microbiota: community of pathogens. Intra-phagocytic: it does most of its harm inside the phagocytes, the lymphocytes, the macrophages, the monocytes of the immune system). And we have shown it to be the cause of most chronic disease.

The genomes accumulate gradually during life, incrementally shutting down the *innate* immune system. They shut down the innate immune system incrementally during life.

Genes from the accumulated metagenome determine the clinical disease symptomology. Depending on what genes are accumulated in the metagenome, that determines which effects they are going to have on the body — which of the metabolites are going to be affected by the pathogenic genomes.

The microbiota is located in the cytoplasm of the nucleated cells, where it has access to both the DNA gene transcription and the protein translation machinery of *Homo sapiens*. In addition, the host DNA repair mechanisms are susceptible to modification by junk from the metagenome.

That is very, very important. Because it is located in the cytoplasm, the microbiota can upset the host DNA repair mechanisms.



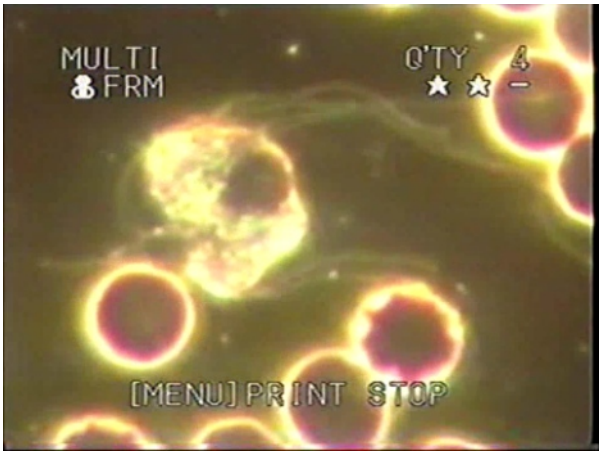
[00:07:20 SLIDE 6]

At ICA2004... (00:07:20)

At the Congress in 2004 in Budapest, I reported that Sarcoidosis had succumbed to an antibacterial therapy that we had developed.

And over the last six years our cohort of over 500 (human) subjects has demonstrated reversibility of many autoimmune diagnosis — **Reversibility** — including Lupus, MS, RA, Type 2 Diabetes and Uveitis. (My colleagues will give details of this later in this session.)

But surprisingly, as the chronic inflammation receded, CFS/ME (Chronic Fatigue Syndrome), osteoporosis, periodontal disease, cardiovascular disease, cognitive deficiencies, obsessive compulsive disorder, bipolar, memory loss — all of these — also disappeared as the chronic inflammatory condition disappeared.



[00:08:21 VIDEO/SLIDE 7]

Bacteria video (00:08:21)

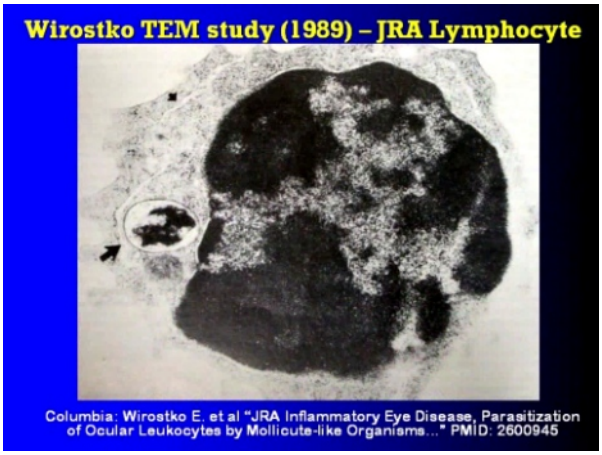
Well, what do these microbiota look like?

Here is a monocyte, an infected monocyte, and the cytoplasm has effectively exploded from pressure of the pathogens and it is throwing out these tiny biofilm tubules.

I am not quite sure why it is not showing up perfectly but you can see here there are probably a dozen tiny biofilm tubules that are very long and they are also extremely small. You can compare them with the size of the cell — the standard cell diameter there of 4 or 5 microns or so — and extremely tiny little biofilm polymer tubules thrown out as the cells disintegrate.

This is untreated blood, this is human blood put between cover slips and allowed to age for six to thirty-six hours.

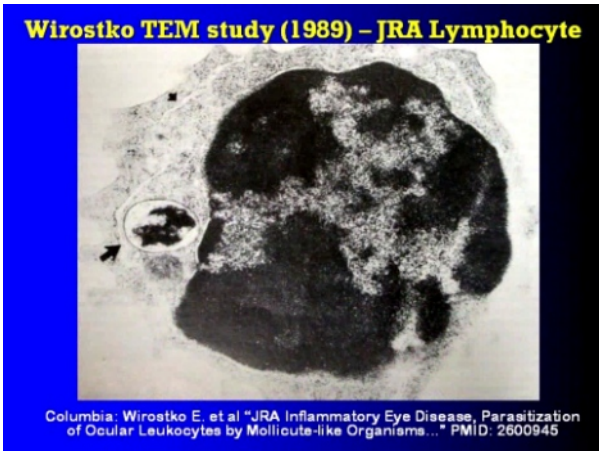
Look at the length of these. This is about 20 cell diameters long. This one is about 10 cell diameters long. It is amazing and you can see them very easily under light microscopy if you are looking for them.



[00:09:32 SLIDE 8]

Wirostko TEM study (1989) — JRA Lymphocyte (00:09:32)

Under the electron microscope they look a little bit different, of



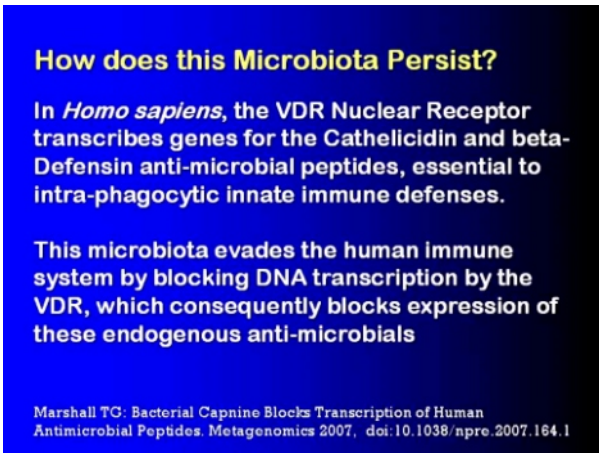
[00:09:32 SLIDE 8]

course, because you can actually look at them in the cells before they can become heavily parasitized.

This is an image from the Emil Wirostko TEM study in 1989 at Columbia University. This is a Juvenile Rheumatoid Arthritis lymphocyte. And what we have is, we have the nucleus area of the lymphocyte and then the cytoplasmic region outside the nucleus, and in the cytoplasmic region there is a staining artifact which is basically nucleic material, DNA material, inside some form of transparent biofilm type of protection and then a very thin exoskeleton to contain the whole lot.

But what is even more interesting are these tiny little elongated structures, which don't come up all that well on the projection here, but when you look up the original paper (which was "JRA Inflammatory Eye Disease, Parasitization of Ocular Leukocytes by Mollicute-like Organisms..." from 1989), when you look at the original photographs you can see the incredible detail of these transparent colonies that are living inside these lymphocytes.

Of course, the lymphocyte should never allow this to happen. But it does allow it to happen because these microbiota have figured out how to overcome innate immunity. They have overcome the last line of defense.

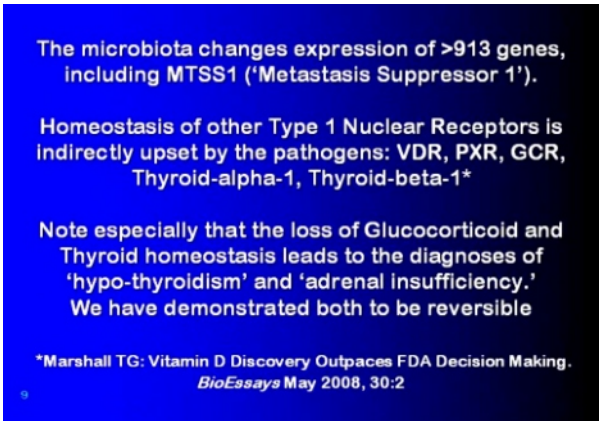


[00:11:19 SLIDE 9]

How does this Microbiota Persist? (00:11:19)

Well, how does it do that? In *Homo sapiens*, the VDR Nuclear Receptor transcribes genes for the Cathelicidin, beta-Definsin anti-microbial peptides. It is also involved in the expression of the alpha-Defensins as well. And these are key to the intra-phagocytic innate immune defenses. When these anti-microbial peptides and anti-microbial proteins get knocked out, then the phagocytes can no longer protect themselves from attack by the pathogenic microbiota.

The microbiota evades the immune system by blocking DNA transcription by the VDR. It blocks the VDR, which consequently blocks expression of these endogenous anti-microbials. The body cannot produce the anti-microbials because the DNA-transcription, the receptor that would do that (express those anti-microbials) is blocked by the pathogens.



[00:12:22 SLIDE 10]

The microbiota changes expression... (00:12:22)

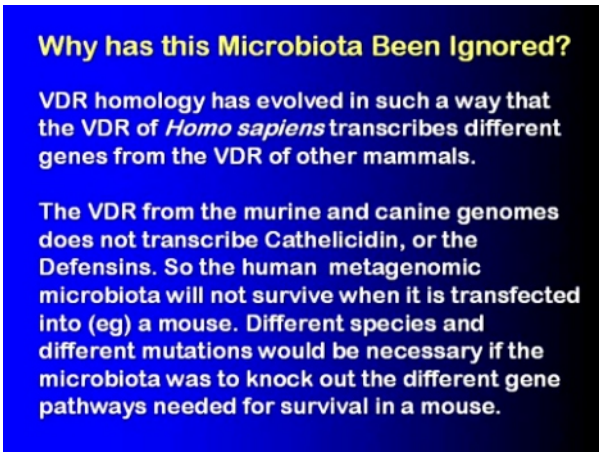
The microbiota changes the expression of greater than 913 genes. And those are genes which do everything from create a parathyroid hormone precursor, right through to create the Missing in Metastasis protein (MTSS1 is the gene) Metastasis Suppressor number 1.

All of these genes, 913 that have already been confirmed, are transcribed by the VDR. The VDR nuclear receptor is a very key nuclear receptor in *Homo sapiens*.

But homeostasis of the other Type 1 Nuclear Receptors is indirectly upset by these pathogens. The VDR, of course, but PXR, the

Pregnane Xenobiotic Receptor (PXR), the Glucocorticoid Receptor (GCR), Thyroid-alpha-1, and Thyroid-beta-1 are all profoundly affected by the elevated levels of the seco-steroid that are caused by the VDR being knocked out.

And obviously, note that especially that the loss of Glucocorticoid and Thyroid homeostasis leads to the diagnoses of hypo-thyroidism and adrenal insufficiency. We have demonstrated both of these are reversible.



[00:13:43 SLIDE 11]

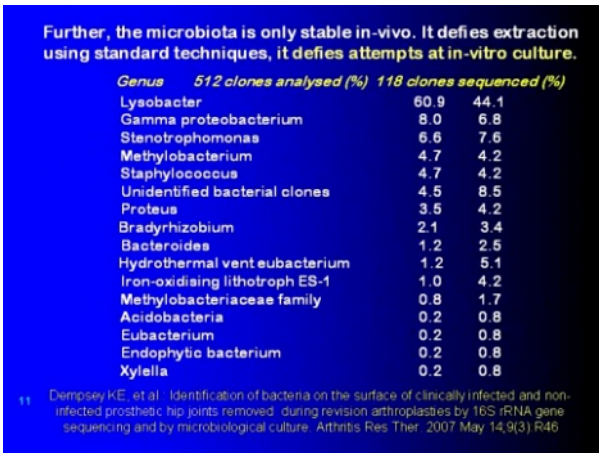
Why has this Microbiota Been Ignored? (00:13:43)

So why haven't we seen this microbiota before? There has been so much study of pathogens in mankind. Well there are a few reasons.

The first one is that the VDR homology, the shape, the amino acids that go together to make up the VDR is a little bit different in *Homo sapiens* to what it is in all of the other mammals and all of the other fish, etc. as well, which have VDR. And it transcribes different genes from the VDR of the mammals.

And you know how much of our work we have been doing in animal models. Well, a very key function of the bacteria that have evaded the human immune system do not appear in mammals because the VDR homology is so unique to *Homo sapiens*.

The VDR from the murine and canine genomes, for example, does not transcribe Cathelicidin or the Defensins at all. So a human metagenomic microbiota would not survive if it was transfected into, for example, a mouse. Because different species and different mutations would be necessary if the microbiota was to knock out the different pathways needed for survival in a mouse.



[00:15:06 SLIDE 12]

Further, the microbiota is only stable in-vivo... (00:15:06)

Further, the microbiota is only stable in-vivo. It defies extraction using standard techniques. You saw how that cell had disintegrated after about 6 hours of aging. You can imagine what it does under centrifuge. Further, most of these species in the biofilm microbiota defies attempts at in-vitro (culture).

This is a study from Dempsey et al., which was a study of biofilm from prosthetic hip joints which were removed during revision arthroplasties. And they did gene sequencing and tried to match up the 740 known genomes that we have for bacteria against what they found — the DNA that they found in the biofilm.

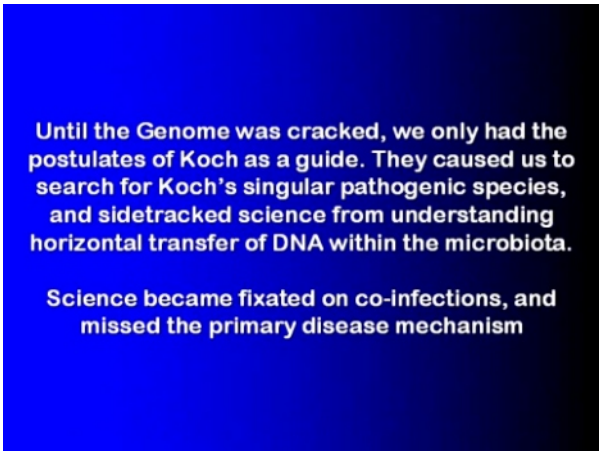
And this is what they found: Lysobacter (Lysobacter was about 44% of the clones that were sequenced), Proteobacterium, Methylobacterium, Staphylococcus — well Staph-aureus, you would actually expect to find that in a human biofilm, that is not unusual. But its size is small, only about 4.2 percent (*That* is unusual) — unidentified clones.

But look at this: Hydrothermal vent eubacterium. This was a eubacterium that was first located in hydrothermal vents under the ocean, and here it is, its DNA is popping up in man, and at high

concentration. 5.1 percent of the clones that were sequenced, which is higher than Staphylococcus genus.

There are some other genus here. You can look up the paper and go into more detail.

This is what the human microbiome project is aimed at setting out. Exactly what species, what genes exist in Man.



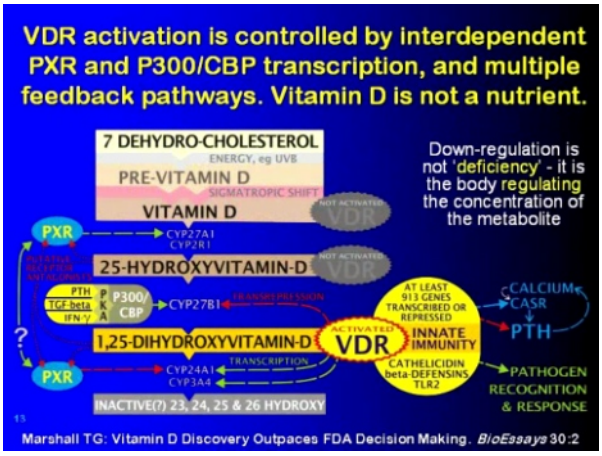
[00:17:03 SLIDE 13]

Until the Genome was cracked... (00:17:03)

Until the Genome was cracked, we only had the postulates of Koch as a guide. They caused us to search for Koch's singular pathogenic species. We kept looking for one pathogenic species. As a result, you can find a paper that will blame poor old EBV for just about every disease known to man, and CMV, and HHV as well.

Because Koch's singular pathogenic species in this age of the genome really means very little, it sidetracks science from understanding the horizontal transfer of DNA within the microbiota, sharing of genes between the organisms which we now know occurs very, very much faster than we ever dreamed could be the case.

Science became fixated on the co-infections, those things that we could see, like the EBV, and missed the primary disease mechanism — the ability of the pathogens to knock out the innate immune system.



[00:18:08 SLIDE 14]

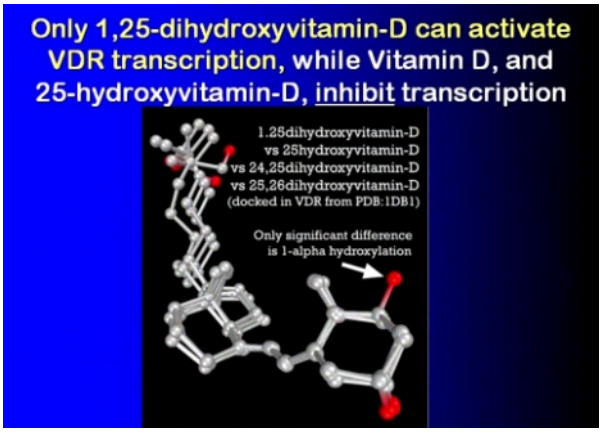
VDR activation... (00:18:08)

Another reason, is that 'vitamin' D is the primary ligand that activates the VDR Receptor.

And at some stage during the 20th century, mankind decided that Vitamin D was a nutrient. Well, Vitamin D is not a nutrient. It is a seco-steroid transcriptional activator. And its concentrations are very closely controlled by a very complex control system, which involves not only the VDR, but also the Pregnane X Receptor (the Pregnane Xenobiotic Receptor) the P300/CBP PKA pathways, and feedback by a number of enzymes: CYP24, -27A1, -27B1. There is transrepression from VDR activation, feedback paths, there is transrepression, or actually antagonism — Receptor antagonism — from the metabolites, and there is also feed-forward pathways. Quite a complex mechanism.

If Vitamin D was a nutrient we would see a simple, first order, mass-action metabolism. We do not see that. We see the complex control system of a hormone, of a seco-steroid transcriptional activator, which is what it is.

When we concentrate on the concentration of this intermediate substrate, 25-Hydroxyvitamin-D, that is the one that medicine has been measuring as an indication of Vitamin D status. That is down-regulated in disease. When the VDR is knocked out the production of the CYP27A1 is downregulated and the production of 25-Hydroxyvitamin-D in the body is down-regulated.



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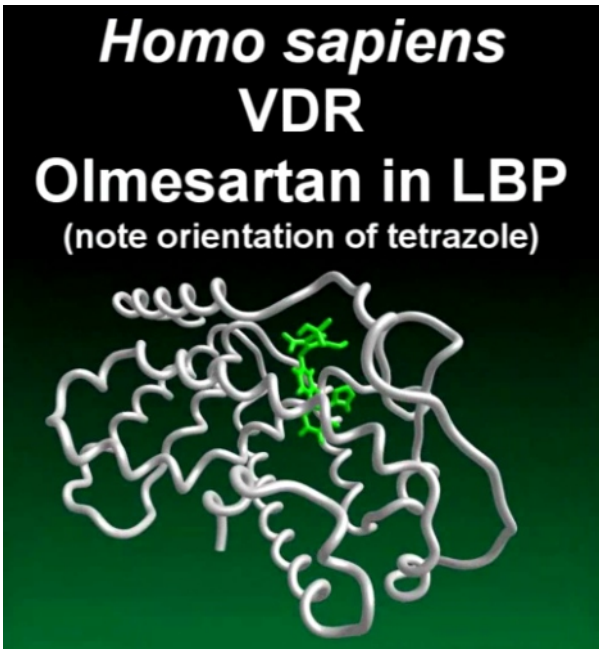


[00:22:00 VIDEO/SLIDE 16]

[00:22:20 VIDEO/SLIDE 17]

Down-regulation is not 'deficiency.' It is the body regulating the concentration of the metabolite. What is happening is, because the VDR is knocked out, no longer can these genes degrade the 1,25-D and the 1,25-D is becoming very high in concentration, affecting the other receptors and so it tries to block the metabolism at this pathway. And in so doing, down-regulates the level of the thing that we are measuring, thinking that it is a meaningful measure of Vitamin D homeostasis. It is not. It is down-regulated in disease.

My paper, "Vitamin D Discovery Outpaces FDA Decision Making" in *BioEssays* last February contains that diagram and all the associated description.

Only 1,25-dihydroxyvitamin-D can activate VDR transcription... (00:20:54)

But, there is another problem too.

Only 1,25-dihydroxyvitamin-D, the doubly-hydroxylated version, can activate VDR transcription, can actually activate to transcribe the genes. While Vitamin D that we ingest, and 25-hydroxyvitamin-D that is hydroxylated from that — both inhibit transcription.

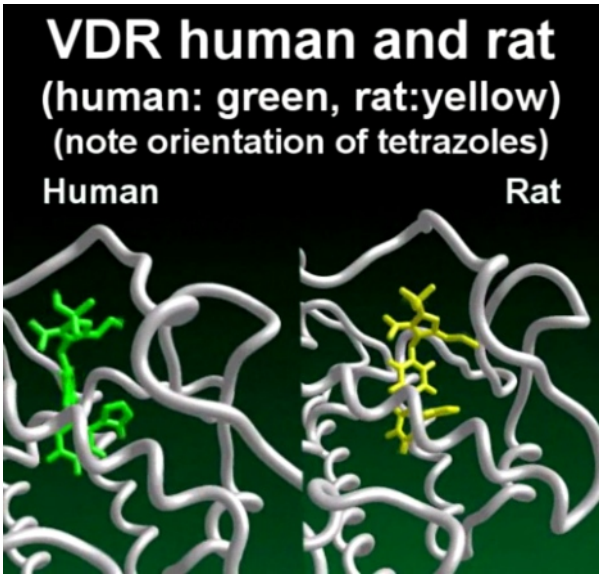
Here we have in-silico data showing how each of these Vitamin D metabolites fit into the VDR receptor. And you can see that only one of them has the 1-alpha hydroxylation which is necessary to actually activate the Receptor so it transcribes genes. And yet all of them occupy similar space inside the VDR and they all have very similar values of Kd as well. So, if you are giving a lot of Vitamin D supplementation, it is actually tending to displace, on a concentration dependent basis, the active metabolite from the VDR.

Homo sapiens VDR... (00:22:00)

Well, luckily, there is an agonist that works in-vivo. It is a drug called Olmesartan. Here we have a molecular dynamics emulation of the human VDR, with Olmesartan sitting in the binding pocket, in an activated position. As you know, all proteins are in motion at all times.

Rattus norvegicus VDR... (00:22:20)

And here we have the same thing in the rat (*Rattus Novegicus*). It looks very, very similar to the VDR, a very similar ligand position, but they are not quite the same. And when we put them side by side, you can really see the difference.



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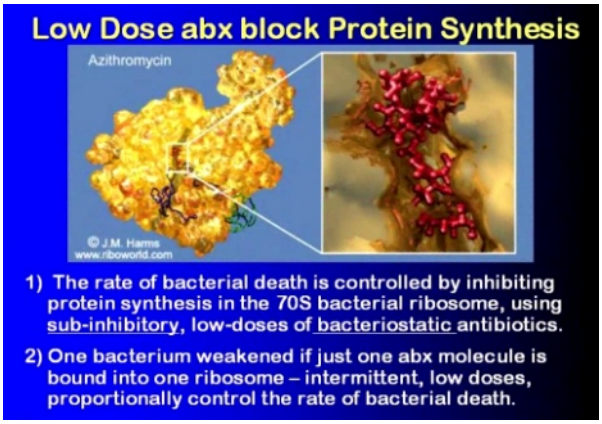

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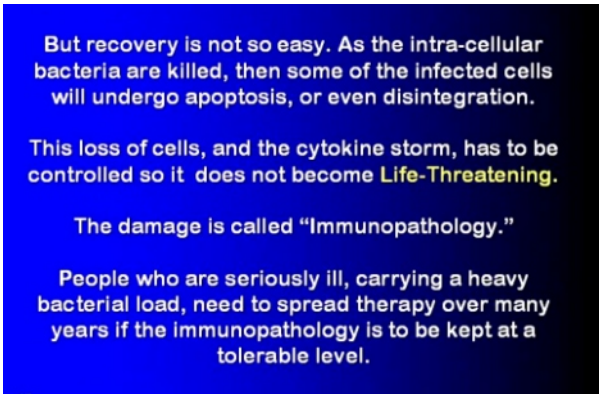


[00:24:11 SLIDE 20]

VDR human and rat... (00:22:35)

In particular, look at this Tetrazole. The Tetrazole ring is in a totally different orientation because it is binding to totally different amino acids in the VDR of the rat and the VDR of the human being.

It is only by getting down to the level of the molecules that we can really understand the difference between our animal models and humans, *Homo sapiens*.

Low Dose abx block Protein Synthesis (00:23:05)

Then, once the innate immune system has been activated again, we can use very low-dose bacteriostatic antibiotics to block protein synthesis.

Here I have got Azithromycin blocking the 70S-ribosome, which translates RNA into proteins in the bacterial organisms, being blocked by Azithromycin.

The rate of death when you are using bacteriostatic antibiotics is controlled by inhibiting the protein synthesis. And we can use sub-inhibitory low doses of bacteriostatic antibiotics. Later on Dr Blaney will talk a little bit about dosing issues.

But just remember that one bacterium is weakened if just one antibiotic molecule is bound into one ribosome, because these antibiotics actually block the functioning of protein generation. **So intermittent, low doses can proportionally control the rate of bacterial death.**

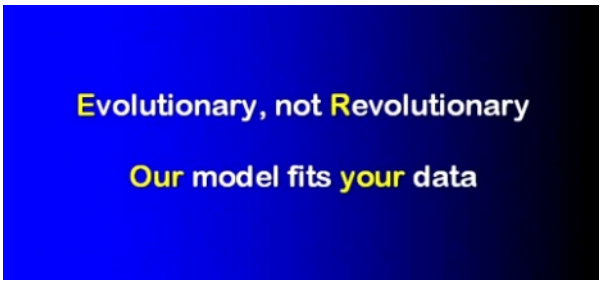
But recovery is not so easy... (00:24:11)

And that is very fortunate, because recovery is not easy. There is a huge bacterial cellular load, whether it is 90% of the body, or not, I would not know but it is huge load. As the intra-cellular bacteria are killed, some of the infected cells will undergo apoptosis. Some even disintegrate.

The loss of cells, both white and red cells, and the cytokine storm which is concomitant with that, has to be controlled so it does not become life threatening.

The damage is called Immunopathology. And people who are seriously ill, carrying a heavy bacterial load — which is just about every patient with an autoimmune diagnosis — they need to spread the therapy over many years if the immunopathology is to be kept at a tolerable level.

You cannot just give the patient the antibiotics, kill the bacteria and send the patient home feeling well. The problem is there are just too many to kill, the load is too high, and just like you have with, for example, anthrax, the patient dies from the cytokine storm. You have got to be very, very careful.



[00:25:29 SLIDE 21]

Evolutionary... (00:25:29)

Finally, I want to point out that what we have done is Evolutionary, it is not Revolutionary. We have built on the people like Wirostko, Yehuda — from back over the last couple of decades — many of you too, I'm sure, in the audience.

Our model, the molecular model of the disease fits your data.

Please, seek out my colleagues over the next couple of days, talk with us about your data, your studies, especially if you think that our model does not stand up to scrutiny. We would love to discuss it with you.

It does fit your data.

[00:26:08 SLIDE 22]

Newton... (00:26:08)

And finally, we will end this presentation contemplating Newton.

Thank you.

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Q/A

Howard Amital: We maybe now have time for two short questions.

Q: I have a question for you. I was surprised how you are finding that Olmesartan, the, I think, angiotensin-II receptor blocker, have impact on D. Is that, in your opinion, a cross-specific effect of or something to do with the angiotensin-II levels?

A: **Dr Marshall:** I published a paper back in 2005 pointing out that all of the small, highly mobile, highly polar drugs, including the statins and sartans, they all target multiple receptors and not just the receptors through which they were generally thought to operate.

And olmesartan just happens to be one that activates the VDR. It is as simple as that.

Its primary target as it was designed was as an angiotensen-II receptor blocker, which it does a very good job of at quite low dose. But as you make more frequent dosing and increase the dosing, you start to allow it to work on the VDR in a concentration-dependent manner and it becomes a very effective and safe VDR agonist.

Q: I am... from Brazil. I would like to know more of this microbiota. Is it just the patients with immunosuppressant disease... do all have this microbiota?

A: **Dr Marshall:** Yes, all human beings have this microbiota. Not all of the microbiota becomes pathogenic.

Q: **Brazil:** Not all?

A: **Dr Marshall:** Not all becomes pathogenic. I mean, in some cases...

Q: **Brazil:** Only those that have disease?

A: **Dr Marshall:** Only those that collect a particular set of genomes that causes the human body to function in a way that is recognized as a disease.

For example, we are working at the moment in medicine with Chronic Fatigue Syndrome, trying to understand "what is that?." It was not known as a disease a year ago, and now the CDC has said it is a physiological disease.

There are many other things that we put down to aging, particularly diseases of the aging. Periodontal disease, which are in fact, directly interrelated with even the cause of, say, Lupus, or any of the autoimmune diseases.

Q: **Brazil**: Autoimmune disease...

A: **Dr Marshall:** Yes. But it depends on the accumulation of the genomes during a lifetime. Which pathogens a person is exposed to. Some, for example, from all sources and how they accumulate.

Howard Amital: Thank you very much.