

Understanding Human Disease requires study of a Metagenome, not just the Human Genome

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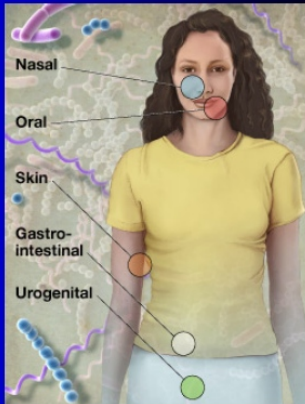


in-vivo, in-vitro, in-silico
Insulin, Human genome, 1078 bacterial genomes
Visiting Scientist, Dept of Surgery, Hospital for Sick Kids, Toronto, 1981
"Modeling and simulation in diabetes care." PhD thesis, University of West Australia, 1984

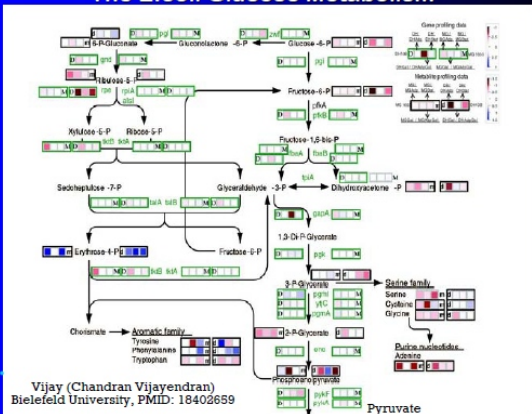
The NIH Human Microbiome Project

10% human cells,
90% bacterial cells

25,000 human genes,
1,000,000 bacterial genes



The E.coli Glucose Metabolism



Understanding Human Disease requires study of a Metagenome, not just the Human Genome

PRESENTED BY PROF TREVOR MARSHALL
Director, Autoimmunity Research Foundation



Video available from: <http://www.vimeo.com/2585394>

My talk is going to be primarily about *translational* medicine, that Dr. Hershko mentioned as being one of the waves of the future. I'm going to be talking at the level of the gene, and I'm going to be talking at the clinical level as well.

I would like to first make an apology to those of you who are scientists here for explaining what in-silico means. But there are three phases of biology, of biological research. There's in-vivo, that means in living persons. There's in-vitro, that means, basically, in the lab in cell culture. And there's in-silico. The first time I came across in-silico research was here at the Hospital for Sick Children in Toronto back in 1981. This is a group of us there at the time. We'd just come back from a conference where IBM had shown how they were using their new supercomputers to produce human insulin. This was Humulin, and it was synthesized with the use of in-silico technologies, primarily done with computers. And it was one of the first of the major drugs that were produced by computers. This was my first introduction to in-silico work. But most of you would be more familiar, of course, with the use of in-silico technologies in the decoding of the human genome and perhaps more importantly, although less well-known, in the decoding now of 1078 (last time I looked it up) *bacterial* genomes, fully decoded.

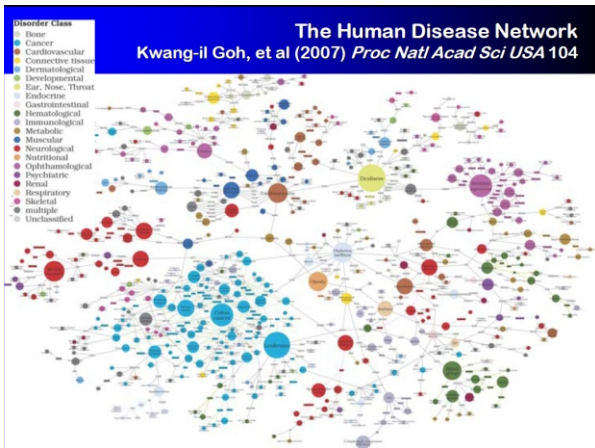
The NIH has just started a large Human Microbiome Project, which over the next ten years aims to define exactly what microbes exist in the organism we call *Homo sapiens*. Basically, you'll all be familiar with the normal areas where microbes are expected—nasal cavities, oral, skin, GI and urogenital. But it's known that microbes are far more prevalent in the human body than anybody, up until now, has even dreamed. The NIH says that in a human body approximately 10% of the cells are human cells and approximately 90% are bacterial cells.

Now remember that bacterial cells are *really* small. You will have hundreds or thousands of them inside each infected human cell. However, those are the numbers from the NIH Microbiome Project, and that's a staggering start. We then go to the next set of numbers. There are approximately 25,000 human genes active in the human body, and about one million bacterial genes. The interaction between the bacterial genes and the human genes, of course, will help define how the organism *Homo sapiens* actually operates.

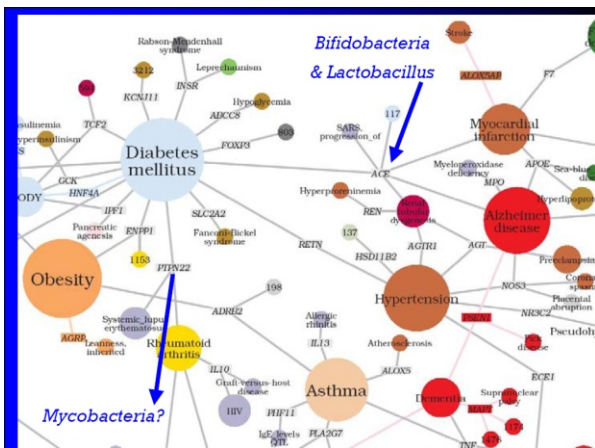
Well, why is this important? If we look at, for example, the *E. coli* glucose metabolism, you can see at the top here we've got Glucose-6-phosphate coming in, and at the bottom I've taken it as

far down as Pyruvate and the citric acid cycle down below. But basically this shows the genes in the *E. coli* organism, the proteins that are produced, and the end products of that protein action on the input glucose-6-phosphate. And the main thing to notice is that it's not all that different from the way the human body utilizes glucose. You can see a lot of names that you recognize. Obviously the end products here adenine, cysteine, the amino acids, also tyrosine, tryptophan, those are very well-known. They're invariable in man, but you'll find also glycogen and a number of others, ribo-5-phosphate, a number of other metabolites that are very common if one studies the human glucose metabolism. This particular analysis was done by Vijay at Bielefeld University in Germany, and he has done a lot more work than just this one slide shows.

But really, what I wanted to point out was, there is not a lot of difference between the way that *E. coli* metabolizes glucose-6-phosphate and the way the human body does. Many of these metabolites are the same. If you could have an interaction between the *E. coli* genome and the human genome, then an entirely new spectrum of transcription and translation is opened up. Now, in fact, published in the Proceedings of the National Academy of Sciences last year by Kwang-il Goh, et al, they actually took all the genes in the gene bank, and they then plotted all the diseases, bone, cancer, diseases right through to skeletal, respiratory, renal. And they plotted from the gene bank all the various diseases and the genes that were common to the various diseases.



Now if we enlarge a section of that, we have the rheumatic diseases here, the rheumatic diseases because those of you who are from Szechuan Province, I'm going to be giving a more extended seminar next Monday at 2PM at West China Medical Center in Chengdu. So I'm focusing on the rheumatic diseases here, but we could focus on the neurological or any of the other diseases. If we take this one gene here, ACE, which produces the angiotensin converting enzyme, it is known to be associated with Myocardial Infarction, with Renal tubular dysgenesis, with Alzheimer's, with progression of SARS, and with Diabetes Mellitus. My particular interest in it was that it was also associated with the first disease that we studied, which was a disease called Sarcoidosis, a rare disease but one which turned out to be a very typical inflammatory disease.



So if you want to study these diseases, you're going to be drawn inexorably toward the study of the gene for ACE, for the angiotensin converting enzyme. And it just so happens that a number of bacteria act on that gene. But in particular, lactobacillus and bifidobacteria, which are what we regard as being *friendly* bacteria, gut bacteria that are in the yogurts and other supplements that some of us eat, these bacteria produce a number of peptides that down-regulate the expression of ACE in the human body. So that is known—that very common bacteria, which nobody would argue are present in the human body, produce peptides that down-regulate a gene that is a *key* gene in

understanding the diseases like myocardial infarction, Alzheimer's, renal disease, progression of SARS, and diabetes.

Therefore, if you're going to study those diseases, you have to study these organisms as well as the host organism *Homo sapiens*. You're looking at a *metagenome*. You're looking at more than one genome that has to be examined. Similarly, we have another one down here, PTPN22, which is related to diabetes mellitus, rheumatoid arthritis, and lupus (SLE). That one is known to be up-regulated as part of the innate immune system's response to mycobacteria. With a surge in latent tuberculosis, with our increasing knowledge of *Mycobacterium avium* and its prevalence in the population, it's very important, if you're going to study these diseases, you need to study how that particular gene behaves in the environment, in the environment that we live in. If it's stimulated by mycobacteria, and probably by other bacterial species as well, that will be up-regulated, and that's known. Once again, we're expanding the metagenome that we need to study when we think about disease.

Back a number of years ago I reported that sarcoidosis had succumbed to an antibacterial therapy based on a VDR agonist. We were actually able to *reverse* that disease process. And in the subsequent five years, we put together an observational cohort of over 500 human subjects with a whole variety of different diagnoses. And we went on to demonstrate the reversibility of many autoimmune diagnoses.

This is from a paper that was presented at the Sixth International Congress on Autoimmunity just two months ago in Porto, Portugal. We have listed here a number of autoimmune diagnoses which responded to an antibacterial therapy which involved a VDR agonist, a small molecule called a VDR agonist. We start at Rheumatoid Arthritis, Hashimoto's Thyroiditis, Uveitis, Psoriasis, type 2 Diabetes, Sjogren's, Celiac, SLE, right down to the fewest number of cases, which were in Diabetes Insipidus. In general, between 18 and 53 months of treatment, 81% of the cohort experienced reduced disease symptoms with an antibacterial therapy involving a small molecule VDR agonist.

But this is the surprising thing. [The therapy was also effective against] Chronic Fatigue Syndrome/Myalgic Encephalomyelitis, osteoporosis, periodontal disease, cardiovascular disease, cognitive deficiencies. At Karolinska earlier this year there was a conference on cognitive deficiency in disease, and we gave papers showing that both CFS and cognitive deficiencies were directly related to inflammatory diseases and, chemically, some of the links. Obsessive Compulsive Disorder, Bipolar, and memory loss—things that we don't think to be associated with inflammation—these also disappeared as the chronic inflammatory condition—the primary inflammatory condition, rheumatoid arthritis, SLE, whatever—disappeared.

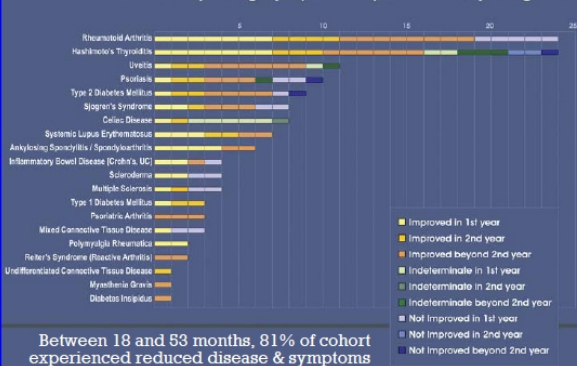
Now what we have done, we have identified, back around the turn of the century, an intraphagocytic metagenomic microbiota, and

In 2003, I reported that Sarcoidosis had succumbed to an antibacterial therapy based on a VDR agonist.

In the subsequent 5 years, our observational cohort of over 500 (human) subjects has gone on to demonstrate **reversibility** of many 'autoimmune' diagnoses.

Waterhouse JC, Perez T et al, 6th Intl Congress on Autoimmunity 2008, Porto

Number of Patients Reporting Symptom Improvement by Diagnosis



Between 18 and 53 months, 81% of cohort experienced reduced disease & symptoms

Surprisingly, CFS/ME, osteoporosis, periodontal disease, cardiovascular disease, cognitive deficiencies, OCD, Bipolar, and memory loss, also disappeared with the chronic inflammation.

We have identified an **intrapagocytic metagenomic microbiota**, and the method by which it evades the immune system.

The genomes accumulate gradually during life, incrementally shutting down the innate immune system. Genes from the accumulated metagenome determine the clinical disease symptomatology.

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

we described the method by which it evades the immune system. And that's where this small molecule comes in. So, firstly, a number of groups in the past had identified that there were microbiota, or communities of microbes, that lived inside the cells. There are many studies; I'll show you one in a while. But what we did was, we said, "Right, but how do you deal with those? What do they do? How do they evade the immune system to persist inside phagocytes?"

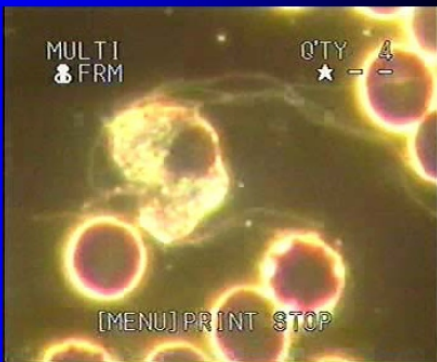
I mean, phagocytes are supposed to be the body's *defense* against persistent infection. What we found was that the genomes in this metagenome, in the microbiota, the biofilm, if you like, the genomes accumulate gradually during life, incrementally shutting down the innate immune system. And, depending on the environment to which you are subjected, which pathogens you become subjected to during life, the metagenome will gradually build up during life. Eventually, genes from the accumulated metagenome will determine a clinical disease symptomatology or just a disease of the aging.

The microbiota is located in the cytoplasm of nucleated cells. Therefore it has access to both the DNA transcription and to the protein translation machinery of *Homo sapiens*. In addition, and this is very important for those of you who are studying mutations, the host DNA repair mechanisms are susceptible to modification by "junk" from the metagenome.

Well, what does the microbiota look like? This is light microscopy. This is a monocyte with a very highly infected cytoplasm. You can see the nucleus, there's the cytoplasm, and the cytoplasm has effectively exploded from expansion of the pathogens in it and thrown out dozens of these tiny, tiny transparent tubules, which stain for bacterial DNA. You can see them along here; there's probably 20 sub-microscopic bacteria tubules through there, in the infected cytoplasm and the nucleus. Look at the length of these tubules. This is about 20 cells long, this one is, absolutely huge. This is optical microscopy of live blood, aged between 6 and 36 hours on a microscope slide, untreated other than that, just live blood aged on a microscope slide. It was taken on a Chronic Fatigue Syndrome patient using a Bradford microscope by Dr. Andy Wright in Manchester.

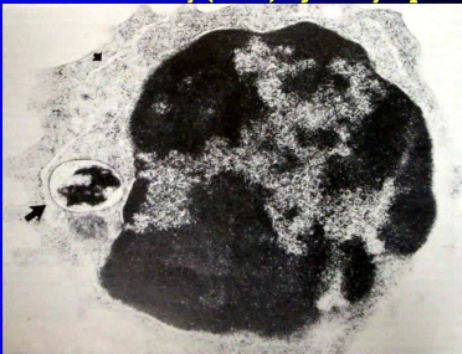
Now there was a TEM study done (Transmission Electron Microscopy) in 1989 at Columbia University by Emil Wirostko and his colleagues. And he looked at the phagocytes, all of the phagocytes, actually, from a number of diseases. He looked at Crohn's, he looked at juvenile rheumatoid arthritis, and he looked at sarcoidosis. And he took the phagocytes from the eyes of these patients, and was therefore able to image them very well with TEM microscopy. And what he found was a number of artifacts inside the cytoplasm which stained for bacterial DNA. This is the stain; unfortunately, this is 1989, and the TEM was all monochrome and not enhanced at that point. Then there are also very small tubular structures, transparent tubular structure throughout the

Light Microscopy of the persistent Intrapagocytic Metagenomic Microbiota



CFS patient
Bradford
Microscope
Andy Wright,
Manchester

Wirostko TEM study (1989) - JRA Lymphocyte



Columbia: Wirostko E. et al "JRA Inflammatory Eye Disease, Parasitization of Ocular Leukocytes by Mollicute-like Organisms..." PMID: 2600945

How does this Microbiota Persist?

In *Homo sapiens*, the VDR Nuclear Receptor transcribes genes for the Cathelicidin and beta-Defensin anti-microbial peptides, essential to intra-phagocytic innate immune defenses.

This microbiota evades the human immune system by blocking DNA transcription by the VDR, which consequently blocks expression of these endogenous anti-microbials

Marshall TG: Bacterial Caprine Blocks Transcription of Human Antimicrobial Peptides. *Metagenomics* 2007, doi:10.1038/npre.2007.164.1

The microbiota changes expression of >913 genes, including MTSS1 ('Metastasis Suppressor 1').

Homeostasis of other Type 1 Nuclear Receptors is indirectly upset by the pathogens: VDR, PXR, GCR, Thyroid-alpha-1, Thyroid-beta-1*

Note especially that the loss of Glucocorticoid and Thyroid homeostasis leads to the diagnoses of 'hypo-thyroidism' and 'adrenal insufficiency.' We have demonstrated both to be reversible

*Marshall TG: Vitamin D Discovery Outpaces FDA Decision Making. *BioEssays* May 2008, 30:2

Why has this Microbiota Been Ignored?

VDR homology has evolved in such a way that the VDR of *Homo sapiens* transcribes different genes from the VDR of other mammals.

The VDR from the murine and canine genomes does not transcribe Cathelicidin, or the Defensins. So the human metagenomic microbiota will not survive when it is transfected into (eg) a mouse. Different species and different mutations would be necessary if the microbiota was to knock out the different gene pathways needed for survival in a mouse.

cytoplasm, not unlike what we were seeing on the other side, but not nearly so heavily developed, so heavily parasitized. And here, of course, you've got the nucleus, and the nucleus itself is starting to break apart and become weak as well. This was from a juvenile rheumatoid arthritis patient.

The important thing is to understand how the microbiota *persist*. Because it's one thing to understand that a microbiota must be present, that it is present, but you can't do anything about it until you understand how it evades the immune system. That was the breakthrough we made back around 2003. We looked at the nuclear receptors. The nuclear receptors are a family of very complex proteins—transcription factors—that transcribe DNA for many of the key functions of the human body. For example, the progesterone receptor, the estrogen receptor, the androgen receptor, the glucocorticoid receptor are all nuclear receptors.

And there's one of them called the VDR. The VDR nuclear receptor is very important for the innate immune system of *Homo sapiens* because it transcribes the genes for the Cathelicidin and beta-Defensin anti-microbial peptides. The primary way that phagocytes themselves protect themselves against pathogens is with Cathelicidin, secondarily with beta-Defensin. So those two genes, and the transcription of those genes, is essential to intra-phagocytic innate immune defenses.

This microbiota evades the human immune system by blocking DNA transcription by the VDR, which consequently blocks expression of these endogenous anti-microbials. In addition, the microbiota changes the expression of the 913 or more other genes that are transcribed by the VDR, particularly including MTSS1. MTSS1 is the Metastasis Suppressor number 1 that gives rise to the protein called "Missing in Metastasis." And when the bacteria, the microbiota, knocks out the VDR, that is one of the other 913 important genes in the body that get knocked out as well.

The homeostasis of other Type 1 nuclear receptors is also indirectly upset by the pathogens. The VDR, PXR (the Pregnane Xenobiotic Receptor), the Glucocorticoid Receptor, the Thyroid-alpha-1 and Thyroid-beta-1 are all Type 1 receptors which are upset when the VDR is knocked out by the microbiota. In particular, when the Glucocorticoid and Thyroid Receptors are knocked out, then we end up typically with diagnoses of hypothyroidism and adrenal insufficiency. We've demonstrated that both are reversible, once the inflammatory disease can be reversed.

Well, why has the microbiota been ignored? There are a couple of reasons. The first one is that VDR homology—the shape, the actual proteins, the amino acids that fit together to make the human VDR—VDR homology has evolved *between* the species in such a way that the VDR of *Homo sapiens* transcribes different genes from the VDR of other mammals. In particular, the VDR from the murine and canine genomes, which is what we've most generally studied when we've been studying diseases, doesn't transcribe Cathelicidin or the Defensins. So if the microbiota knocks out the

VDR in the murine or canine genomes, it cannot persist. The phagocytes will get it, because the Cathelicidin and Defensins are transcribed by different receptors in other genomes. It's only *Homo sapiens*, and one or two of the other major primates, where the VDR transcribes cathelicidin.

So a *human* metagenomic microbiota will not survive when it's transfected into a mouse. Koch's Postulate fails. Different species and different mutations would be necessary if the microbiota was to knock out the different gene pathways needed for survival in a mouse. Of course, you would know from evolutionary statistics that the likelihood of a pathway developing in other mammals and other animals is high. And indeed, there was a conference a month ago from the American Association of Microbiology in San Diego where a presentation was given by the Max Planck Institute in Germany, who have just found the same microbiota, the same biofilm inclusions, in the phagocytes from worms that were recovered from barrier reefs, from coral reefs. So it's pretty certain that other species have developed, that microbiota have developed so that they can overcome the immune system of other species, but it's a *different* mechanism to the one that they use to overcome man.

Now, secondly, the microbiota is only stable in-vivo. It defies extraction using standard techniques. You saw what happened with that blood that was just aged under a slide. If you try to extract the cells, cells that are infected disintegrate. It's very, very difficult to culture or to identify these pathogens in-vitro. But you can do it using DNA shotgun techniques. You can take the DNA from *Homo sapiens*, and you can actually use shotgun techniques, the same way as we decode the human genome or the bacterial genomes, to figure out what genomes are there. And because we now have a roadmap of the 1000+ bacterial genomes, when we find DNA from those genomes in our human sample, we can say, "Aha! We're looking not only at human DNA in our shotgun sequencing, we are looking at bacterial DNA as well, and probably from X species", one of the 1078 that we have defined.

This is a study that was done by Dempsey, et al, that actually used 16S RNA rather than shotgun, but it doesn't matter. They took infected prosthetic hip joints, which were removed during revision arthroplasty, and they used ultrasound to get the biofilm off of the removed hip joints. And these are the species that they found in that biofilm, removed from people. *Lysobacter*, a typical bacterium that you expect to find in a biofilm, had about 44% of the clones sequenced and about 60% of the clones analyzed.

Methylobacterium, *Staphylococcus*, well, you'd expect *Staph. aureus* actually, in biofilms, and it's there. *Proteus*, look at this—hydrothermal vent *Eubacterium* was present at a little bit higher number [percent] of clones [sequenced] as *Staphylococcus*. The iron-oxidizing lithotroph—a number of species here that nobody dreamed existed in man until we were able to use molecular gene technologies to figure out that, "hey, what we're looking at is not *Homo sapiens*. It's not anything we've looked at

Further, the microbiota is only stable in-vivo. It defies extraction using standard techniques, it defies attempts at in-vitro culture.

Genus	512 clones analysed (%)	118 clones sequenced (%)
<i>Lysobacter</i>	60.9	44.1
<i>Gamma proteobacterium</i>	8.0	6.8
<i>Stenotrophomonas</i>	6.6	7.6
<i>Methylobacterium</i>	4.7	4.2
<i>Staphylococcus</i>	4.7	4.2
Unidentified bacterial clones	4.5	8.5
<i>Proteus</i>	3.5	4.2
<i>Bradyrhizobium</i>	2.1	3.4
<i>Bacteroides</i>	1.2	2.5
Hydrothermal vent eubacterium	1.2	5.1
Iron-oxidising lithotroph ES-1	1.0	4.2
Methylobacteriaceae family	0.8	1.7
Acidobacteria	0.2	0.8
<i>Eubacterium</i>	0.2	0.8
Endophytic bacterium	0.2	0.8
<i>Xylella</i>	0.2	0.8

Dempsey KE, et al.: Identification of bacteria on the surface of clinically infected and non-infected prosthetic hip joints removed during revision arthroplasties by 16S rRNA gene sequencing and by microbiological culture. *Arthritis Res Ther.* 2007 May 14;9(3):R46

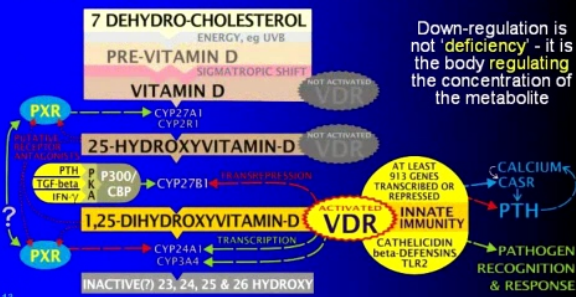
Until the Genome was cracked, we only had the postulates of Koch as a guide. They caused us to search for Koch's singular pathogenic species, and sidetracked science from understanding horizontal transfer of DNA within the microbiota.

Science became fixated on co-infections, and missed the primary disease mechanism

before." The computer matches it up with DNA from hydrothermal vent *Eubacterium*.

Until the genome was cracked, we only had the postulates of Koch as a guide. Koch, in 1897, produced a set of postulates which defined when you could claim that an infectious disease was present. They caused us to search for a singular pathogenic species, because the postulates of Koch said, "one bug, one disease," and sidetracked science from understanding the horizontal transfer of DNA within the microbiota. Science became fixated on co-infections, things that the immune system couldn't knock out, but which we could culture, we could observe, and we missed the primary disease mechanism—the metagenome.

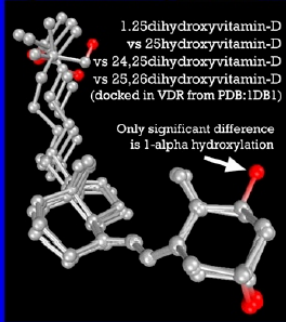
VDR activation is controlled by interdependent PXR and P300/CBP transcription, and multiple feedback pathways. Vitamin D is not a nutrient.



Marshall TG: Vitamin D Discovery Outpaces FDA Decision Making. *BioEssays* 30:2

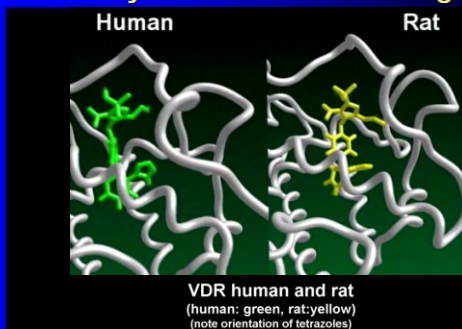
The other reason that this has escaped study by science is that the VDR stands for Vitamin D Receptor. It is activated by a substance which we know as Vitamin D, which is a transcriptional activator, a secosteroid. Vitamin D is *not* a nutrient. There is a very complex control system by which 7-dehydro-cholesterol is synthesized into 1,25-dihydroxyvitamin-D, which then activates transcription by the VDR nuclear receptor. There are multiple feedback pathways, there is transcription of degrading enzymes, there is trans-repression of an enhancing enzyme, and then, via the PXR (the Pregnane-X Receptor), other enzymes are affected and finally this primary CYP27B1 second hydroxylation enzyme is activated by PKA P300/CBP pathway—very complex mechanism, which you would expect. It's at the heart of innate immunity. A paper we published this year goes into the control system in some detail.

Only 1,25-dihydroxyvitamin-D can activate VDR transcription, while Vitamin D, and 25-hydroxyvitamin-D, inhibit transcription



And this is the interesting thing. Here's in-silico technology. We've taken the X-ray structure of the VDR, actually a number of VDRs from different groups, and we've docked the various types of Vitamin D, the various hydroxlations, into that receptor. Only one of them has the 1-alpha-hydroxylation here, which is capable of activating transcription. But all of the others overlay the same region in the receptor. In other words, they are *antagonists* for that transcription process.

Functioning of innate immunity is partially restored by Olmesartan – a VDR agonist

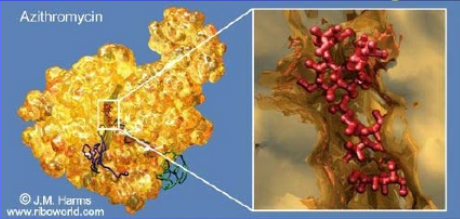


If we take the VDR agonist which we have found, which is a drug called olmesartan, we see here olmesartan in the human VDR. This is a molecular dynamics emulation using a large computer array computing real-time atomic force interactions, and, of course, like all proteins, everything is in motion, there's total motion all the time. And here we have a rat VDR with the same drug in it. Now what's particularly interesting is the orientation of the tetrazoles and the functioning of the drug is different in the two VDRs. If you look at that tetrazole orientation and the other one, they're different—there are 7 hydrogen bonds there, and there are only 5 hydrogen bonds in the VDR of a rat. So the *drug itself* doesn't behave in the same way in the VDR of the rat versus the VDR of the man.

Here is a basic problem that we have missed in our biological sciences. We have missed trying to go to the level of the gene and understand the difference between species. And we have assumed

Marshall TG: Molecular Static and Dynamic Analysis Reveal Flaw in Murine Model used by US FDA to Detect Drug Carcinogenicity. *Abstract, Days of Molecular Medicine, 2007*

Low Dose abx block Protein Synthesis



- 1) The rate of bacterial death is controlled by inhibiting protein synthesis in the 70S bacterial ribosome, using sub-inhibitory, low-doses of bacteriostatic antibiotics.
- 2) One bacterium weakened if just one abx molecule is bound into one ribosome – intermittent, low doses, proportionally control the rate of bacterial death.

But recovery is not easy. As the intra-cellular bacteria are killed, some of the infected cells will undergo apoptosis, or even disintegration.

This loss of cells, and the cytokine storm, has to be controlled so it does not become **Life-Threatening**.

The damage is called “Immunopathology.”

People who are seriously ill, carrying a heavy bacterial load, need to spread therapy over many years if the immunopathology is to be kept at a tolerable level.

that any mammalian species is a reasonable emulation of *Homo sapiens*’ genome.

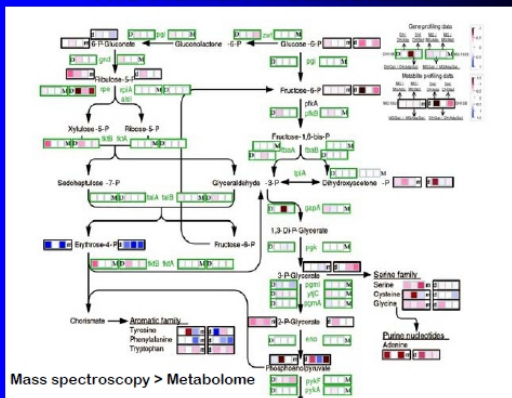
The other thing that you can do to go after the microbiota is to use low-dose antibiotics which block protein synthesis. Proteins that create biofilms that protect the community can be attacked by a number of simple antibiotics.

This one is azithromycin and the 50S side of the ribosome, the protein translation area of the ribosome, and you can block the ribosome. The rate of bacterial death is controlled by inhibiting protein synthesis in the 70S bacterial ribosome using sub-inhibitory, low doses of bacteriostatic antibiotics. We’re talking about very low doses here, where azithromycin—typically patients with inflammatory disease once you activate the VDR, those patients can only withstand one tenth of the normal dose of something like azithromycin. One bacterium is weakened if just one antibiotic is bound into one ribosome.

So intermittent, low doses proportionally control the rate of bacterial death. We can control the rate of bacterial death by using the protein synthesis inhibitors, and that is just as well, because there is a phenomenon called “immunopathology.”

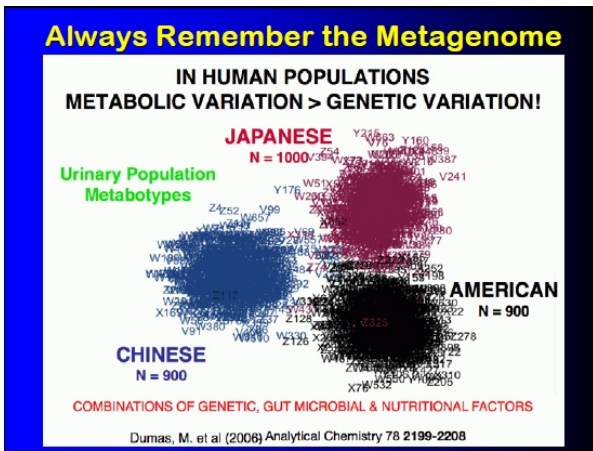
Immunopathology is the damage done to the rest of the body when the immune system is doing its job. When the immune system is phagocytosing dead cells, phagocytosing pathogens, then it releases a cytokine storm, it release chemokines, it activates the nuclear factor Kappa-B pathway, all of the normal ways that the body signals amongst itself that something is desperately wrong. When you start allowing the human immune system to find the huge bacterial load we’re carrying, ten times the number of bacterial cells to human cells (that’s the NIH number), then the immunopathology can become life-threatening. The loss of cells, the cytokine storm, *has* to be controlled, so it doesn’t become life-threatening. So what we do is, we use very low dose antibiotics, sometimes just the immune system itself, and spread the therapy over many years.

The Human Metabolome



There is one other way that we can visualize the metagenome—the way that the human experience is a metagenome, the product of many, many genomes working in tandem. Now I pointed out earlier this *E. coli* glucose metabolism, and what I was pointing out to you there was that a lot of the products that are produced, fructose-6-phosphate for example, and [it is] very common in both *E. coli* and the human genome. Both produce those substrates. But there are some of the substrates, some of the proteins that are produced by *E. coli*, which I called “junk” earlier on in one of my other slides, but they are exudates, they are by-products of the bacterium itself, working. And there are some of those that are unique. And we can use mass-spectroscopy to image them.

At the Imperial College in London that is what they’ve been doing. They’ve come up with a concept called the “metabolome”—the Human Metabolome—or the spectrum of metabolites that is associated with *Homo sapiens*. And for my final slide I want to look



at this particular image which is from Dumas, et. al., in *Analytical Chemistry*, 2006. What Jeremy and his colleagues at the Imperial College did here was to take those non-human metabolites that were present in urine, and they produced a method of displaying on a two-dimensional surface, the specific measures of those non-human metabolites in such a way that you could see that different populations were producing different metabolites. So the American population has different non-human metabolites in its urine to the Japanese, to the Chinese—a very large number, about 1000 patients. And you could see that, due to their environment, due to their food, due to everything that is associated with their metagenome, these three populations are different, and we can measure the way that their bodies are operating differently.

One particular artifact which you can just see here, you can just see a violet number stuck here in the American group. There were actually five Japanese that moved to America. When they did that, their metabolomes suddenly took on the American characteristics rather than their native Japanese characteristics. In other words, it's *environmental* factors that drive our metabolome. So that's my final thought—always remember the metagenome. The human experience is based on a metagenome—ten times as many bacterial cells in the human body as human cells is the current estimate. Over the next ten years we'll be able to firm up exactly what that number is and exactly what the function of those—I hesitate to call them pathogenic—let me call them symbiotic cells rather than pathogenic cells.

Thank you very much.

Q: I'm from India, [inaudible] and I've been working since a long time with the bacterial pathogens as well as the human genome relic [inaudible].

So, my question is very relevant to MHC gene, Major Histocompatibility Complex gene, in relation to bacterial pathogens. How they actually react, I do not understand actually, even today, how the Major Histocompatibility gene would actually function with pathogenic and non-pathogenic selectively of the microbes which are living in our system. And that is [they are] the queries that I wanted to ask you, whether you have something in relation to MHC and human pathogenicity as well as the microbiome infection and how they actually [interact]...

A: I don't have a *micro*-answer, I have a *macro*-answer. The answer is: we're dealing with approximately one thousand genes, which are estimated to be in the human body, compared to the 25,000 genes of *Homo sapiens*. When there is interaction, such as you would definitely have in the cytoplasm of nucleated cells, where there is interaction, the interaction is not on the scale of one protein affecting MHC, it is on a number of proteins and enzymes and, in fact, also small molecules from multiple species affecting MHC. Because MHC is so important to the adaptive immune response, you can expect that the pathogens are going to have to develop some way of overcoming the adaptive immune

response and therefore, some way of mutating, under-expressing, or whatever, the MHC.

So the macro answer is: we don't know, but it makes sense. The micro answer is: we don't know.

Q: Actually, this is a very interesting new phenomenon—discovery—that, [for example,] like a computer, IBM is not very compatible with the Apple system. But now gradually, the two systems, they have some micro-programs that can be integrated with one another. Would that be possible, that the microorganism's genome can, sometimes, in some mechanism—can dynamically integrate directly with the human genome?

A: Well, we have done that randomly. In the past there have been... For example, smallpox vaccination was very strong at one point in history. Polio vaccination with the different vaccines, for example, has been different for different generations of people living today. So, we have actually done that. We have also used BCG, a live vaccine, for example, in trying to combat tuberculosis. And we know BCG is related to causation of other diseases, sarcoidosis being one of them that is up-regulated by BCG. So, randomly we have sort of done that.

But at this point we have no idea [of] how to introduce another genome in order to try and make the metagenome on the whole positive rather than being negative. The Phage therapy, that was very popular in Russia and in Eastern Europe, for example, is, in many ways, a way of trying to change the metagenome in such a way that it wasn't harmful, [so that] it wasn't making the harmful substances anymore. And in the short term it worked reasonably well, but in the long term it didn't produce very much extended longevity or any of the things that you would expect from it. Does that answer your question?

Q: Yes. But from the perspective of retrovirus, there are retrovirus genomes integrated into our human genome from time to time, like HIV.

A: Yes, very common, in the telomeres, from our early study into the telomeres and then working upwards. And if you look into these sicknesses, the inflammatory sicknesses, the rheumatic diseases, the telomeres are different, they are ragged. There is something going on there that we don't understand yet, and that's where we have got to start looking.

Q (last question): Very interesting for us, we do immunology. This is for me a completely new world, [a] novel concept. I mean, we are trying to use a lot of the TLR ligands as our adjuvants.

But on the other hand, you are telling us that we are full of bacteria all over, so we should be hypersensitive or sort of tolerant, but we still use the TLR ligands and we get very strong responses. So how do you put this together? Because you show—I don't remember the gene—that inside of our cells we have a protective gene not to be [to keep us from being] hypersensitive.

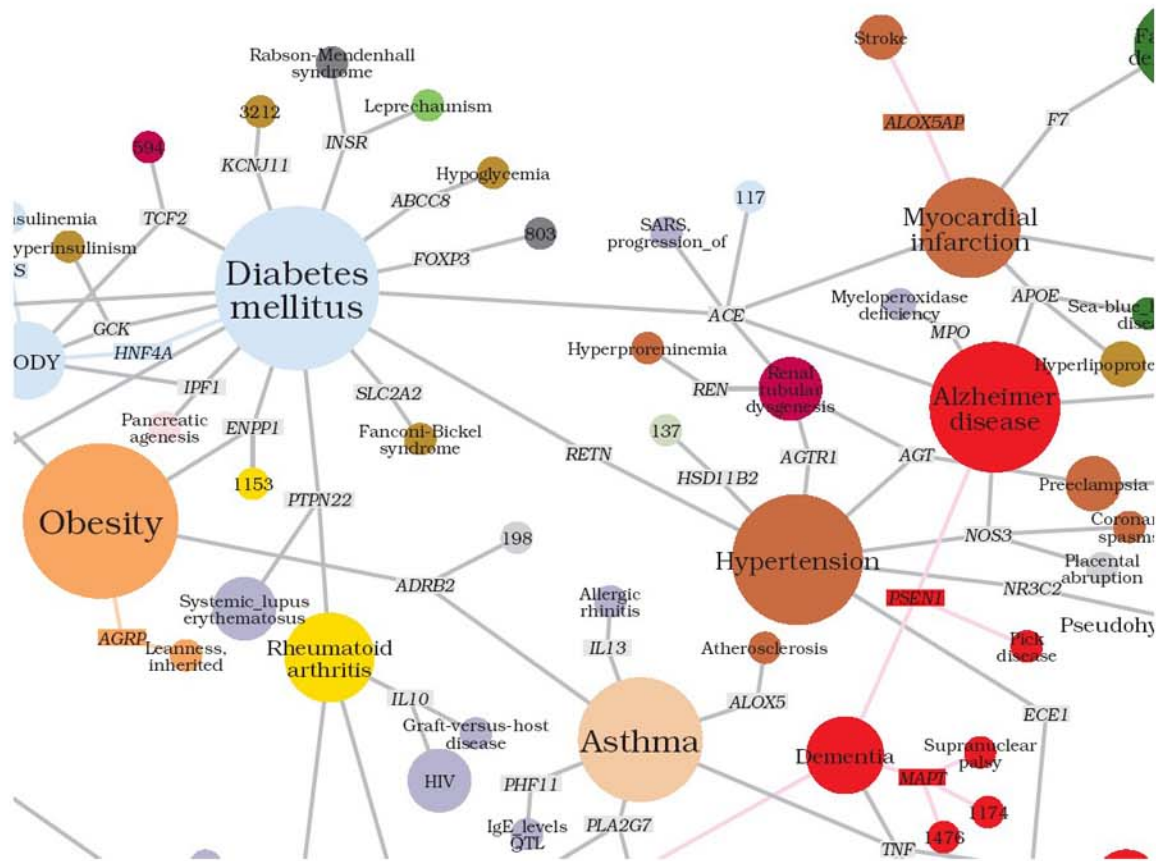
So how do you put this together while still [bearing in mind that our patients] respond very well like [to] CPG, flagelin, or whatever, other TLR ligands?

A: Well, if you look at autoimmune disease in general, the focus in autoimmune disease research for the last five decades, the last half century really, has been on the adaptive immune response, on the things we observe, on the antibodies. And one of the diseases that I mention there, Chronic Fatigue Syndrome—which is thought to be a neurological disease or just a psychosomatic disease by some physicians—but there are actually distinct antibodies which were identified in CFS patients. This was reported at the Porto 6th International Congress in September [2008].

Because what happens, is when the innate immune system fails, you get a cytokine cascade and a chemokine cascade which signals to the adaptive immune system that something is wrong. And it tries to figure out what is wrong. It tries to take the fragments of whatever is available, whatever DNA has been left after phagocytosis and figure out what to do with it.

And when we think that we are producing antibodies to the human body itself, we are actually producing antibodies that is [are] the adaptive immune system's best guess at what the pathogen looked like. But because we have not identified the pathogens enough yet, at the level of the gene, at the level of the DNA, we don't realize that we are looking at a pathogen.

You would know that antibodies, for example, are very non-specific. A typical antibody will react to anything from a range of small molecules right up to a number of larger proteins. Some are very specific but very few are very specific. So if you look at the adaptive immune system, it is downstream from the innate immune system failure. And we have just always assumed that the innate immune system just worked and it hasn't been just working. It's been gradually getting worse and worse in its operation, particularly during the last century. Although these diseases have been around... The Neolithic Iceman that was dug up in Austria/Italy a decade ago, for example, shows signs of stroke and also arthritis, which are the same diseases. So it looks as though a metagenome has been around for quite a long time, just changing, as each successive generation comes along.



Number of Patients Reporting Symptom Improvement by Diagnosis

