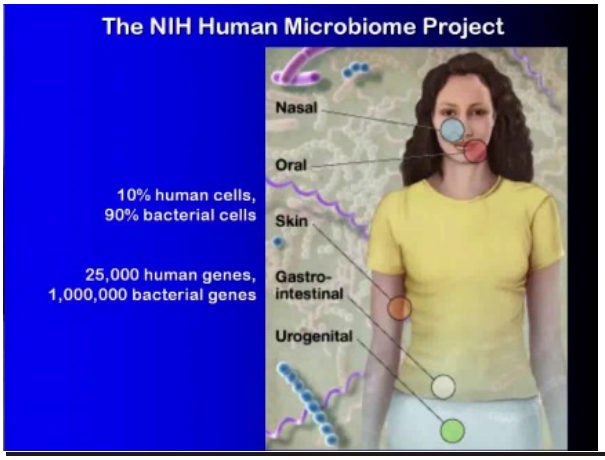


It is time to bury Koch — Infectious disease transitions to an understanding of the Metagenome

PRESENTED BY PROF TREVOR MARSHALL

Prague, Czech Republic, Workshop on Chlamydial infection, April 18, 2009.
Transcript of http://vimeo.com/4307469 video.

Transcript

Well thank you very much for inviting me. It is great to be here in Prague. It is my first visit to Prague. And I must say I am enjoying the city.

Slide # 1: Topic introduction

What I am going to talk about is a new concept called the "metagenome."

As we understand more about the human genome and the pathogenic genomes we are starting to understand more about how the various pathogens — be they *Chlamydia, Mycobacteria,* and *Mycoplasma* — how the various pathogens interact with our own genome in order to cause disease.

And so my title is based on "Infectious Disease transitions to an understanding of the Metagenome."

Slide # 2: In vivo, in vitro, in silico

There are three types of biology that are pretty common these days. The first type, in vivo, of course, in animal or human models.

In vitro is where a lot of the work on antibiotics is being done; in cell culture, in the lab.

In silico is very new. The first time I came across in silico was at this gathering here in Toronto back in 1981. Human insulin had just been synthesized using mathematical formulae, using the IBM supercomputer that could simulate the insulin molecule at the level of the mathematics. And that [in silico] is really what I have been doing over the last decade.

Slide # 3: The NIH Human Microbiome Project

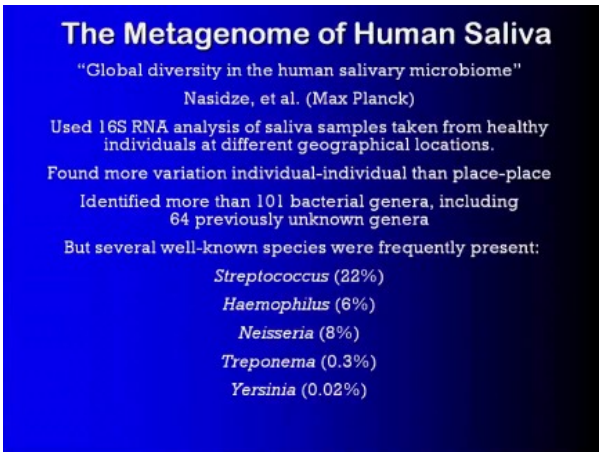
There is a new push going on at the moment. The NIH in the USA has started the Microbiome Project. The goal of the Microbiome Project is to characterize all of the places in the human body where genomes, other than the human genome, are also present.

And NIH has estimated that about 10% of the cells in the body are human cells, and about 90% of the cells in a normal healthy individual's body are bacterial cells. Now, remember that bacteria cells are very, very small. And in most cases the bacterial cells — many, many hundreds of bacterial cells — can live within infected human cells.

But we are starting to get to an understanding now that the

human body does not work — the human genome does not work — in isolation.

It works in concert with a symbiosis of other genomes that have gathered throughout a person's lifetime, and indeed throughout the ages.



Slide # 4: The Metagenome of Human Saliva

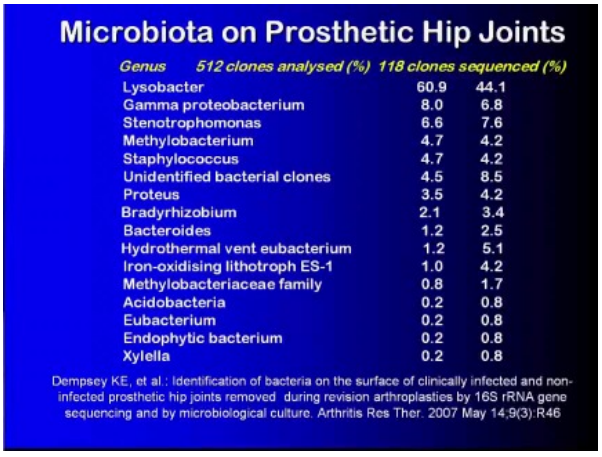
This study has just been published. It is a metagenome of human saliva.

It was a study done by Max Planck Institute in Germany. They took samples of saliva from 10 different places in the world, all over the world geographically, and individuals in those 10 places. And then they sequenced the genes.

Because now, with in silico technology, we do not rely on being able to culture the organism any more, we can actually sequence the sample and then find these little segments of DNA we can match up with our known database of 800 or more identified pathogens.

What they [study] found was that there was more variation individual-to-individual, than between geographic locations. But they found that there are a hundred bacterial genomes, including some previously unknown genera — 64 unknown genera, in this human saliva from healthy human individuals.

Streptococcus was most common, of course, at 22%. *Haemophilus* at 6%. *Neisseria* at 8%. But look, *Treponema* and *Yersinia* were in trace amounts. It is interesting actually that *Chlamydia* was not found. The body can apparently deal with the *Chlamydia* organism and reject it, or at least a healthy body can.

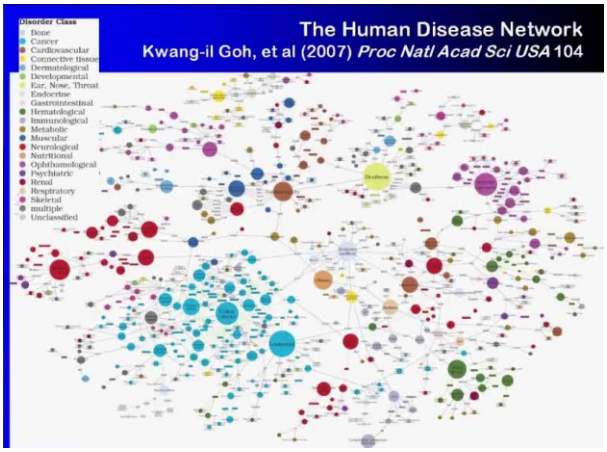


Slide # 5: Microbiota on Prosthetic Hip Joints

A similar study on prosthetic hip joints, this is hip joints that were being replaced during surgery.

They used a special procedure with ultrasound to shake the biofilm off the hip joints, and see what species were present. Of course, you have got all the normal ones. You have got *Staph*. You have got the gliding bacteria you would expect to find in a biofilm, *Lysobacter*.

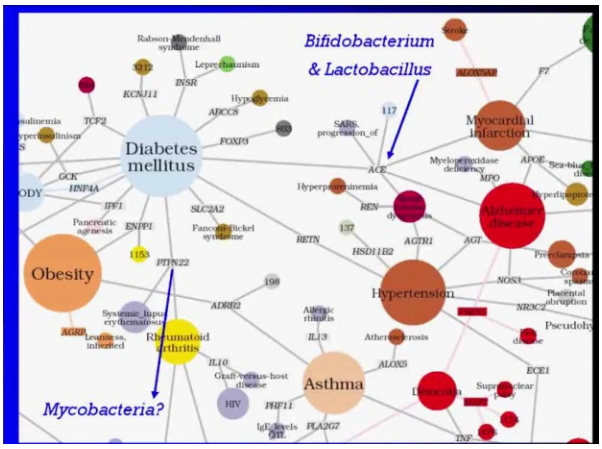
But look here. Down here we have got hydrothermal-vent *Eubacterium* at about 5% of the samples. And that is the same level as *Staph*, *Staph aureus*, a very common biofilm pathogen which is at about 4%. The hydrothermal-vent *Eubacterium,* the genome for that had been isolated in bacteria that had been extracted from hydrothermal vents at the bottom of the ocean, what is it doing in man? Well, that is what we are about to find out in the next decade. The next decade we will see a huge change in how we understand how the human organism interacts with the other organisms that are within the human body.



Slide # 6: The Human Disease Network

Now there are two ways of looking at disease. One way of looking at disease is saying, "Well, this person is sick. She cannot put her weight on her leg. She has to lie down all the time." In other words, focus on the symptoms and categorize diseases based on symptoms. And that is the way that medicine has done it for the last century.

But now we are starting to view diseases based on the genes. On the common genes that affect the various diseases. This [referring to slide] is a gene map that was produced a year or two ago now, 2007, and it has all of the diseases: neurological diseases, deafness; the autoimmune diseases; cancers. It has them all linked together based on common genes.



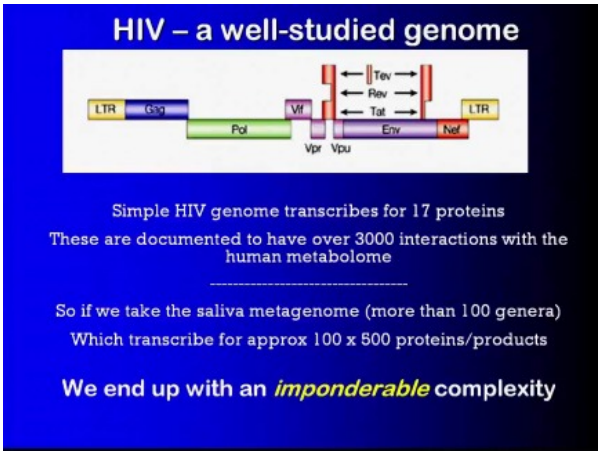
Slide # 7: Human Disease Network, close-up of area around the autoimmune diseases

If we look at a close-up of the area around the autoimmune diseases, you can see that this one gene here, called ACE — which is involved in the progression of SARS, the infectious disease SARS. It is involved in myocardial infarction, it is involved in Alzheimer's, it is involved in kidney disease, and it is also involved in Sarcoidosis and some of the other granulomatous diseases.

What is interesting about this common gene, ACE, it is not only involved in so many of what we would think of as being different diseases — kidney disease, cardiac disease, Alzheimer's — but also that that particular gene we know is affected by some of the bacteria which are present in every person's body. In particular, *Bifidobacterium* and *Lactobacillus*, which are species which you can find in yogurt. You certainly find them in probiotics. Those effect the expression, by the human genome, of the gene ACE. They also effect all the diseases, or the disease states, whose balance is dependent on ACE. I mean, healthy body is healthy because everything is in balance. When certain proteins start being downregulated, others start being upregulated, you start to get a disease state setting in.

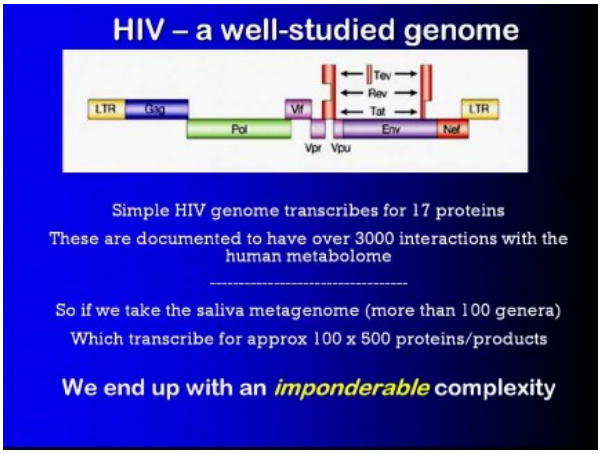
Down here we have another gene, PTPN22, and that one is associated with Lupus-SLE, Rheumatoid arthritis, and Diabetes. And that one is one of the body's primary responses to *Mycobacteria.* When the body senses *mycobacterial* infection, one of the first things it does is upregulate this gene, PTPN22.

So by looking at the genes in disease we can get a totally different picture from trying to work basically on symptoms, and differentiating symptoms as we have done for the last century.



Slide # 8: HIV — a well-studied genome

Well, *Chlamydia* is not a very well-studied genome. I'll give you some data on *Chlamydia* in a little while, but the one that I want to focus on, because it illustrates the problem that we have — we being *Homo sapiens*, as a species have — and that is the HIV genome.

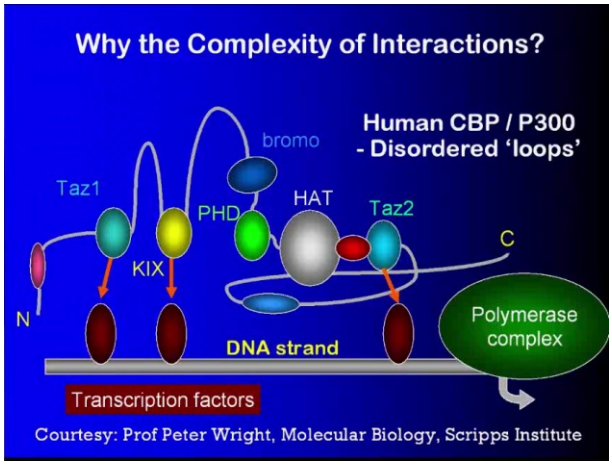


The HIV genome is very small. It is a small strand of RNA. From that genome 17 proteins, 19 including cleavage, but 17 proteins that are generated from this one strand of RNA, which is the HIV genome. So, HIV does all its damage by generating only 17 proteins. You compare that typically with a bacterium which certainly generates hundreds of proteins, usually close to a thousand proteins.

The HIV genome transcribes for 17 proteins. And with the billions of dollars that we have spent analyzing HIV, finding out what it does, we have identified — "we" being science — has identified, that there are over 3,000 interactions between those 17 proteins and the human metabolome, the human genome.

So if we take the saliva genome, which we saw on slide [4], which has got more than a hundred species and they transcribe for approximately 50,000 protein products, we compare 50,000 here to 17 here [referring to slide]. And then we look at 3,000 [interactions with HIV proteins]. Well, how large is that 3,000 going to rise if you have got that many more proteins being generated by the salivary metagenome?

The answer is, it is imponderable. You get to a point where you are just looking at noise. You are looking at stochastics. The body is trying desperately to deal with the species that it has got there, the DNA at the level of the transcription is trying to ignore all of the proteins and the enzymes that are coming from the pathogenic genomes and still produce good quality proteins from the human DNA, and it is just an imponderably complex problem.



Slide # 9: Why the Complexity of Interactions?

Why is it so complex? This is a slide courtesy of Professor Peter Wright at the Scripps Institute. This is a human transcription factor called CBP/P300. This transcribes some very important enzymes and proteins. I will be dealing with one of them later in the presentation.

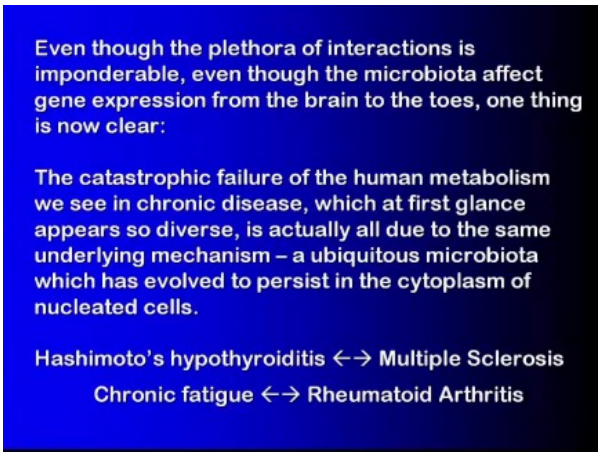
What happens is, you have got a strand of DNA here [referring to slide] and the transcription factors look for areas of that DNA where they are attracted, where their active areas are attracted, and then the DNA is transcribed; split into RNA strands and transcribed by the normal transcription mechanism. But the reason that the viruses affect so many different human gene transcriptions is because they are disordered. They have disordered loops.

This loop here [referring to loop in CBP/P300 on slide] will bind to other substances; it binds to other proteins. Depending on whether that loop is squashed up or stretched out, you will be decoding a totally different region of the DNA. So this one transcription factor actually transcribes thousands of human genes.

But the important thing to note is where this human protein has got areas that are very well structured — and these are the colored areas that are defined, very well structured — and the disordered loops are relatively small in size and number.

In a virus, the viral proteins are almost all totally unordered. So they have no shape until they wrap themselves around a protein or an enzyme from their host — from the human body.

Then, depending on which molecules attract each other — which atoms attract each other — they take on the shape of the host protein. They can change very quickly. They can mutate very quickly. As you all know that is one of the biggest problems with HIV.



Slide # 10: A common underlying mechanism

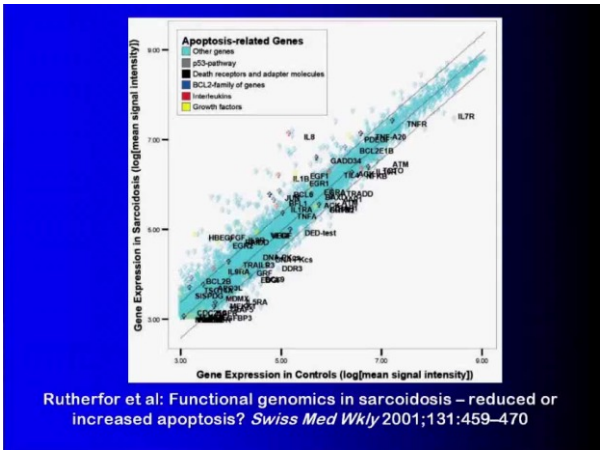
But even though the plethora of interactions is imponderable, even though there are so many of them that it is imponderable, even though the microbiota — the communities of microbes — affect gene expression from the brain to the toes, we can say one thing, and that is:

The catastrophic failure of the human metabolism we see in chronic disease, which at first appears so diverse and so different between the various disease diagnosis, is actually due to the same underlying mechanism — a ubiquitous microbiota which has evolved to persist in the cytoplasm of nucleated cells.

It is very important that these bacteria persist by overcoming the innate immune response. The very cells that are supposed to kill the bacterial pathogens, they actually overcome, and they live within those phagocytes — and live within those phagocytes for quite a long time.

So the same cause is behind Hashimoto's hypothyroiditis and Multiple Sclerosis. The same cause is behind Chronic fatigue and Rheumatoid Arthritis. Diseases that we would not normally associate if we are looking at them as symptoms.

But when we look at them as genes we can see how they are associated.



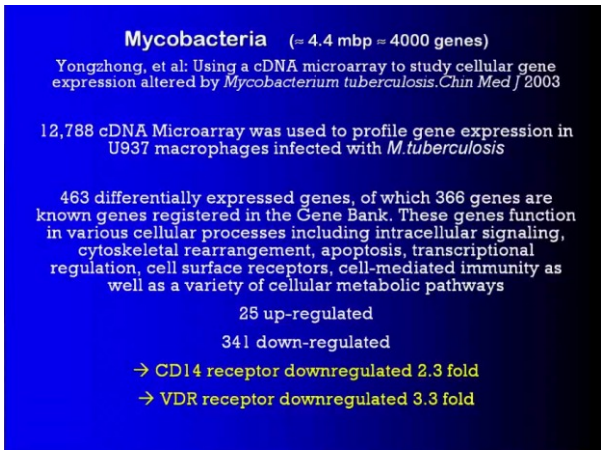
Rutherfor et al: Functional genomics in sarcoidosis – reduced or increased apoptosis? *Swiss Med Wkly* 2001;131:459–470

Slide # 11: Gene Expression in Sarcoidosis

In fact, when you do a genome-wide study — this is one of apoptosis in Sarcoidosis, which was done by Novartis many years ago, 2001 — what you find is basically there is nothing that jumps out at you. There are no genes up here fully on [referring to slide]. There are no genes down here that are fully off. There is just a whole lot noise around the expected region [referring to the mid-line in the diagram]. Yes, there is some like interleukin-8 that stick out, but basically it is just noise.

It is the total sum of all that noise, each one of those proteins, that is affected by the infection; each one of those proteins, that is affected by the microbiota.

Together, the sum total of them is what gives rise to the symptoms of chronic disease.



Slide # 12: *Mycobacteria*

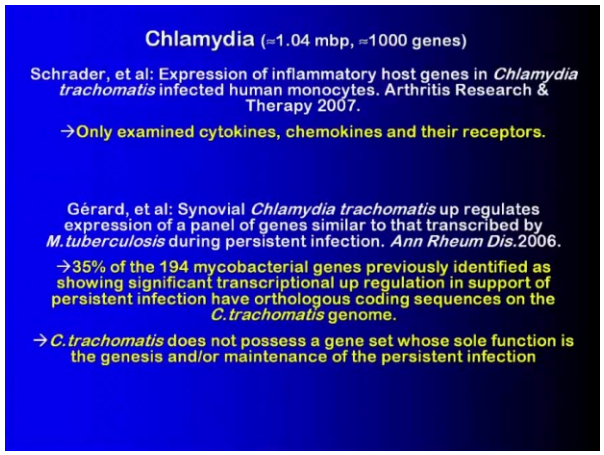
If we take *Mycobacteria,* that has been fairly well studied, there are about 4,000 genes in *Mycobacteria.* There is a very good study, published in the *Chinese Medical Journal* back in 2003, where they infected cells in culture, in vitro, with *Mycobacterium tuberculosis,* and they tracked which genes actually changed in the cell when they infected it with this one strain of pathogen, *Mycobacterium.*

They are not talking about one toxin. There is not one toxin. There were 463 genes whose expression were changed. 366 of them were known genes. The other genes were unknown, mutations.

And the genes function in various cellular processes including intracellular signaling, cytoskeletal rearrangement, apoptosis, transcriptional regulation, cell surface receptors, cell-mediated immunity as well as other cellular metabolic pathways. In fact everything, effectively, that determines how the human body operates is affected in that cell when it is infected with *Mycobacteria tuberculosis*.

25 were up-regulated and 341 were down-regulated. Two that I am going to point out were the CD14 receptor, was downregulated 2.3 fold, and the VDR receptor was downregulated 3.3 fold.

Downregulated three times in size. That is a very significant effect of Mycobacterium on the VDR.



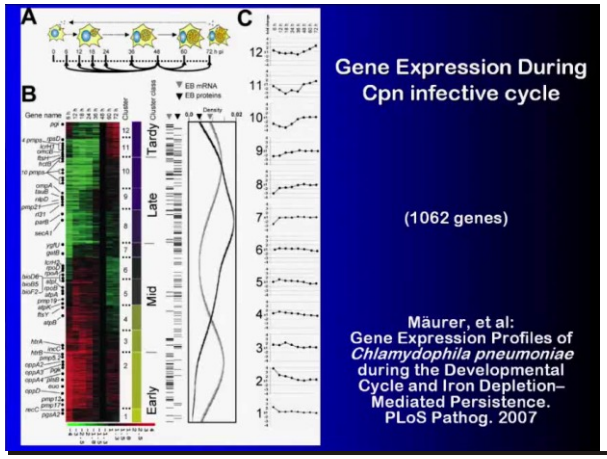
Slide # 13: *Chlamydia*

Chlamydia has about a 1,000 genes. It is a smaller genome. There have been a few studies.

The first I cite here only examined cytokines, chemokines, and is really of very little interest. Then there was another one that was published in 2006 which found that of the 194 *Mycobacterial* genes in that previous study that I was talked about, 35% of them have similar coding sequences in the *Chlamydia trachomatis* genome.

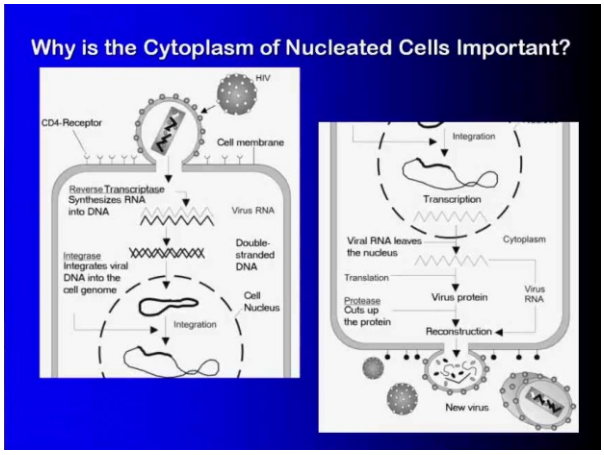
In other words, of the genes in *Mycobacterium* which are responsible for it being persistent, it being able to become chronic, about 35% of them have very, very similar genes in the *Chlamydia* genome. But the interesting thing about *Chlamydia* is it did not posses a gene set whose sole function is the maintenance of persistent infection.

Mycobacteria does. *Mycobacteria* has two defined states. Active and latent. But *Chlamydia* does not have genes which are solely responsible for that [persistent infection].



Slide # 14: Gene Expression During CPN Infective Cycle

Gene Expression During CPN Infective Cycle was published in *PLoS Pathology* in 2007.

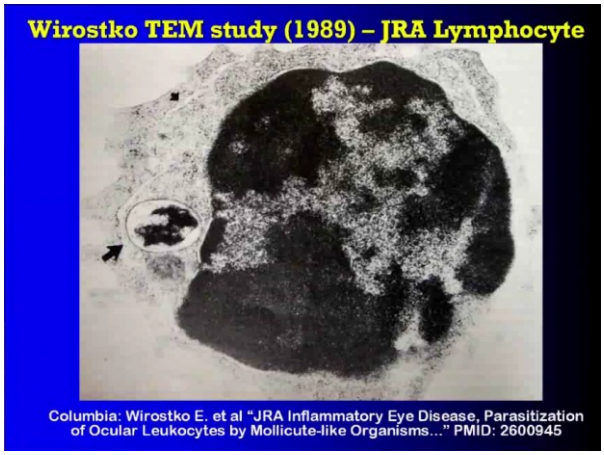


Slide # 15: Why is the Cytoplasm of Nucleated Cells Important?

Why is the cytoplasm of nucleated cells — that means cells with a nucleus, the phagocytes in particular — why is that so important? Once again, we will go back to our old friend HIV, because it is pretty well studied. [Illustration split to fit on horizontal slide.]

HIV infects through the cell membrane, through the CD4 receptors typically, and then the RNA of the HIV is reverse transcripted into DNA, double stranded DNA, which then goes into the nucleus where it becomes integrated with the human genome, and then transcription occurs in the normal way. The viral RNA leaves the nucleus, is assembled once again, reconstructed, and then leaves the cell.

Now the important thing to notice is a lot of the work is done in the cytoplasm in the region around the nucleus. In the nucleus you have the integration, you have the DNA repair, you have a number of mechanisms. But the cytoplasm is very important. Because in the cytoplasm, that is were all the proteins are generated. That is where most the enzymatic activity takes place. So the cytoplasm is very, very important.

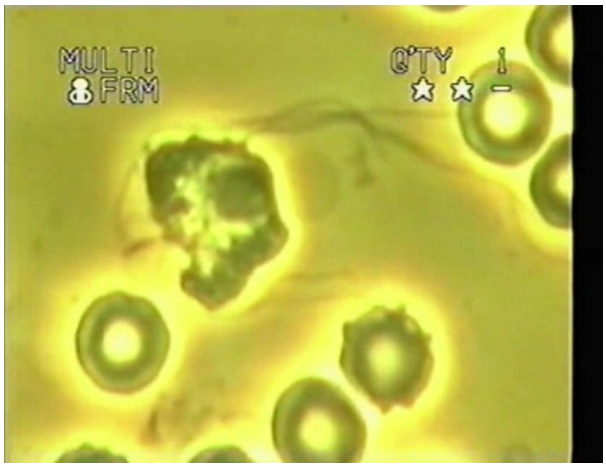


Slide # 16: Wirostko TEM study

Here we have a picture from a transmission electron microscopy study at Columbia University back in the 1980's by Emil Wirostko.

Emil's group studied lymphocytes, monocytes, macrophages, and neutrophils from patients with Juvenile Rheumatoid Arthritis, Sarcoidosis, and Lupus.

And they found the same thing. In all of those diseases there were infectious colonies of bacteria, which stained as bacteria, that were living within the cytoplasm of these phagocytic cells. The very cells, the lymphocytes that are supposed to get rid of the pathogens from our body, are actually being parasitized in these chronic diseases.

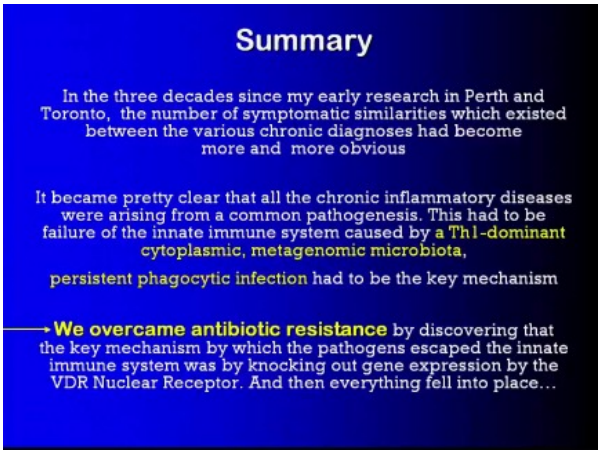


Slide # 17: Video from optical microscope

If we look at it with an optical microscope, we can see we have a nucleus here [left of center screen] and a cytoplasm which has just swelled and exploded as a result of these small colonies of biofilm-like pathogens. These huge long tubules are being thrown out from the degrading cell. These are very, very thin tubules caused by bacterial protein. This one is about 20 cell diameters long — extremely long.

That is what happens when the cytoplasm of the cell becomes so infected that the pathogens start to break out. The biofilm starts to break out to try and find more suitable hosts other than that particular cell.

Here we can see [referring to video on the screen], zoomed out, the length of this huge long biofilm tubule that is put out.



Slide # 18: Summary

In the three decades that have gone past since my early research in Perth, Western Australia, and Toronto, Canada, the number of symptomatic similarities which existed between the various chronic diseases become more and more obvious to me.

It became pretty clear to me that all the chronic inflammatory diseases were arising from a common pathogenesis which, I figured, had to be a failure of the innate immune system. And we found that it had to be a Th1-dominant cytoplasmic, metagenomic microbiota. And in particular, that persistent phagocytic infection had to be the cause.

How did I figure that out? I just did. Sorry. [smiling]

There is no other way that the biochemistry all fits together. If you look at the number of changes to the biochemistry, the molecular chemistry which occurs in these chronic disease states it is imponderable, it is just huge. There is no other way that the human genome could go that wrong. People are not born with that many mutations in their genome. Basically, the genome of Homo sapiens is fairly uniform. It had to come from somewhere else, and in fact, it had to come from pathogenic genomes.

But the thing that is really important is, for decades, chronic disease patients have been given antibiotics and have responded to the antibiotics differently from the way healthy people responded to the antibiotics. One of the reasons for that is because the postulates of Koch, from 1897, said basically, “Look, you have got to be able to examine the bacterium out of the body, in the lab.”

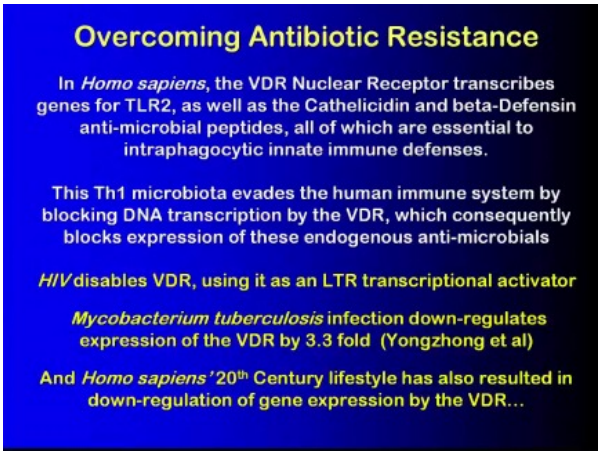
The moment you take it out of the body, you get rid of a whole lot of things that happen inside the cells of the human body. For example, if you take the antibiotics clindamycin, minocycline, and rifampin — rifampin was the primary antibiotic used against tuberculosis, minocycline you know, and clindamycin you know — all of those activate a nuclear receptor in the human body call the PXR nuclear receptor, which is right at the heart of the human immune system. We will get to that in a future slide.

So that when those antibiotics are in the human body, they have additional actions to what they have in a petri dish. And this is something that we were really only able to understand once we could understand the genes and how the genes interacted. And what I figured they had to be doing is knocking out gene expression by the VDR Nuclear Receptor.

The VDR Nuclear Receptor in man is responsible for some key endogenous antimicrobials. That means, antimicrobials that are produced in the human body itself. There are 24, approximately, families that have been identified and about 17 of them are affected by the VDR directly or indirectly. So it is absolutely key.

In particular, the Cathelicidin antimicrobial peptide, the receptor TLR-2, that is the one that has been on the previous slides of all the other speakers as the one recognizing *Chlamydia,* that gets knocked out when you knock out the VDR.

You knock out Cathelicidin and you knock out beta-defensins. At that point, the cell's immune defenses have been virtually knocked out. Just by the bacteria figuring out how to knock out that one nuclear receptor, out of the thousands and thousands of proteins that are in the human body.



Slide # 19: Overcoming Antibiotic Resistance

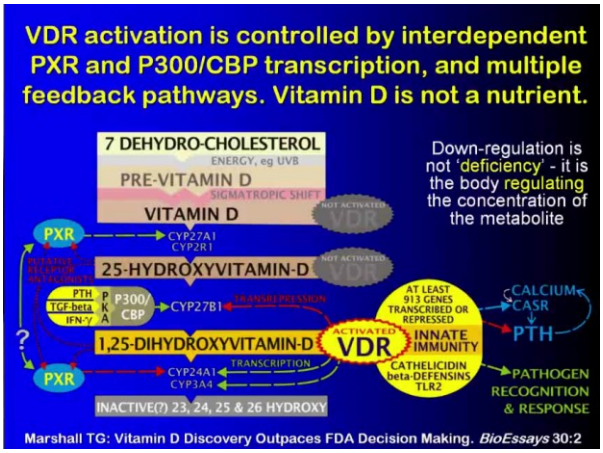
In *Homo sapiens* — and this is different from animals, animals have different function in their DNA transcription levels — but in *Homo sapiens* the VDR Nuclear Receptor transcribes genes for TLR2, Cathelicidin, beta-defensin antimicrobial peptides; all of which are essential to intraphagocytic innate immune defenses.

So, a microbiota has evolved which we have called a Th1 microbiota, because one of the common factors is interferon-gamma which is produced when the innate immune system is attacked by these persistent pathogens.

The Th1 microbiota evades the human immune system by blocking DNA transcription by the VDR, and that consequently blocks expression of these endogenous antimicrobials.

So it comes as no surprise, then, that we now know that HIV totally disables VDR. HIV takes VDR and actually uses it to help transcribe its own RNA genome, its LTR transcription. The tat protein from HIV actually takes away one of the human body's main innate defenses and uses it as part of the viral replication. We saw earlier that *Mycobacterium tuberculosis* downregulates the VDR by 3.3 fold. So these pathogens know how to get around human innate defenses.

Unfortunately during the 20th century, *Homo sapiens* changed their lifestyle in several ways that together has resulted in further downregulation of gene expression by the VDR and which made their bodies more susceptible when infections came along, like HIV or TB that required weak VDRs in order to be persistent.



Slide # 20: VDR Activation

The VDR is called the VDR because it is short for vitamin D receptor. Now, vitamin D is not a nutrient, despite what we have thought for 100 years, certainly back to the 1930's. Which is what? 80 years. For the last 80 years we have thought vitamin D was a nutrient, but it is not a nutrient.

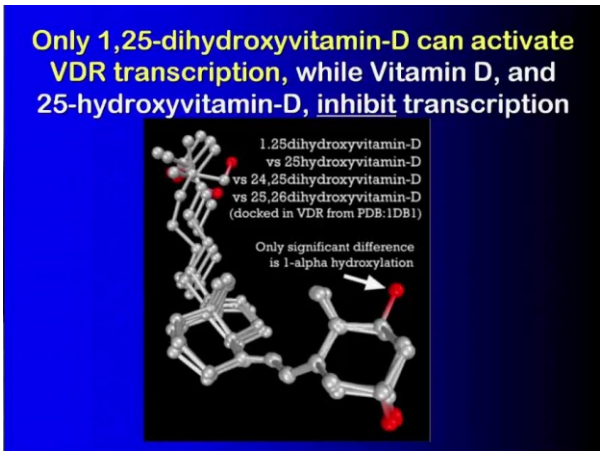
The body manufactures vitamin D. There has been no human study on whether any vitamin D is necessary. There has certainly been studies in other animals. A very elegant study in fish showed that the body manufactures all the vitamin D it needs.

Vitamin D is not a nutrient. It is a transcriptional activator. It is a secosteriod hormone.

There is a very complex control system here which involves the P300/CBP that I was talking about earlier, as well as the VDR, to synthesize from 7-dehydrocholesterol the cathelicidin, beta-defensins, antimicrobial peptides, and the toll-like receptor 2.

In fact, the VDR is responsible for at least 913 confirmed genes, which range everywhere from Down's syndrome to cancers to the calcium sensing receptor and PTH downregulation.

Very, very important receptor indeed.

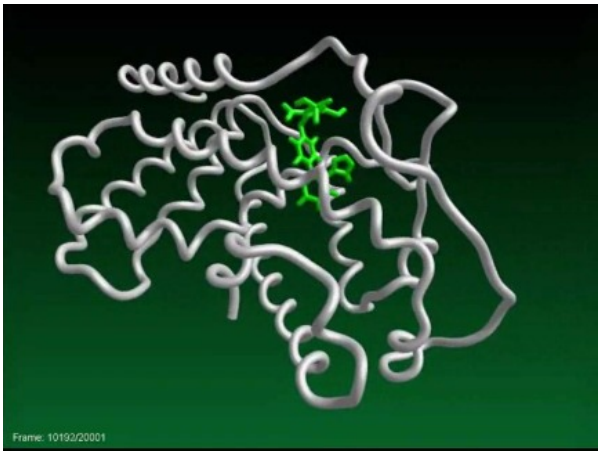


Slide # 21: Only 1,25-dihydroxyvitamin-D Can Activate VDR Transcription

Now, what we found was that only the active metabolite [1,25-dihydroxyvitamin-D], produced by the body, activates the VDR; while the 25-hydroxyvitamin-D — which is produced when vitamin D is ingested through food or through supplements, or through vitamins — that [25-D] actually stops transcription.

All exogenous forms of vitamin D have to be removed if you want to overcome chronic infections. Because all you are doing is aiding the pathogens in their ability to overcome the innate immune system.

We have got the various forms of vitamin D docked here as they exist within the molecules — [referring to slide] this is some in silico work — and only one of them has got the 1-alpha hydroxylation needed to activate the VDR [see arrow on slide]. All the others will take up space in the VDR, but they will not activate genes. They just get in the way.



Slide # 22: Homo sapiens VDR Olmesartan in LBP (Ligand Binding Pocket)

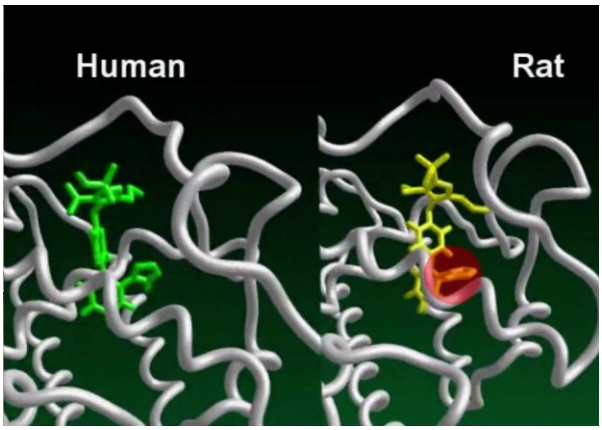
So we can, luckily, restore innate immunity by using a VDR agonist. I identified one called olmesartan. And here we have a protein [referring to animation on screen] this is a human VDR. It is moving all the time — all proteins are in motion all the time. In the binding pocket, there is the olmesartan drug [shown green].

Slide # 23: Rattus norvegicus VDR Olmesartan in LBP

This is the olmesartan drug here in yellow [referring to animation on screen].

This is actually the rat VDR shown on this particular slide.

[Next,] I will show you the human and the rat side by side, and show you that the two are not the same.

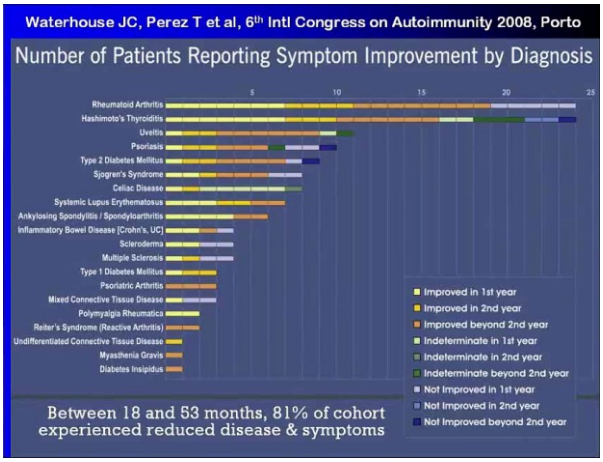


Slide # 24: VDR human and rat

The drug behaves differently in the rat and in the human. If you look at this tetrazole ring [see red highlight on slide], you can see it is in different orientations in the rat, and in the human. In fact, there are two less hydrogen bonds and that is stabilizing, or not stabilizing, a critical helix in the structural assembly.

So this drug does not perform in the same way in the rat as it does in the human.

Big problem of course, because most our studies are still being performed in animals, even though we have the capability of examining in silico, at the molecular level, there are very few people doing that at this point.



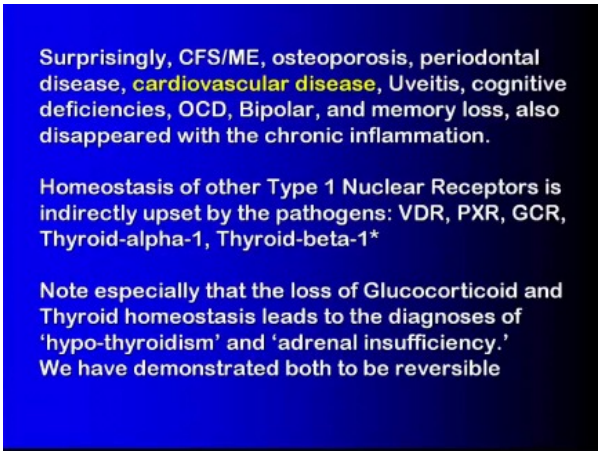
Slide # 25: Data from Observational Cohort sample of response in Autoimmune Disease

So what happens when you address the problem of the VDR and remove exogenous, that means from outside the body, remove exogenous vitamin D and just allow the body to make the vitamin D it needs, and then at that point, the body becomes susceptible to very small amounts of antibiotics — the antibiotics which really did very little when the VDR was overcome by the pathogens?

Once the VDR has been reactivated again, then the patient starts to respond to antibiotics in the same way that a normal, healthy individual would do. And I will be talking more about that this afternoon when I talk about the protocol that we have put in place to reverse the mechanisms that the bugs have used to overcome our immune systems.

This is the data that was reported at the *Autoimmunity Congress* in Portugal last September [2008]. It shows a small portion of our clinical cohort, observational cohort*:* Diseases from Rheumatoid Arthritis, Hashimoto's Thyroiditis, Uveitis, Psoriasis, Type 2 Diabetes, Sjogren's, Celiac, SLE, right down to Undifferentiated Connective Tissue Disease, Myasthenia Gravis, Diabetes Insipidus*.*

All [diagnosis types] responded favorably in all cases, with reversal. 81% of the cohort experienced reduced disease and symptoms between 18 and 53 months, and the trial is ongoing at this point.



Slide # 26: Notable outcomes

But what was surprising was, it was not just the autoimmune conditions that responded to the antibacterial therapy, but also Chronic Fatigue Syndrome/Myalgic Encephalomyelitis, osteoporosis, periodontal disease, cardiovascular disease, Uveitis, cognitive deficiencies, Obsessive Compulsive Disorder, Bipolar, and memory loss; they also disappeared as the chronic inflammation disappeared.

So as these people got better, all of these things that we never really associated with pathogens — osteoporosis, cardiovascular disease — well, actually, this is a Chlamydial infection conference,

so you would know that *Chlamydia* has been implicated in cardiovascular disease.

There is a study going on at the moment. One of our collaborating physicians is doing a study where he is actually tracking the reduction in arterial wall thickness, year by year, of patients as they recover on the antibacterial therapy. That is unheard of. Even when you are using statins, there is still some gradual increase in the wall thickness.

When you actually combat the pathogens, the walls go back to normal.

The homeostasis of other Type 1 Nuclear Receptors is also indirectly upset by the pathogens, and in particular, the thyroid receptors. You will notice that we had a lot of people with Hashimoto's Thyroiditis diagnosis. Partly that is because a lot of people with chronic disease have thyroid problems. Because one of the first things that happens when the VDR stops working, the level of 1,25-dihydroxyvitamin-D rises in the body and that shuts down the thyroid metabolism.

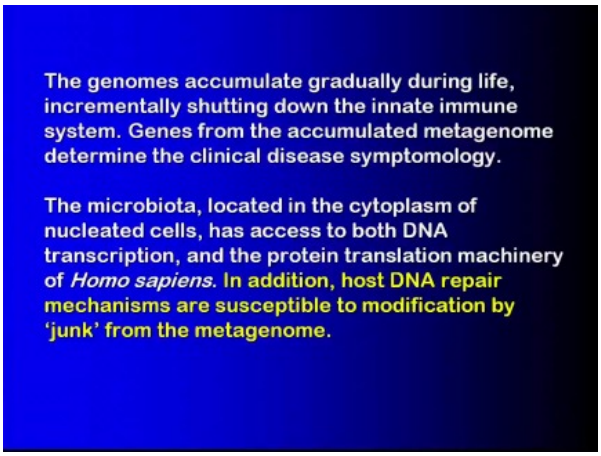
It also shuts down the adrenal axis, the glucocorticoid receptor is also overcome by these high levels of 1,25-D.

The reason you cannot get rid of chronic disease by giving people vitamin D is because that vitamin D collects in the body and it starts to hit the other receptors at the same time as it is overcoming the bacterial effect on the VDR.

So when people get sick to a certain level, they will no longer respond to conventional therapy. At that point the bacteria are in control. And usually that occurs late in life, except with people that come down with chronic disease, and tragically these days we are seeing even kids coming down with the chronic diseases that they used to not get.

But typically they [bacteria] just cause the "diseases of aging:" Dementia, osteoporosis, muscular problems, you know them. Normally the microbiota just stays dormant and pops up in late life and people say, "oh yes, I am getting old."

Well, no, the bacteria are starting to take charge.



I have already mentioned that the loss of Glucocorticoid and Thyroid homeostasis leads to diagnosable disease states.

Slide # 27: Gradual loss of genome integrity

The genomes accumulate gradually during life. Very important, you were born with this microbiota.

Depending on what you come in contact with during life, from food, from saliva, from aerosols through the air, and from infections, of course, actual acute infections, your metagenome will gradually be built up. And so at any point in life it will be different than it was a decade earlier.

The microbiota has access to the DNA transcription machinery. And that is what I have been talking about, taking a strand of DNA and turning it into proteins that can actually do something.

But what is more important, the DNA repair mechanisms become susceptible to all of this plethora of imponderable effects from the bacterial DNA. So you get modification of the human DNA repair mechanisms by 'junk' from the metagenome. HIV integrates itself into the human genome. HHV6 integrates itself into the human genome.

There is a lot of work being done to show that bacteria do the same thing.

But you saw in the *Mycobacteria* study that there were a whole lot of genes that were identified that were not previously documented as being in *Mycobacteria* or in *Homo sapiens*. And that is because the interaction between the genomes.

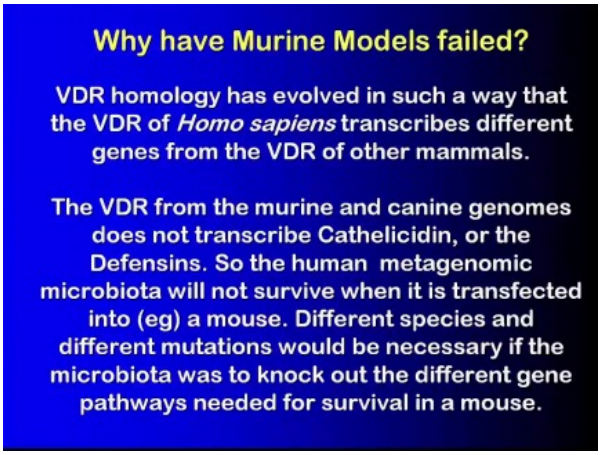
Slide # 28: "Germy mouths linked to heart attacks"

This came out just last week on *Reuters*, Wednesday, April the 1st, and no, it is not an April 1st joke.

It was reporting a study on heart attacks. I love this word, "germy," mouths linked to heart attacks, study finds. But what they found was, "people who had the most bacteria of all types in their mouths were the most likely to have had heart attacks."

That is not a surprise to us. If the body cannot keep the number of species down to the so-called friendly, or at least, mostly friendly bacteria, then the disease mechanisms are going to take over and people will get sick. And that is exactly what this particular study on cardiovascular disease found: It was not that *Chlamydia* was present. It wasn't that *Mycobacteria* were present. It was not that *Mycoplasma* were present. The total number of species that could be identified in the saliva was the best indicator that somebody was likely to have had a heart attack.

We are talking about a metagenomic, very many genomes, microbiota.



Slide #29: Why have Murine Models failed?

So, the last question. Really, second to last is, "Why have murine models failed?" Now what is a murine model? That means mice and rats. When we test these diseases out in mice and rats, which we nearly always do, before we give it to mankind.

Unfortunately, the immune system of mice and rats is very different to the human immune system. It has evolved totally differently. Mice and rats live in a different environment to us. And that seems to have been forgotten by science for the last 50 years.

In particular, if you look at the VDR homology, and that means the shape in the VDR — the shape in the VDR that is produced, the

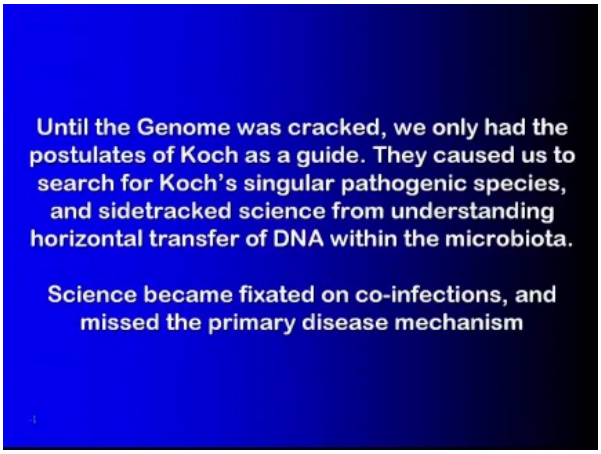
VDR of Homo sapiens transcribes different genes from the VDR of other mammals, even the VDR of higher primates.

And in particular, the VDR from the murine and canine, or dog, genomes does not transcribe Cathelicidin or the Defensins. So if a bacteria evolves to knock out the VDR, it makes human beings sick. But it is not going to make rats and mice sick, because it does not knock out their primary defenses. It does not knock out Cathelicidin or Defensins like it does in man.

So the human metagenomic microbiota will not survive when transfected into a mouse.

If you take the human microbiota, put it into a mouse, the mouse will be able to deal with it because its VDR is not really as important as man's is.

Different species and different mutations are necessary if the microbiota are to knock out the different gene pathways needed for survival in a mouse. That is one of the fundamental reasons why chronic disease has remained 'of unknown cause' for the last 50 years, in my opinion, it is the reliance on mouse and rat models, and to a lesser extent on other mammal models; without really questioning whether a mouse has the same immune system as a human being.



Slide #31: Missing the primary disease mechanism

Until the genome was cracked — until we could crack genomes, until we could sequence the DNA, and figure out exactly what we were dealing with, what made this organism tick — and whether the organism is *Homo sapiens*, or bacteria, or a virus like HIV, something makes that organism tick, and now we have just started to figure it out.

We have decades, maybe a century, of figuring out this imponderable problem that I talked about earlier.

But we have just started. But until the genome was cracked, we only had the postulates of Koch as a guide. The postulates of Koch have formed the basis for infectious disease, clinical infectious disease at least, for a century. And they caused us to look for a single species that was causing the disease process.

Koch basically said, "you have polio virus, and it causes polio." Koch basically said, "you have single pathogen, and it causes a singular disease." And that is not what we are finding. What we are finding is that the human body is a whole plethora of pathogens, a whole plethora of genomes; a metagenome.

So science became fixated on the co-infections. *Chlamydia, Bartonella, Borellia,* HHV6, EBV have all been implicated in widely varying diseases. If you look at the literature on EBV, you will find that just about every disease has been blamed on EBV at some point in time — Epstein-Bar virus.

And the same with *Chlamydia.* So many different diagnosis are supposedly due to *Chlamydia.* Because these are the pathogens, that when the body's own immune system becomes weak, these are the pathogens that the body cannot get rid of. So they stay there. And they can be seen, and observed, and measured. And people say, "Ah! That person has a *Chlamydia* infection! The *Chlamydia* is making them sick."

Well yes, the *Chlamydia* might very well be making them sick, because *Chlamydia* has got some very nasty toxins itself. But in order for the immune system to allow that *Chlamydia* to flourish, they first had to have a suppressed immune system from the metagenomic microbiota.

So for the last century, science became fixated on what are predominantly co-infections and they have missed the primary disease mechanism. The primary disease mechanism being the intraphagocytic, intracytoplasmic metagenomic microbiota.

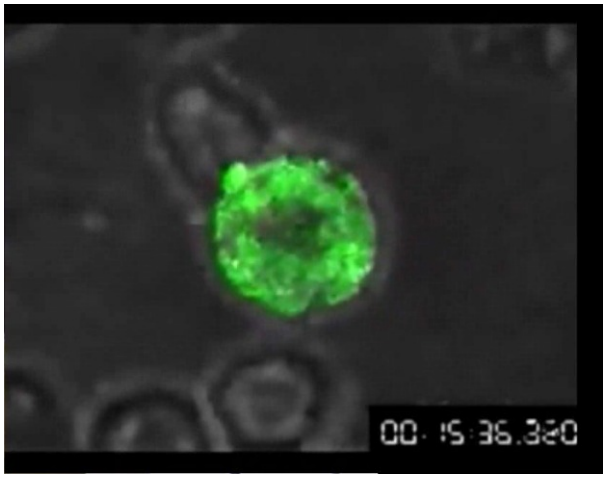
QUESTIONS

Question: What are the methods for measuring the antimicrobial peptides in humans, in the blood?

Marshall: The antimicrobial peptide work that I am relying upon was done by Brahmachary's group in Singapore. I cite it in all of our papers, actually, I think you will find it cited. [1] And they used an in silico analysis.

What they did was look at the DNA and they figured out what activated the DNA whether it is a VDR receptor, whether it was a P300 receptor, and from that they figured out what was responsible for each of the antimicrobial peptides. Cathelicidin, beta-Defensins, and TLR2 have all been confirmed in vitro as coming from the VDR.

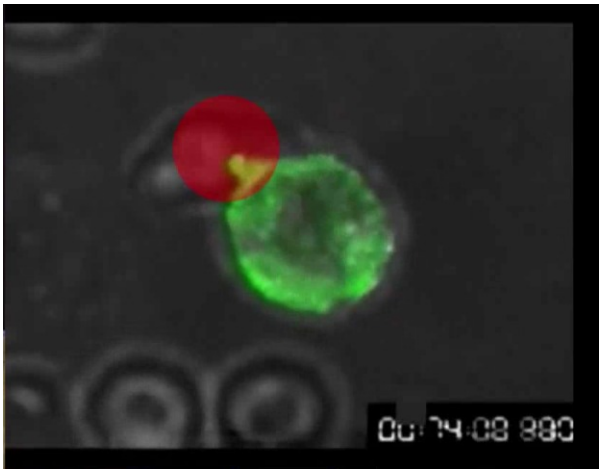
So the 24 families in silico and the actual Cathelicidin, the key metabolites, have been confirmed in vitro by various groups, more than one group.

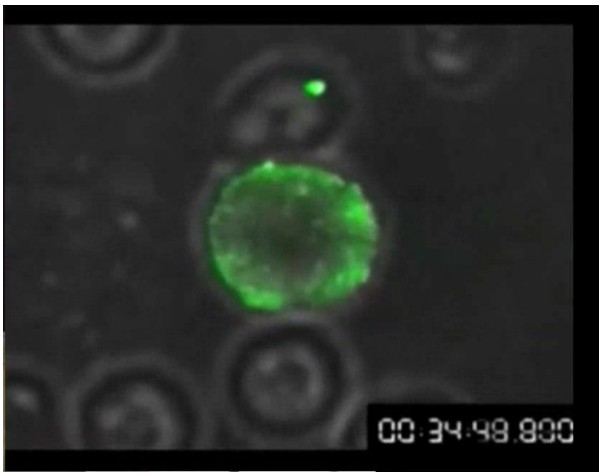


Slide #32: "Microscopy of HIV Transfer Across T-cell Virological Synapses"

This is a slide that just came out last week from a group studying HIV. And what we have got here [referring to the animation on the screen] is we have got two cells, well a number of cells, but there is a cell which has been infected with HIV. We have a cell which is brushing up against it. And at the junction of the two cells, the HIV is trying to get through the cell membranes into that [uninfected] cell. And in fact, that is exactly what is going to happen.

This HIV infection is able to cross the membrane. You can see it frozen there as it is starting to cross. In a little while you will find there will be fluorescent staining inside that cell, or fluorescent artifacts inside that cell to indicate that the tat protein has — there it is, it has broken off, it is now inside the other cell.





One of the reasons that we do not find these pathogens in the blood is because they do not need to be in the blood. They can pass from cell to cell. That has now been shown in HIV with this very elegant study from March 2009, "Microscopy of of HIV Transfer Across T-cell Virological Synapses" [2] (which means across the membranes). And of course, that cell now also will become infected and will eventually bud into virions.

All of this without the bacteria having to have to deal with what is in the bloodstream. It has not had to deal with any antibiotics the patient has been taking. Because it is existing totally inside the cytoplasm of the cells.

Question: When you follow the people for saliva samples in the world, do you see local similar profiles, like people from Prague would have certain profiles, but either people from New York would have different samples, or do they look the same?

Marshall: Firstly, I don't follow them. But there have been studies done. The two that come to mind are the study out of Imperial College in London, where they looked at uria. In the urine, they looked at the various proteins that were found in the urine that could not be produced by the human body itself but were being produced by bacteria which were present in the urine, present in the kidneys, one assumes.

And what they found was that there were very distinct groupings. The Japanese population, the Chinese population, and the US population were all different. And when Japanese moved to the US, their microbiota changed to the US population. Because the food changed, their environment changed, everything changes. So we very much are a product of the bacteria that make us.

Now, that was not specifically looking at saliva. The study from Max Plank that looked at saliva did, in fact, look at 10 locations throughout the world to make sure that they had good geographic diversity. And if you look up the paper that I cited there you will find the detailed data.[3]

Question: Because there is now a new field of human genomics, then you can track the 'mother of mothers' in Africa, you can see how people moved in Europe, several passages, you can also follow certain tendencies to diseases, such as high blood pressure?

Marshall: Right. Well, for example, *Helicobacter Pylori*, has been used to track migrations in the South Pacific. Because it has been isolated from very ancient skeletons and other DNA fragments in various regions of the South Pacific. And depending on the particular genome that they found at these various points, they were able to figure out how the populations migrated in history based on the *Helicobacter Pylori* genomes that they found.

Borellia was recently located in an Egyptian mummy. They were able to isolate some *Borellia* genome.

So we have an incredible opening up of discovery over the next decade or two as we start to get our minds around this concept of a microbiota, a community of pathogens

Comment: Yes, I think that it is always about the interaction between humans, and that we are exposed to selection criteria, so if we are in an environment where bacteria are and we cannot cope with them we die. Only the people that can deal with them survive.

Marshall: Yes. Many people are exposed to *Borellia.* And to *Chlamydia*. But only a fraction of them become very ill. Only a fraction of them become ill, in fact. Some of them can get rid of the organism immediately. And that is because it is the innate immune system that is key.

It is the status of the immune system when that person gets challenged by the acute pathogen that is so important in determining what is ultimately going to happen during their lives.

And ,therefore, it is very important to study the family, to study the maternal line — because these bugs are primarily passed down the maternal line — to study the maternal line, and you will find that a kid who has got Lupus had a mother who had arthritis, and grandmother that had thyroiditis. You can see the diseases.

Once you realize that all these diseases, the chronic diseases, are inter-related from the same cause, you can track them from within a family, and also horizontally within families as well.

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