NeuroTalk 2011
Expo Center, Dalian, China, 22-25 May, 2011

Presentation of Prof. Trevor G Marshall:
"The Human Microbiome is the mechanism fuelling Neurodegenerative Disorders"

The Human Microbiome is the mechanism feuling Neurodegenerative Disorders

PRESENTED BY PROF TREVOR MARSHALL
Director, Autoimmunity Research Foundation

Expo Center, Dalian, China, NeuroTalk 2011, May 22, 2011.
Transcript of http://www.youtube.com/watch?v=OAwxzQCjXM4 video.

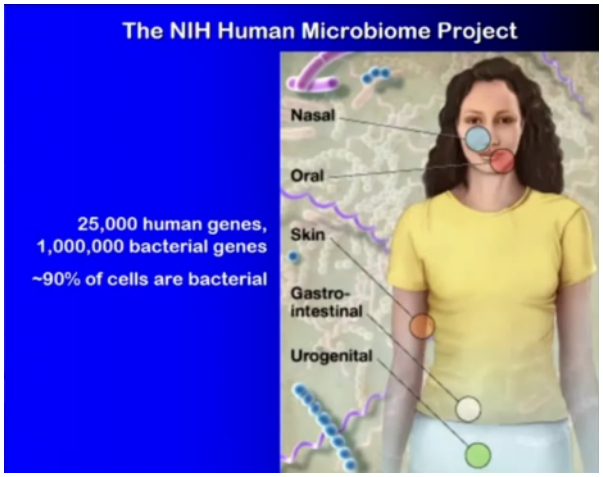
Transcript

00:04
The Human Microbiome is the mechanism fueling Neurodegenerative Disorders

Prof. Trevor G. Marshall
Faculty of Health Sciences, Murdock University, West Australia
Autoimmunity Research Foundation, California
Revised: 22 May 2011

Now our next speaker is Trevor Marshall and he is currently in the United States, the Director of Autoimmunity Research in Thousand Oaks in California. Since he is an Australian, he also keeps one foot in the door in Australia and he is adjunct professor in the faculty of Health Sciences at Murdock University, and that is in Western Australia. And I think he just went from the South to the West.

TM: Right, but we are still in the antipathy, so to that is something. But most of my work now is based in California with the Autoimmunity Research Foundation.



00:51
The NIH Human Microbiome Project

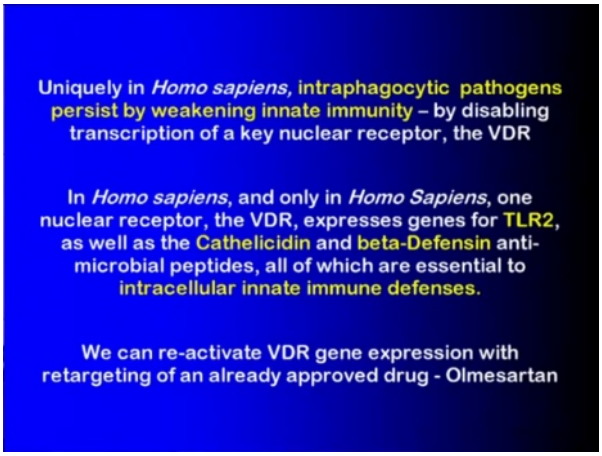
So I am going to talk about the Human Microbiome. Well, what is the Human Microbiome?

This is a Web page or images off a web page which was produced by the National Institutes of Health about three or four years ago when they kicked off the Human Microbiome Project. And the Goal of the Human Microbiome is to look for microbes in and around the human body by using the DNA sequencing technology which is available these days.

So instead of trying to culture the microbes, and we know that only about 2-5% of microbes are capable of being cultured, the DNA of the microbes was going to be able to be used to identify just what commensal or a pathogen with *Homo sapiens*.

The data is starting to come in. What we have found is that in *Homo sapiens*, there is in fact, thousands of species that are living in and around the human body. Whereas we have thought for so long that the human body is a sterile compartment, it is not sterile. It is not even close to being sterile.

I am going to show you some of the science in a short time. I'm going to go first right quick and show you what we have actually done, and then hopefully, that will make you a little more interested in the science and keep your attention later on.



02:23

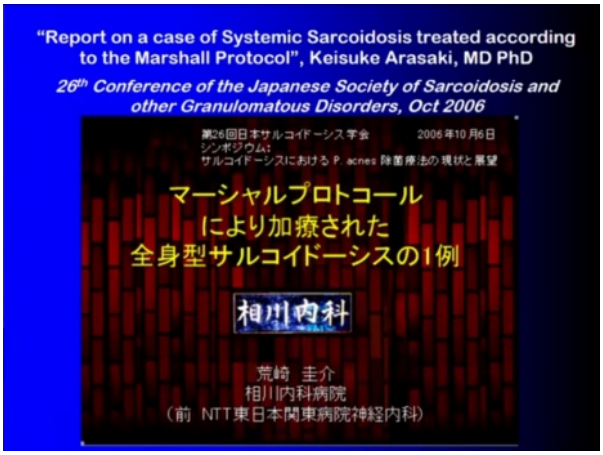
Uniquely in *Homo sapiens*

What we contributed was that we noticed back about a decade ago, that uniquely in *Homo sapiens*, not in the great apes, but uniquely in *Homo sapiens*, intraphagocytic pathogens can persist by weakening innate immunity—innate immunity, not adaptive immunity—by disabling the transcription of a single key nuclear receptor, the VDR, type 1 nuclear receptor.

Because in *Homo sapiens*, and only in *Homo sapiens*, one nuclear receptor, the VDR, expresses genes for TLR2, as well as the Cathelicidin and beta-Defensin anti-microbial peptides (actually Cathelicidin is a protein that is broken down into peptides through microbicidal action), and all of which are essential to intracellular innate immune defenses.

So if you are a microbe that wants to persist inside the nucleated cells of the human body, it makes sense that you have evolved to knock out that receptor [VDR]. And in fact, that is what we found has been happening.

Because we found a drug that was already approved that we could retarget by changing the dosing. We could retarget this to reactivate VDR gene expression, or certainly, expression of genes by the VDR.

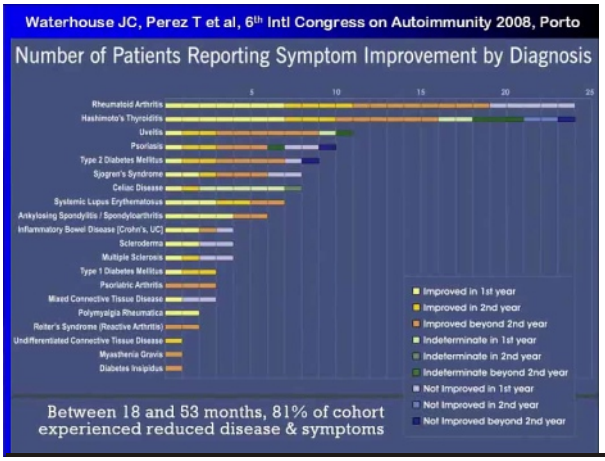


03:52

Japan Sarcoidosis study by Keisuko Arasaki, MD Phd

One of the first signs that we had been successful was this paper that was presented in Japan at the 26th conference of the Japanese Society of Sarcoidosis and other Granulomatous Disorders, in 2006.

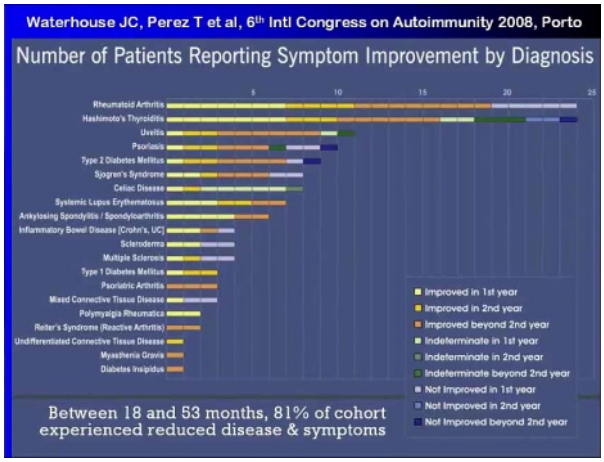
This was presented by Keisuko Arasaki. Arasaki-san was reporting on the case of systemic Sarcoidosis treated according to the Marshall Protocol. I did not choose that name, I am sorry, but the therapy we have developed was being used by Arasaki-san on a very sick patient in Japan, with Neurosarcoidosis and extremely disabled. He was reporting very good results and in fact, that patient is back at work practicing as a lawyer (matter of interest).



04:48

Number of Patients Reporting Symptom Improvement by Diagnosis

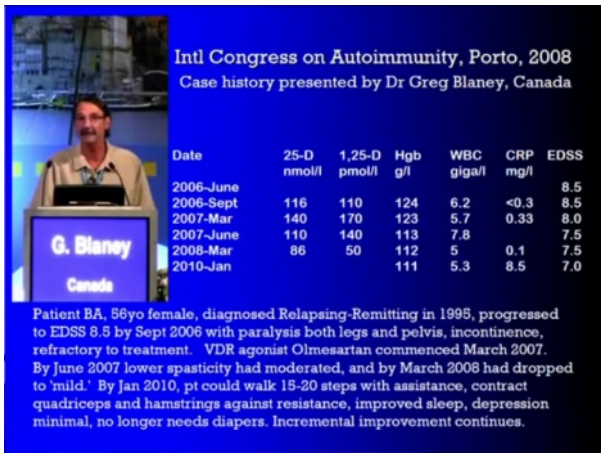
Then, by 2008, we collected some more data, just a quick retrospective analysis of it. We presented at the International Congress on Autoimmunity in Portugal, in 2008, about 100 patients with various diagnoses that were being treated with this VDR agonist.



A whole range of chronic diseases were found to be responding extremely well. We have a very unusual way of presenting this data, because **we have a very low rate of failure**. In fact, the rate of failure is only about 3 or 4 per 100 on this chart.

So we have actually spelled out year-by-year how patients responded to therapy. Rheumatoid Arthritis and Hashimoto's Thyroiditis, of course, are up at the top. Uveitis, Psoriasis, Multiple Sclerosis down here, Psoriatic Arthritis, Diabetes and then we just had a few patients, one patient in fact, with Myasthenia Gravis and Diabetes Insipidus diagnoses.

But by an average of 36 months into treatment, 81% of the cohort were reporting reduced disease and symptoms. Many of them have gone on beyond that point.



06:17
Intl Congress on Autoimmunity, Porto, 2008

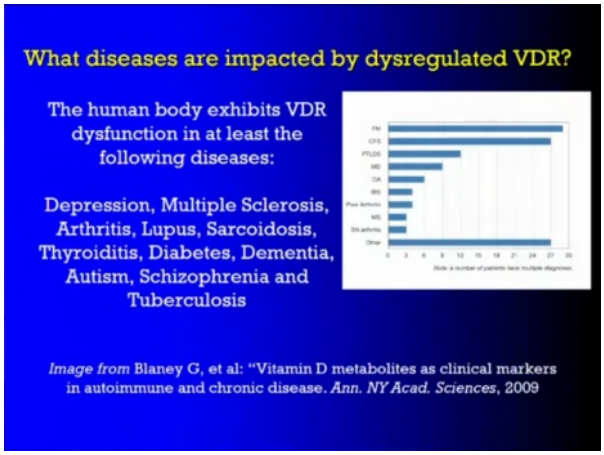
This is one of the MS patient case histories, which we also presented in Porto separately, by Dr Greg Blaney from Vancouver in Canada, one of our worldwide collaborators.

It is tracing the case of a patient with Multiple Sclerosis, starting off with an EDSS of 8.5 (you actually can not see that for some reason, well it does not matter, believe me). The EDSS started at 8.5 it got to 8.5, 8.0, 7.5, 7.5 and 7.0 over a period of four years, roughly, from 2006 to 2007.

The patient was a 56 year old female—the patient is a 58, probably now, year old female—diagnosed with relapsing and remitting in 1995, progressed to EDSS 8.5 by September 2006, which resulted in paralysis in both legs and pelvis, incontinence and refractory to treatment.

The VDR agonist olmesartan commenced March 2007. By June 2007, lower spasticity had moderated, and by March 2008 had dropped to 'mild.' By January 2010, patient could walk 15-20 steps with assistance, contract quadriceps and hamstrings against resistance, and improved sleep, depression was minimal, and no longer needs diapers. The Incremental improvement continues.

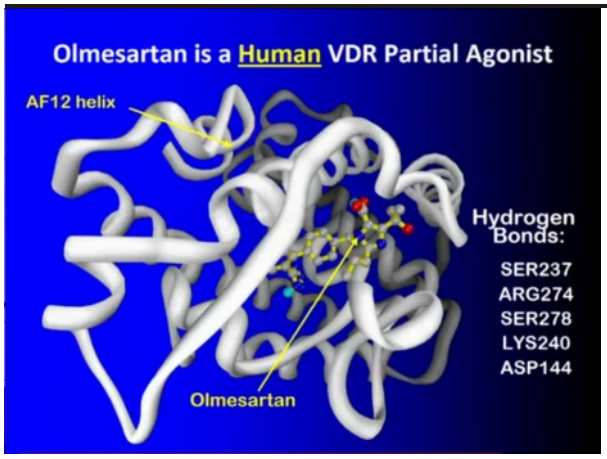
Quite an amazing case history, but it is not just Multiple Sclerosis.



07:56
What diseases are impacted by dysregulated VDR?

Dysregulated VDR has been documented in a number of conditions: Depression, Multiple Sclerosis, Arthritis, Lupus, Sarcoidosis, Thyroiditis, Diabetes, Dementia, Autism, Schizophrenia and Tuberculosis.

This is from a paper by Greg Blaney, featured in the earlier slide, which is in the *Annals of the New York Acadamy of Sciences*, a year or two ago.

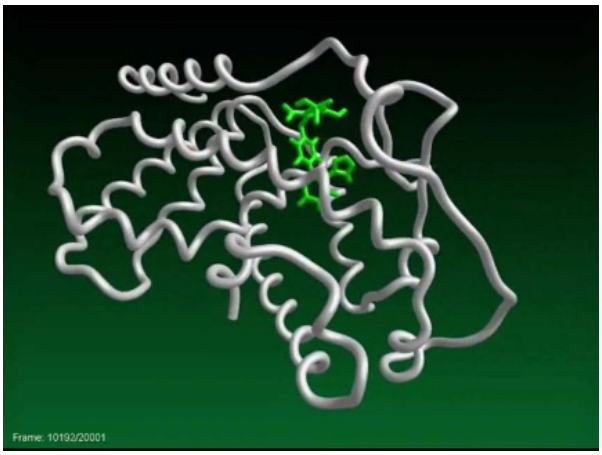


08:26

Olmesartan is a Human VDR Partial Agonist.

So if we now start looking at the science and looking at this drug, you have got a receptor and you have got a drug ligand which sits in the binding pocket. In this case it is olmesartan.

We have ways of studying to show that it is an agonist, but the way that I used was a very, very strong methodology. We used molecular dynamics.



08:54

***Homo sapiens* VDR Olmesartan in LBP (note orientation of tetrazole)**

We actually simulated the operation of the VDR in real time with the drug in the binding pocket.

09:20

Video

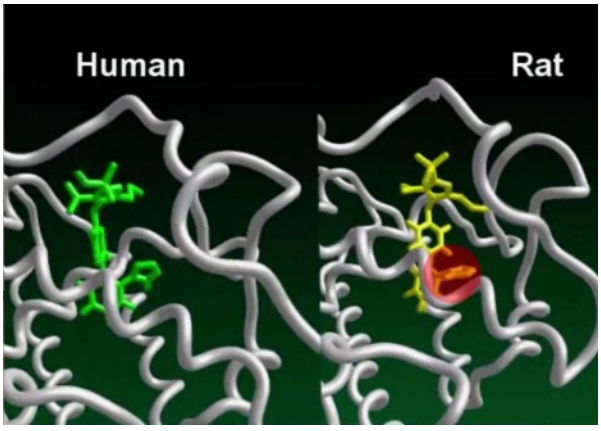
This is the [human, *Homo sapien*] VDR. All molecules move at all times, usually fairly vigorously. It is a fairly large molecule. There are about 200 amino acids shown on screen there, and four or five major helixes.

09:34

***Rattus norvegicus* VDR Olmesartan in LBP (note orientation of tetrazole)**

But we were also able to do this using *Rattus norvegicus*, the rat VDR, because there was an x-ray model available for the rat VDR.

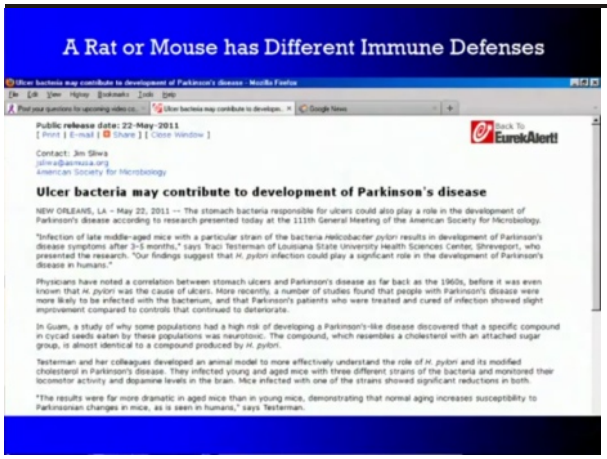
What we found was **the drug does not work in a rat**, which is why probably no one else would know that [olmesartan] could be retargeted.

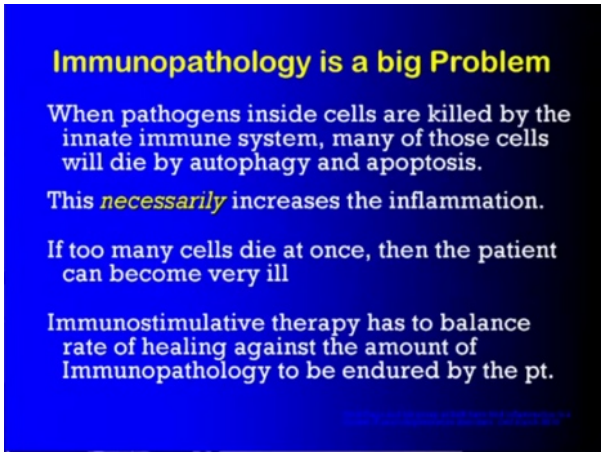


09:54

Video [comparison video split screen of *Homo sapiens* and *Rattus norvegicus*]

If you look at the tetrazole ring, here, there are two strong hydrogen bonds in the human. Whereas in the rat, the tetrazole is not hydrogen bonded. It does not create the forces necessary to activate the VDR receptor.





10:15

A Rat or Mouse has Different Immune Defenses

In fact, a rat or mouse has different immune defenses as well. This a press release that just came out the 22nd of May 2011: "Ulcer bacteria may contribute to development of Parkinson's..." where they were able to induce Parkinson's in a mouse strain of middle age mice with a delay of about 3-5 months.

But I want to caution that the immune system in a mouse is totally different, probably needed a totally different species [pathogen mix] [than] what is needed [to induce Parkinson's] in *Homo sapiens.*

10:54

Immunpathology is a big Problem

Well, there are always problems, and there is a huge problem when you start to stimulate the immune system and that is Immunopathology. All of our bodies are carrying around very large loads of microbes. Most of those microbes are not pathogenic, they do not cause diagnosable disease.

The microbiome project you may have seen on the first slide has now estimated that about 90% of the cells in the human body are bacterial in nature. Now, admittedly, bacterial cells are very, very small so the volumetric analysis would not be 90%, but by numerical analysis, about 90%.

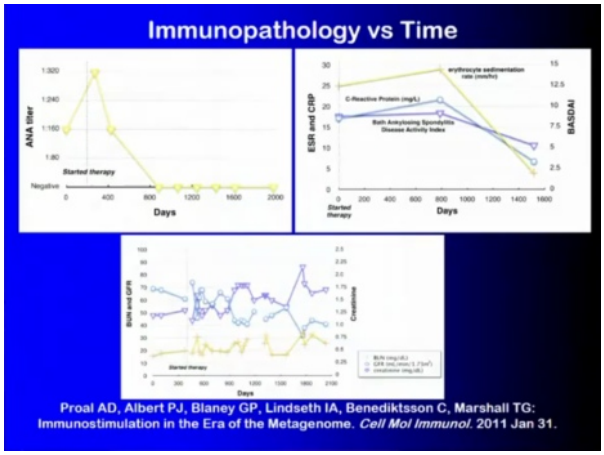
When the immune system is enabled to start killing the pathogens inside cells, then many of those cells will die by autophagy and apoptosis and that necessarily increases the inflammation.

So the inflammation, the level of inflammation, be it subclinical or clinical, that the individual was experiencing necessarily increases when you start killing off the microbes.

If too many cells die at once, then the patient can become very, very ill, so immunostimulative therapy has to balance the rate of healing against the amount of immunopathology that is going to be endured by the patient.

There are many groups that have tied inflammation to neurodegenerative diseases. Fred Gage's group has worked down at the Salk Institute. [It] is the one that I have been following the most.

But the thing that we all fail to realize, at least as far as I know, we all fail to realize that once you start dealing with the source of the inflammation, rather than suppressing the inflammation—actually trying to allow the body to deal with the microbes at the heart of the inflammation—then you get immunopathology and you get a surge in disease symptoms.



13:25

Immunopathology vs Time

Here are some of the graphs from our most recent paper which is "Immunostimulation in the Era of the Metagenome," *Cellular and Molecular Immunology*, just January 2011.

The first one is a RA patient. We have the ANA titre vs time, 2000 days on therapy. Initially, the ANA titre goes to 160, it doubled after about a year, and then gradually fell away.

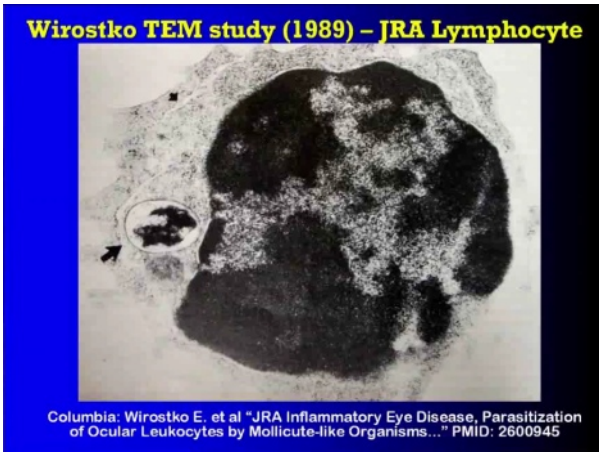
The initial response is an increase in autophagy, an increase in apoptosis, increase in cell death, increase in disease symptoms.

Similarly over here for an Ankylosing Spondylitis patient, the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)—actually that remained fairly stable and then it started falling away—but the sedimentation rate rose and the C-reactive protein rose, peaking at about the 800 day or two year level, roughly, into therapy.

And finally, we have a Sarcoidosis patient where we have plotted BUN (Blood Urea Nitrogen) and GFR Glomular filtration rate and creatinine against a number of days, up to about 2100, about six or seven years and you can see that they fluctuate.

What you probably can not see from the back is if you look at the GFR, the GFR started at 70, which is not really good, and it started at about 70 and by about three years it had dropped down to 40 and it has maintained stably at about 40 for the subsequent three or four years of therapy.

When you have a patient with a GFR of 40, you would normally be pressing the panic buttons, but **when you are changing the paradigm of the therapy that you are using, the panic buttons change. It is a very difficult problem that we have to solve.**



15:45

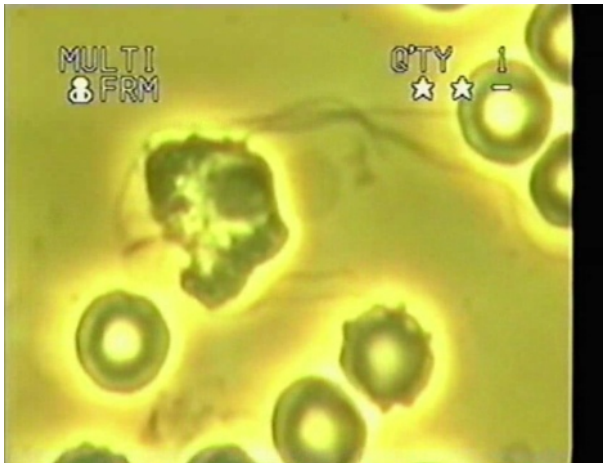
Wirostko TEM study (1989)—JRA Lymphocyte

What do these pathogens look like?

These [images] are from a study by the Wirostko group. It is a transmission electron microscopy [TEM] study in the year of 1989. It was done at Columbia University in the US.

This is a lymphocyte from the eye of juvenile rheumatoid arthritis patient, but they did a number of other patients as well. You can see that there is a nuclear staining for bacterial DNA which clearly has not been properly phagocytized and there are also longer artifacts as well, fine film-like artifacts that stain up quite nicely.

You can see them better if you look up the original article by the Wirostkos.



16:16
Video

Under light microscopy, what you see is the occasional cell—about one in 100 monocytes, typically, in a very ill patient—are infected. And this one the cytoplasm is heavily infected.

[It is] a monocyte and it is already starting to throw out these long polymers to try and spread the infection to other cells, that are still relatively potent.

You can see it with optical microscopy. It is very difficult, we are right at the limits of microscopy, and you really can not see as much detail as we would like to see to understand exactly how these pathogens survive.

16:59
Salivary microbiome Source: Nasidze et al., 2008 Genome Research, Max Planck Institute.

But the DNA analysis gives us a lot more information.

Here is the Salivary microbiome which was produced by the Max Planck Institute as part of the Human Microbiome Project. The size of the text is roughly proportional to the percentage of that particular species or phyla that they found. This is very small (pointing to Yersinia), but on the other hand, it is Yersinia.

Yersinia pestus, one of the subspecies, is responsible for the black death. Yersinia was found in the saliva of healthy human beings from twelve different places on the planet, along with Neisseria, Strep, and a number of other enterobacteria, another number of organisms that we would regard as being pathogenic.

Yet very few of these could actually be cultured. Some were cultured, but very few could be cultured because when the microbes move into the microbiome, there is a lot of sharing of genomes—actually genes, and especially plasmids, disappear—and you are dealing with a community of microbes rather than individual infestations of microbes. This community builds up gradually during [human] life.

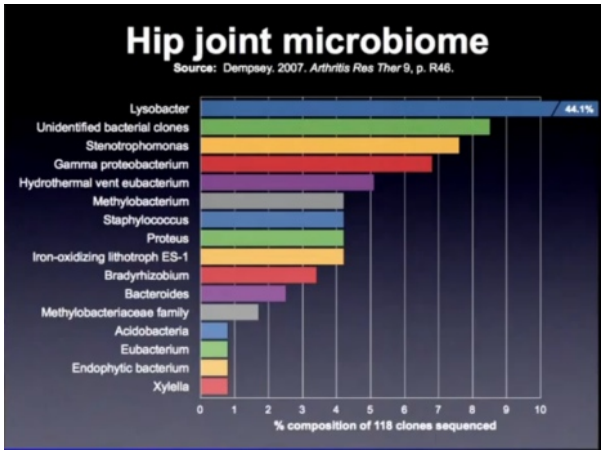
18:23
Select bacterial genera detected in commonly smoked cigarettes

So where do the microbes come from? Everywhere!

Here is the cigarette microbiome, which was published last year, I think, yes, 2010. It shows that when somebody smokes a cigarette, they take in a very large load of Pseudomonas, Acinetobacter, Klebsiella, Clostridium, Bacillus, Proteus, and Staph is down here at the bottom at about the 5% level. There are other species of course as well, that could not be identified or smaller than the 5% level.

Think about it. The other thing that impressed me was that the initial data from the COPD lung found that Pseudomonas is very

strong in the COPD lung. The microbiome work is opening an entire new insight into how the human body functions and in particular, how it dysfunctions.



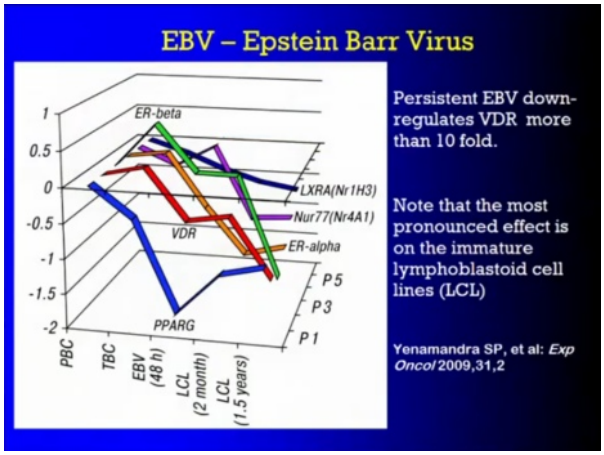
19:25

Hip joint microbiome

Some of you will say "It is contamination," so let us go to an internal sample rather than external sample—saliva and skin and things.

These were the species that were shaken off a hip joint that was replaced with revision arthroplasty. A number of species [were found]: Lysobacter, it was a biofilm that had collected on the collected on the old joint, so you would expect Lysobacter to be there.

But this one [highlighting Hydrothermal vent eubacterium], I want to point out. [It is] a bacteria that has been previously identified as living in hydrothermal vents at the bottom of the ocean. Here was their DNA turning up inside a human being. That is not the sort of bacterium that you typically would find floating around surgery as a contaminant.



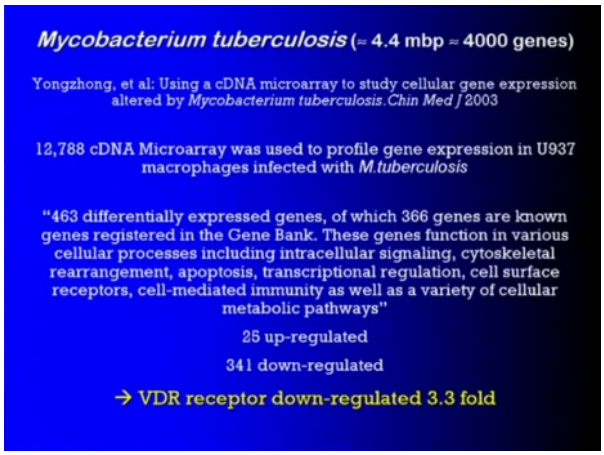
20:14

EBV-Epstein Barr Virus

Some of the bacteria that we already know downregulate the VDR as their survival mechanism: Epstein Barr Virus, which of course is associated with just about every inflammatory disease, you will find something in PubMed pointing to EBV.

But if you look here at the VDR, you will see that it is very heavily downregulated in the lymphoblastoid cell lines. After one and a half years, expression of the VDR has been attenuated down to extremely low levels. Even after two months, it is very well attenuated as well.

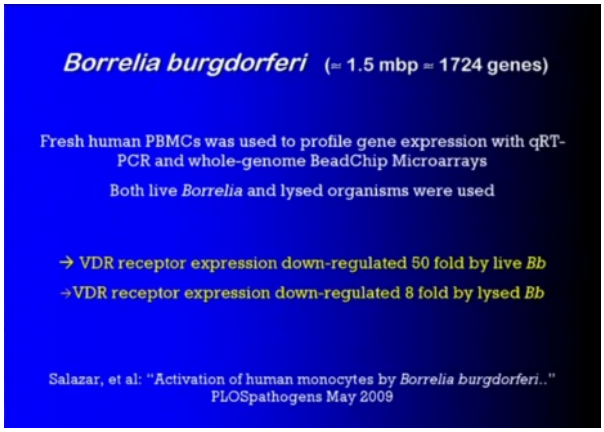
Whereas, in peripheral blood cells, there is not that much activity by the EBV. That is not surprising. EBV is certainly a very persistent pathogen.



21:06

Mycobacterium tuberculosis

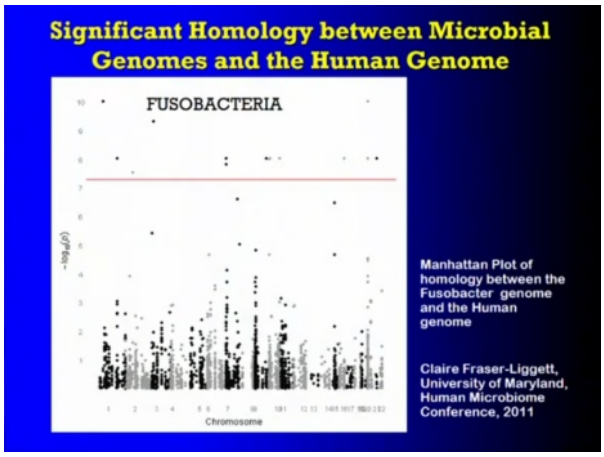
Also, *Mycobacterium tuberculosis;* some very good work done here in China, in 2003, which showed that the VDR receptor was downregulated when a cell was infected with *Mycobacterium tuberculosis*.



21:26

Borrelia burgdorferi

And similarly, *Borrelia burgdorferi* (Bb), very heavily, live Bb fifty-fold downregulation of VDR—a very strong one indeed.



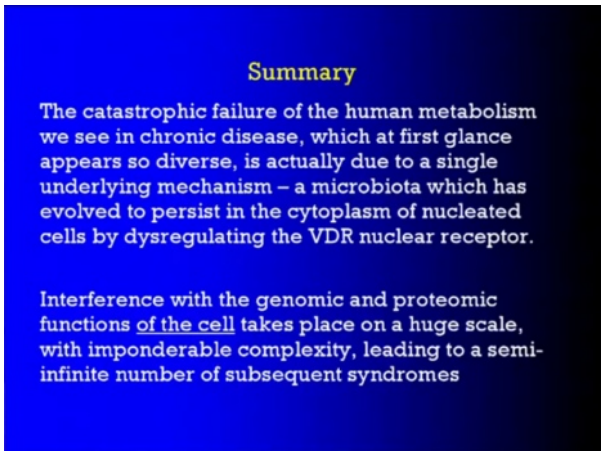
21:35

Significant Homology between Microbial Genomes and the Human Genome

If you look at species you do not really regard as pathogens, this is a Manhattan Plot of the genomes of Fusobacter and the twenty-two human chromosomes, and what it shows is the degree of homology, the degree of similarity between the genome of the bacterium and the human genome.

You can see some of the similarity points are really, really high. There is very likely to be interference between the proteome and the genomes of these pathogens when they become part of the microbiome.

Fusobacter is a very common constituent of the microbiome.



22:21

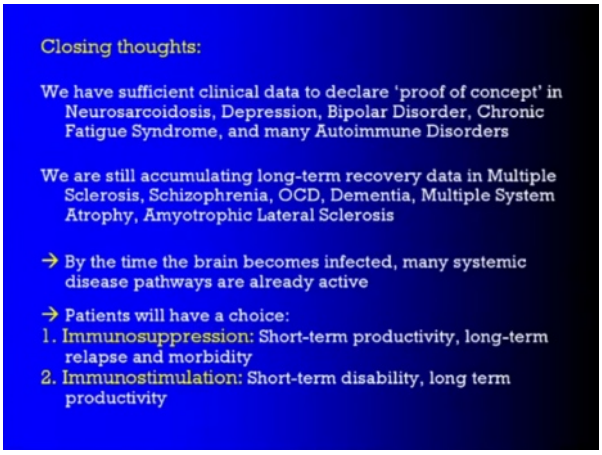
Summary

So, what we have to say is that the catastrophic failure of the human metabolism we see in chronic disease, which at first glance appears so diverse—hundreds of separate diagnoses for typical chronic inflammatory disease—is actually due to a single underlying mechanism, which is a microbiota which has evolved to persist in the cytoplasm of nucleated cells by dysregulating the VDR nuclear receptor.

Interference of both genomic and proteomic functions of the cell takes place on a huge scale, with imponderable complexity, leading to a semi-infinite number of subsequent disease syndromes.

In other words, if you have got fifty or six hundred that was in the slide of the microbiome, but let us just take fifty genomes that are in the cell and persisting in the cell, each of them holding around between five hundred and five thousand genes, and then each of those could interact with the twenty five thousand human genes, the SIRNA's all of the other internal cell operations (and remember, everything happens at the level of the cell, it does not have to happen in the tissue).

All the proteins, all that the human body needs to operate come from the cell—we have to focus our thinking down from the level of the tissue to the level of the cell.



24:00

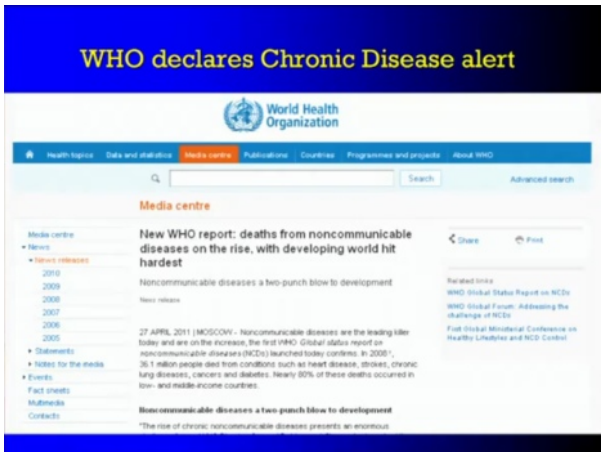
Closing thoughts:

So in closing thoughts, you would have noticed that there was relatively little data. We had good data on Rheumatoid arthritis and Thyroiditis back there, we also have good data on Neurosarcoidosis, Depression—particularly Manic depression/Bipolar disorder, Chronic Fatigue Syndrome and many autoimmune disorders—to be quite happy with the proof of concept.

But we are still accumulating from long-term recovery data in Multiple Sclerosis, Schizophrenia, OCD, Dementia, Multiple System Atrophy, Amyotrophic Lateral Sclerosis, but all in very low numbers at this point.

The other thought I want to leave you with which is the thought that the previous speaker had, which is, that **by the time the brain becomes infected, many systemic pathways are also already active, and vice versa, by the time systemic disease becomes manifest, the brain is very often affected, with a few exceptions**, like ALS, which I am obviously intrigued with.

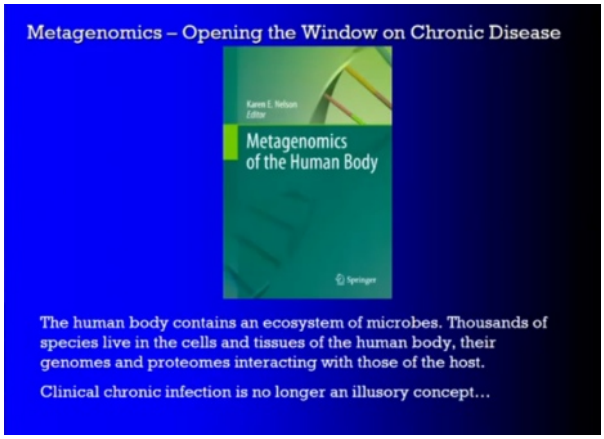
As we move to the future, **there really has to be a choice made, by the physician or by the patient, for immunosuppression to give short term productivity/long term relapse and immobility—and immunostimulation to give short term inability and the long term productivity.**



25:29

WHO declares Chronic Disease alert

The World Health Organization has just declared a chronic disease alert saying that deaths from non-communicable disease is on the rise with the developing world hit hardest. I have got news for them, "non-communicable" is arguable at this point in time.



25:47

Metagenomics—Opening the Window on Chronic Disease

If you want a book to read what is happening, the textbook has just been written on "Metagenomics of the Human Body," which is edited by Karen Nelson PhD who heads up the Maryland campus of the J Craig Venter organization. We wrote the chapter in there on autoimmunity. It is a *Springer* textbook and it is one that I certainly recommend you pull from the library and read.

Thank you very much.

Questions

Q1: Well, thanks. There is something on my mind that I would like to start. What do we look for? Like you say, these bugs, we are looking within the cells so there is not so much external. Normal septic person apparently as I understand it. So what, how can we test for this purpose?

TM: It is even more complex than that. That optical microscopy that I showed you, what we do there is we put blood between two cover slips and then allow the blood to age. Between about six and thirty-six hours the cells that are infected start to explode like that. So you look at them after thirty-six hours and there is just debris, there are no signs of cells. So **in typical sampling operations that one does in a clinical basis, you do not see this**.

Q1: Yes, you have access to a regular microscope slide so you can look at brain tissue? When you are looking at a Parkinson's brain, you can not see anything.

TM: But you can sequence it and you can send it to the Argonne National Lab metagenomics analysis. You just upload your raw data and it compares the RNA that it finds. Not just against the human genomes but against the six-thousand odd complete genomes that are available out there for microbes.

Q1: What I am trying to understand is if these little bugs are actually causing diseases, like you know, disease processes, there should be something you could see if you looked carefully at individual brain cells, for example, some sign of them?

TM: Yes, yes, yes. There was one I—there was an image I got from Karolinska when I was there, a Parkinson's brain. You see the cell, and under black light illumination, you see usually bright areas—the proteins that protect the colonies.

If you remember there was that vacuole type colony? They tend to come up under back light illumination. You can just see the blobs there if you use the highest magnification of your optical microscope. It is about one in one hundred cells that are infected, so you have to look a fair bit, it is only the nucleated cells, you got a red check full of the red cells, but then you can see them, yes.

Q2: I have another question. The patients you show, you followed through years?

TM: We have followed right through from 2002 right through to today, most of them. We have lost some to follow up, but we have followed every one that we can.

Q2: Your efficacious times use from one to two years, then you have follow on?

TM: Response time, well, **there is the initial response of immunopathology which is instant, you know, a month or less, and then there is the initial turning the corner/light at the end of the tunnel which is one and a half to two and a half years, typically. I think we said like thirty-six months, 81% [recovered] is up to that point. Then there is a recovery period. Depending on how ill they are, they become functional again, they get back to work get back to the community at some point beyond that.**

Q2: Right. So if you designed the study for the FDA to get approval for this drug, for your indications called chronic degenerative indications that you have mentioned, how will you design the study and how long will it be?

TM: Well, we are discussing that with the FDA at the moment. **The problem is that FDA doesn't like any studies going out longer than about 18 months.**

Q2: They have a lot of pull.

TM: Right, but in terms of the Phase 2 studies for the NDA, they like them to be closed-ended. We would really not be getting results until a longer term. We are trying to persuade them at the moment that immunopathology is an endpoint, which in and of itself is important. In time, we may be able to do that, I think. How quickly? I have no idea.

Incidentally, the drug we are putting through the FDA is a purified form of the drug that is already on the market, so the drug is already available on the market.

Q3: I am sorry, I am sorry I am encroaching but I will be quick. Very strong question: olmesartan that you have VDR on the gene, how does it actually work in this autoimmunity? (is one [question]) And this VDR gene has been found in Multiple Sclerosis in ..... ? Have you not tried to identify [phosphorylation?] in disease?

TM: I would want to talk with you about that later and have a look at them in neural disease, because I frankly have not looked at it.

Q3: How quickly are old symptoms brought back by olmesartan?

TM: Very quickly. The body changes. The hormonal balance changes very quickly within two to three weeks.

If people are on thyroid medication, that has to be continuously modified, because usually within 18 months they are off thyroid medications. **The whole balance changes very quickly with the immunopathology setting in and with the therapy.**