The Microbiome, which feeds a myriad of Autoimmune Diseases

PRESENTED BY PROFESSOR TREVOR G MARSHALL
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Transcript of http://youtu.be/6My9p6munj8 video.

Chairman (Noel R Rose, USA and Cees G M Kallenberg, NL): Thank you very much. This is the last presentation this morning. We would like to invite Dr Marshall to discuss 'The Microbiome which feeds a myriad of autoimmune diseases.'

Transcript
00:00:22

Dr Marshall: Thank you Mr Chairman.

And now for something completely different.

So a very provocative title talking about "The Microbiome," the microbes which inhabit, or cohabit, our human body, "which feeds a Myriad of Autoimmune Diseases."

"Which feeds?" Causality is implied there. Can I support that? And "a myriad of Autoimmune Diseases." What are we talking about there?

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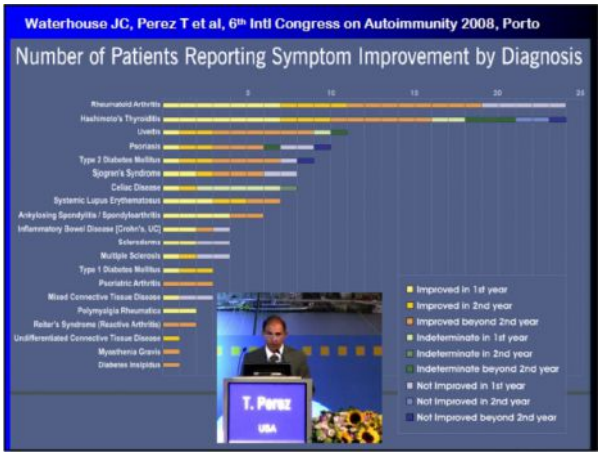
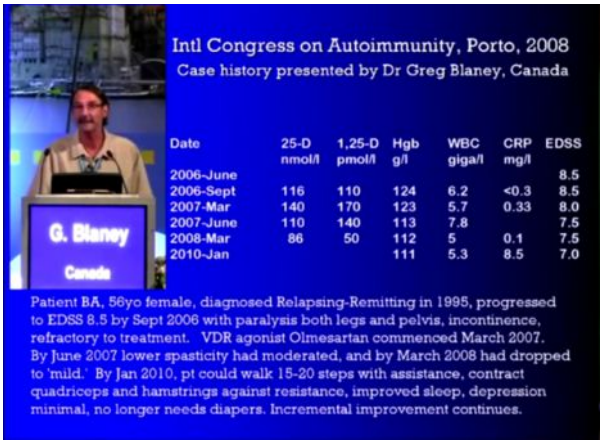
Number of Patients Reporting Symptom Improvement by Diagnosis



Well, let us look back at a little bit of history first, at each of the last several Autoimmunity Congresses my colleages, my clinical collaborators, have given clinical results showing how sections of our cohort—case series and case studies—have been responding to the therapy that we developed in order to counter the effects of the microbiome.

This is from Porto. Captain Tom Perez, just retired after 25 years at the FDA, presented a small retrospective of our cohort—in this case, about 103 patients if I remember correctly—with conditions ranging from Rheumatoid Arthritis through to Myasthenia Gravis and Diabetes Insipidus to Psoriatic Arthritis.

You can see there are more and more patients as you go up through Rheumatoid Arthritis, Hashimoto's, Uveitis, through Psoraisis—the more common conditions—with a detailed status of how the patients were getting along. Most of them were resolving quite nicely.



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Intl Congress on Autoimmunity, Porto, 2008

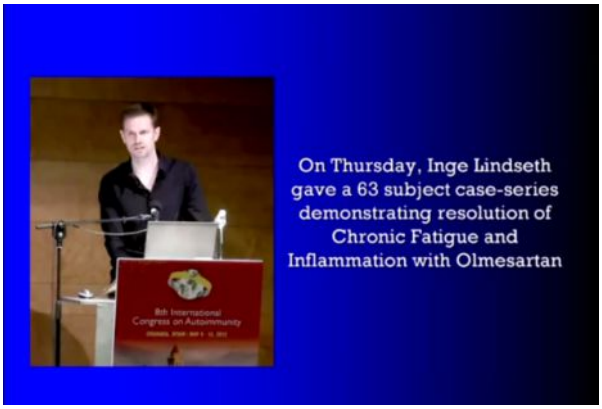
Also in Porto, Dr Greg Blaney from Canada reviewed a couple of particularly difficult cases that he had been working with. This one was an MS patient who started with an EDSS of 8.5, was wheelchair bound, and incontinent, and through to 2008 improved the EDSS, was no longer incontinent and no longer needs diapers, in fact. To this day that patient is continuing to do well.

00:02:41
And at Ljubljana in 2010

And then again in Ljubljana, 2010, Dr Greg Blaney gave another couple of cases, another couple of case histories.

00:02:50
Roswitha Goetze-Pelka, at ACA 2011 (Singapore)

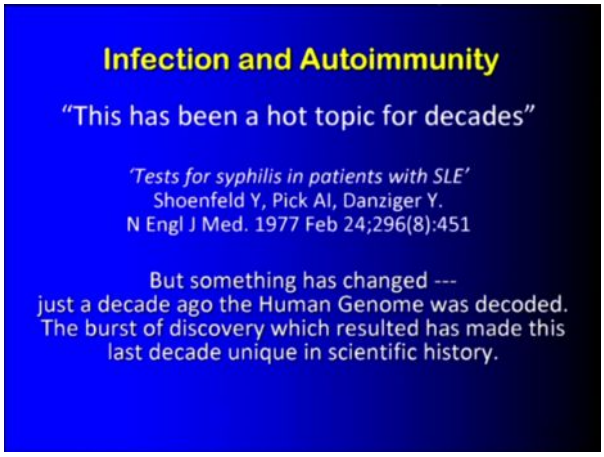
In Singapore, at the Asian Congress last September, Dr Goetze-Pelka, a Psychiatrist from Germany, was talking specifically about how psychiatric manifestations—particularly, Depression, Manic Depression, and to some extent, Schizophrenia—were responding at the same time that the inflammation, the chronic inflammation, was resolving.



00:03:19
On Thursday, Inge Lindseth

And then just a few days ago, Inge Lindseth from Norway—a collaborator from Norway—gave a case series of 63 subjects, both Canadian and European, who were demonstrating resolution of Chronic Fatigue. Chronic Fatigue of course being one of the most difficult diagnoses that we have found to deal with.

And so what is this all about?



00:03:56
Infection and Autoimmunity

Well, let us go back in time even further.

The discussion on infection in Autoimmunity came up on the Autoimmunity Network, Yehuda's Autoimmunity Network.

And those of you who are not members, please join and engage in the discussions there, online.

One of the comments that was made was, "This has been a hot topic for decades."

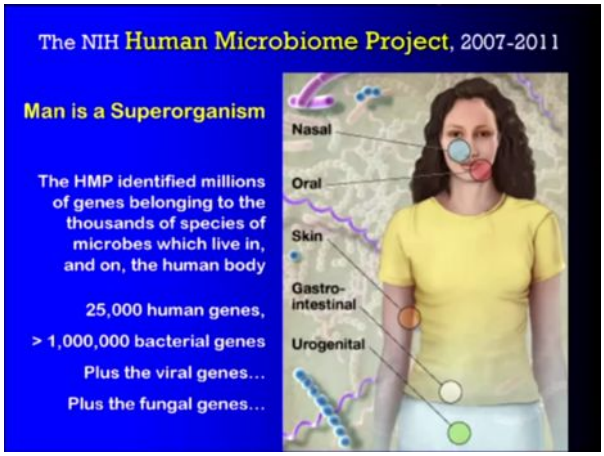
Yes, indeed.

And in fact, if we go back three and a half decades, we will find that the father of this family of autoimmunity, Yehuda himself, was already looking at the links between infection and autoimmune disease.

And I think a couple of years after that, Yehuda had identified Mtb as being one of the potentially key pathogens. And at that time, I was just fooling around with insulin infusers and infertility. But nevermind.

But you know, something has changed in the last decade. Something very important happened a decade ago, and that was we got a complete, working transcript of the Human Genome.

Now I have to admit that nothing much has come out from that yet, in terms of direct Genetics. But another field that sprang from the field of Genetics, **the field of Metagenomics,** is turning medicine on its head—turning medical knowledge on its head—it will take several decades to turn 'medicine' on its head, so think 'medical knowledge' on its head.



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The NIH Himan Microbiome Project, 2007-2011

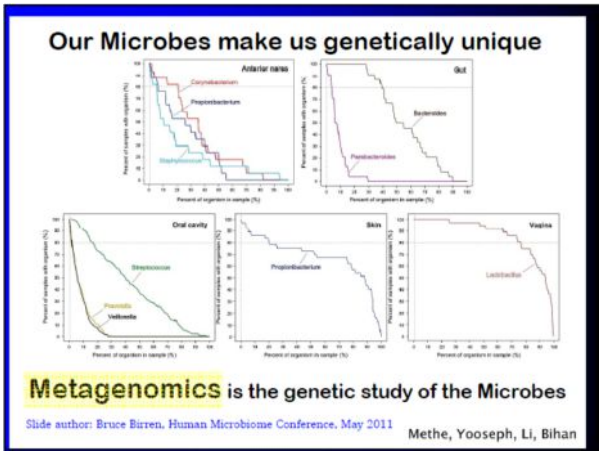
The first discovery is the Microbiome, the Human Microbiome.

Now NIH initiated the project in 2007, and it wound up late last year or earlier this year, called the Human Microbiome Project (HMP). And this was a project which was to define exactly just what microbes were associated with *Homo sapiens*, and to try and figure out what they were doing in and on *Homo sapiens*.

And what was concluded by the project was "man is a super-organism." We all carry microbes not only on external surfaces, but also in our bloodstream, in our tissues, in our cells.

And millions of genes belonging to thousands of species of microbes interact with the 25,000 genes of the human body. And that interaction gives the potential for dysfunction. The microbes

can cause the human body to dysfunction, dysfunction in extremely complex ways, because you are dealing with 25,000 human genes being worked on by millions, potentially millions, of the microbiome genes. And then, of course, you also have also got the viral genes and then the fungal genes (because there are also persistent fungi).

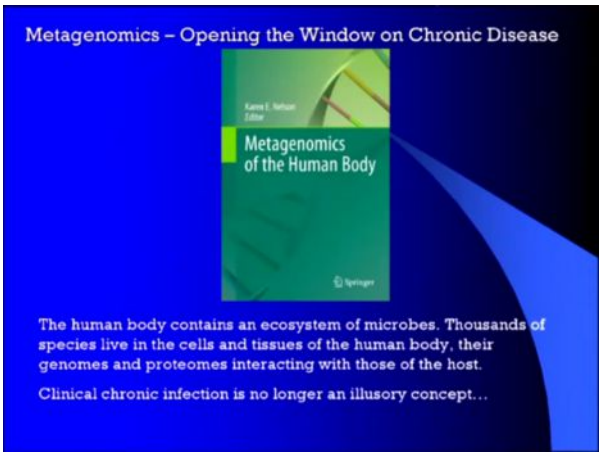


00:07:00
Our Microbes make us genetically unique.

And one of the conclusions that came from the Microbiome Project is that it is our microbes that make us genetically unique.

Every one of us has a different microbiome to the person sitting next door. Every one of us has a unique microbiome. It varies between people and it varies from month to month and in some people, from week to week. It varies when we travel to a different region of the world. **The microbiome changes.**

Metagenomics is the study of the genetics of the microbes and how those microbes interact with the human body.



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Metagenomics—Opening the Window on Chronic Disease

This book was written, it was edited, by Karen Nelson, who was really the instigator behind the HMP. We wrote the chapter on Autoimmunity. It goes into a lot more detail, I think about 35-40 pages, than I am going to go into in this talk.

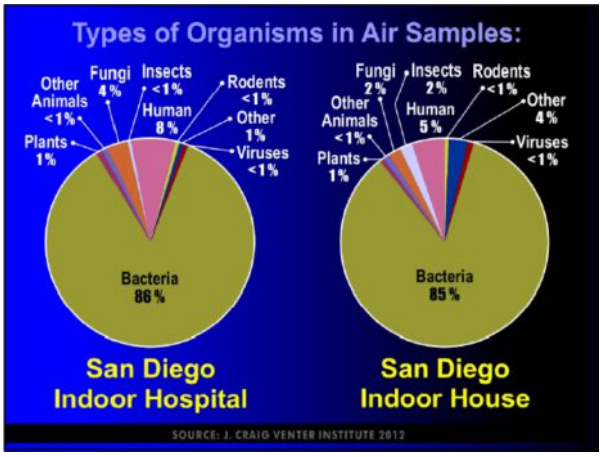
But if you want to find out more about exactly what the microbes do, and how the microbes make us genetically unique, then that is the source for you to go to.

00:08:17
New Scientist Health

But here is a quick summary.

Firstly, babies are born "dirty." They already have a gut full of microbes. But in addition to a gut full of microbes, they also have a body full of microbes. They bring many of the microbes of the mother, some of the microbes of the father, and within a few weeks after birth they have accumulated most of the microbes of the extended family (I guess grandmother has to kiss the baby).

This is the key start to our life. We start our lives with the maternal heritage, with the maternal line. We already see—those of you that have been studying autoimmunity clustering in families—you can already start to see how this occurs. So we start with a microbiome.



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Types of Organisms in Air Samples:

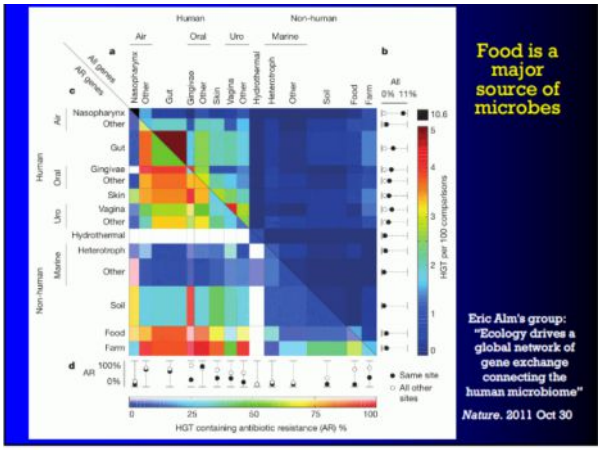
Then, we cannot avoid it from that point on.

From that point on, even the air is full of bacteria.

This is the air at a San Diego Indoor Hospital. This is from the J Craig Venter Institute, work done by Craig Ventor—the first person to get a full Human Genome transcript and one of the leaders of the Metagenomics initiative.

And you can see in a hospital, OK, bacteria are 86% of DNA in the air. That is not really surprising. But look! you go into a house and it is almost identical.

The human DNA in a house is a little bit less dense than it is in the hospital, but we get exposed to microbes, microbial DNA and also fungi and insects also in the air. You can not get away from them there.



00:10:16
Food is a major source of microbes

We get it in our food!

This is a wonderful study that was done by Eric Alm's group at MIT.

What they did was look at the microbes in the human body (which is up in this region of the chart [upper left corner highlight]) and plotted a heat map for the correlation between those microbes in the human body and microbes from other sources.

For example, farm food sources here [highlight lower left corner of heat map], and wheat and other agrarian food sources here and soil and phenotrope, hydrothermal, etc.

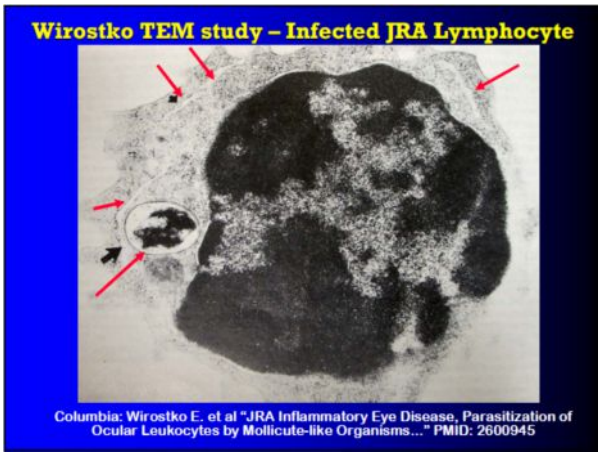
But the thing that is really interesting is this particular section of the heat map [full lower left corner highlighted] is a plot of all the genes which are thought to confer antibiotic resistance.

In other words, there are certain genes in existence which we, or science, have identified as being associated with conferring **antibiotic resistance** when they are transfered from one host to another.

You can see that those genes that cluster in man also cluster very heavily in the farm and in the food categories. In other words, we get our bugs—the genes that we least want—from our food.

00:11:38
Travel, and International transport of food

That is of course exaserbated by today's modern international transport of food and of course, modern forms of international transport of human beings, including ourselves.



00:11:50
Wirostko TEM study—Infected JRA Lymphocyte

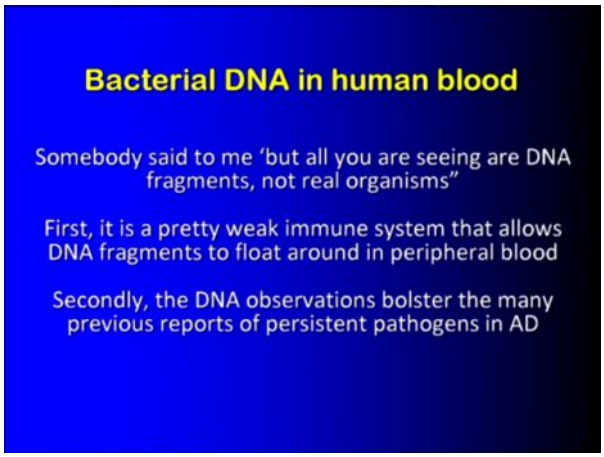
So where do these microbes cause the most trouble?

They cause trouble when their genes can interfere with the transcription of the human genome. When they get inside the nucleated cells of the human body, that is when they can cause trouble.

This is a slide from two and a half decades ago from the Wirostko group at Columbia University, who did a study of several diseases: Crohne's, Juvenile RA and Sarcoidosis—looking at lymphocytes, macrophages and monocytes, specifically—for infections-nucleated staining within the cytoplasm.

In this JRA lymphocyte you can see there is clearly a vacuole here which has not undergone phagocytosis [small black arrow]. And quite a reasonable clump of DNA there [highlight upper left slide at parallel red arrows]. There is also DNA linking these longer, tube-like (well, they could also be plate-like because we do not have 3D) objects as well.

When the microbes can persist in the macrophages and the monocytes of the immune system then, as far as *Homo sapiens* is concerned, it is 'game over'.



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Bacterial DNA in Human Blood

And indeed, that is what seems to be what is happening in chronic disease.

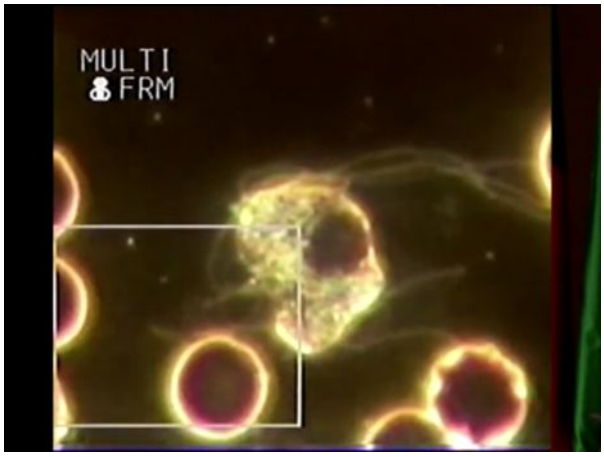
Somebody said to me the other day, "All you are seeing in blood..." because anybody that has the capability of measuring the microbes with the metagenomics tools can look at the human blood and they will find many, many microbial DNA samples from that blood. Human blood is not sterile, human tissue is not sterile.

But of course, most of the microbes cannot be cultivated which is why we have not seen them before.

Somebody said to me, "But all you are seeing in blood are DNA fragments, not real organisms, not live organisms."

Well, that is a fascinating critisism, because firstly, you have to have a pretty weak immune system if that were to allow DNA fragments of microbes to be floating around in peripheral blood.

And secondly, these DNA observations really bolster the observation of, for example, of Wirostko's on the previous slide and many others that have gone before.

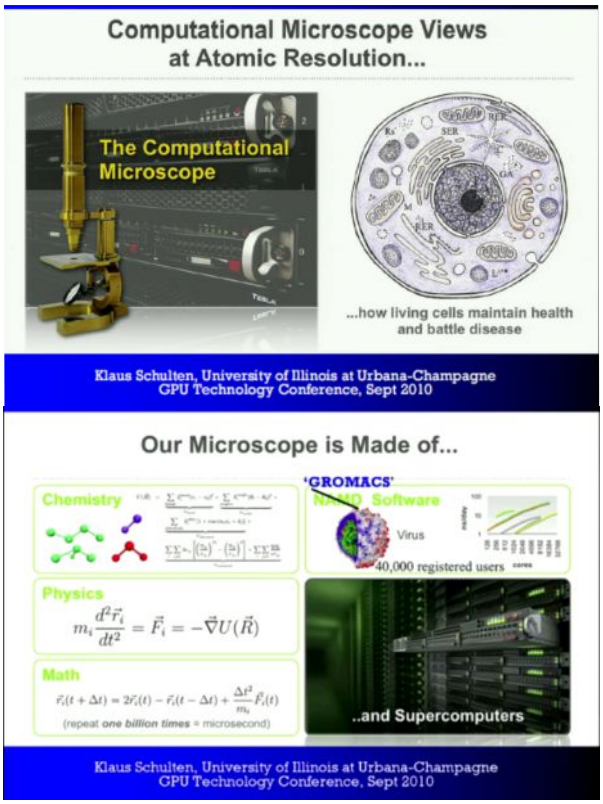


00:14:27
[Video: microbes in blood]

And indeed, you yourself can observe the... Ah! It is moving now.

You, yourself, can observe these infected cells in whole blood if you use the correct processing and make sure you are looking at very fresh blood. Because these cells—monocytes, lymphocytes—very, very quickly disintegrate following removal from the blood stream. And that is another reason you do not see them very much in vitro, all you see is fragments left.

The microbes get out of the... (let us see if I can go back), the microbes get out of the infected cytoplasm very quickly with these long filopodia—characteristic of microbial migration in blood.



00:15:11
Computational Microscope views at Atomic Resolution...

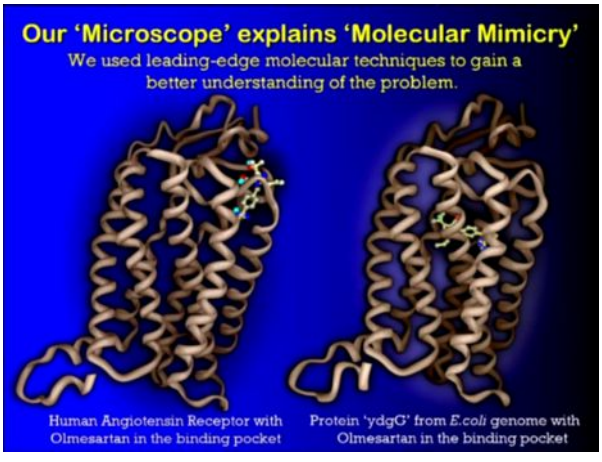
So, it was pretty obvious to me back a decade or so ago, that we needed a different type of microscope. And at that stage, groups at the University of Illinois, also in Europe, were looking at the possibility of doing 'Computational Microscopes'.

In other words, microscopes that take a look at examining 3D structures at the level of molecules.

00:15:40
Our Microscope is Made of...

And in fact, these microscopes are made of chemistry, physics, math, and software. Oh, and of course, supercomputers.

Basically, what the software does is emulate every force, every location of every atom in a molecule, thousands at a time, in order to figure out how the molecules interact and how the molecules stay in homeostasis.

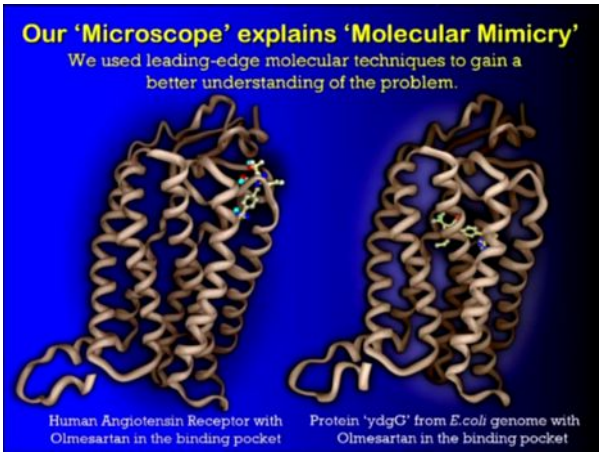


00:16:12
Our 'Microscope' explains 'Molecular Mimicry'

That tool allowed us to look at molecular mimicry.

And what we found with molecular mimicry was something quite different from the concepts that had sprung up by observing it in connection with antibodies.

Firstly, what we have on the screen are two proteins. They are transmembrane proteins (GPCRs). This one [left] is a human Angiotensin II type 1 Receptor, it has a drug in the binding pocket. [Right] is 'ydgG' from *E.coli.* It is one of the many, many proteins in *E.coli* and it happens to be essentially identical with the human Angiotensin II Receptor.

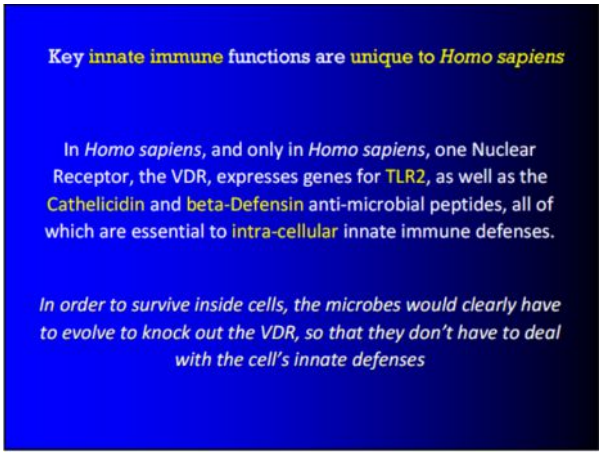


It would quite easily embed itself in the cell wall. Heaven knows what it would do. And Heaven knows what the cell's mechanisms, the Golgi mechanisms etc., would do when faced with this type of protein in the cytoplasm of the cell.

But you can see, it is identical. The shape is identical. I have indicated helixes here, rather than each individual atom because there are thousands of atoms on each of those receptors, but you see it is identical.

There are slight differences. You can see the drug binds in a different spot... But wait a minute! The drug binds into a bacterial protein? **Drugs affect the microbes? Wow! That is an interesting observation in and of itself.**

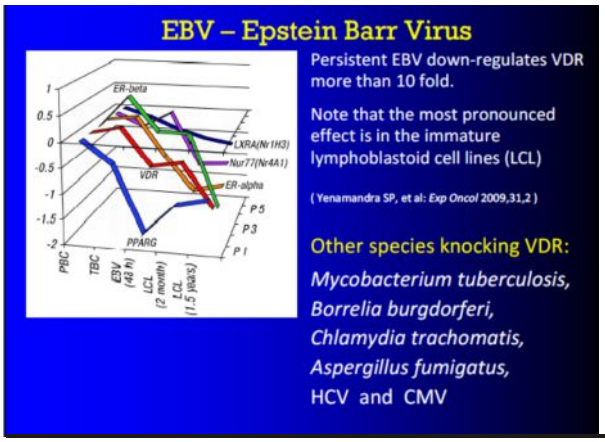
So working on the basis of a better understanding of what microbes are and what they do—I mean, many of these proteins are needed for the microbe to work. *E.coli*, for example, produces energy from glucose, and it produces energy from glucose with almost exactly the same intermediate products *Homo sapiens* does. So when you look at the genes associated with that energy conversion, you find that almost identical to *Homo sapiens.* You put those into a mix in the cytoplasm of the cell, you are going to get dysfunction.



00:18:23
Key innate immune functions are unique to *Homo sapiens*

And another key thing we found by using a computer microscope was the key innate immune functions are unique to *Homo sapiens*. There is one weakness in *Homo sapiens* innate immunity which does not exist in any of the other mammals, including the higher primates. And that is that one Receptor is responsible for Cathelicidin—LL37—the key intracellular microbial defense, beta-Defensins, and TLR2—TLR2 is a key intra-cytoplasmic Toll-Like Receptor—and these are essential to the intracellular innate immune defenses.

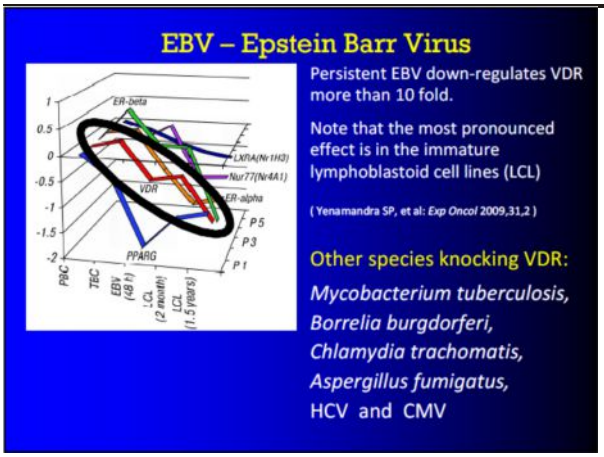
And in *Homo sapiens*, and in only *Homo sapiens*, there is one Nuclear Receptor that is key to expression of all those key innate immune fuctions and it is the VDR Nuclear Receptor. And in order to persist inside cells, microbes have to knock out the VDR, so they do not have to deal with the cell's defenses.



00:19:32
EBV — Epstein Barr Virus

And in fact, that is exactly what they do. Let us take a classic microbe for chronic disease, EBV.

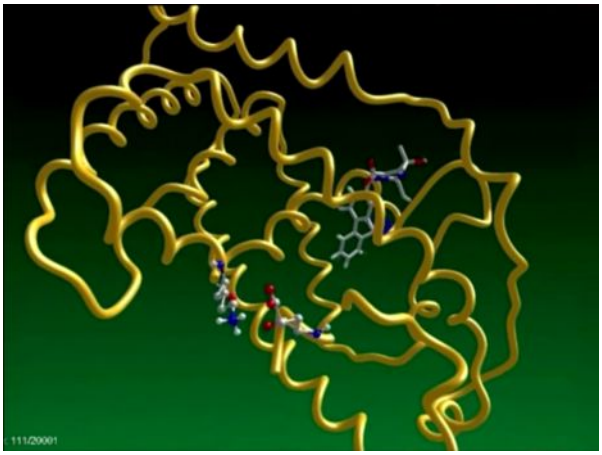
EBV knocks out VDR very, very effectively, by about 15 times the magnitude, looking at the lymphoblastoid cells lines after one and a half years, for example.



Peripheral blood cells, not a big deal, but by the time you get through to the lymphoblastoid cell lines after 2 months and one and a half years, EBV is really knocking down the expression of the human proteins, and particularly the VDR.

Other microbes that are already known to also knock out the VDR function: *Mycobacterium tuberculosis*, *Borellia burdorferi, Chlamydia trachomatis*, *Aspergillus, HCV* and *CMV.*

Reads like a Rogue's Gallery of the microbes that we see in autoimmune disease.



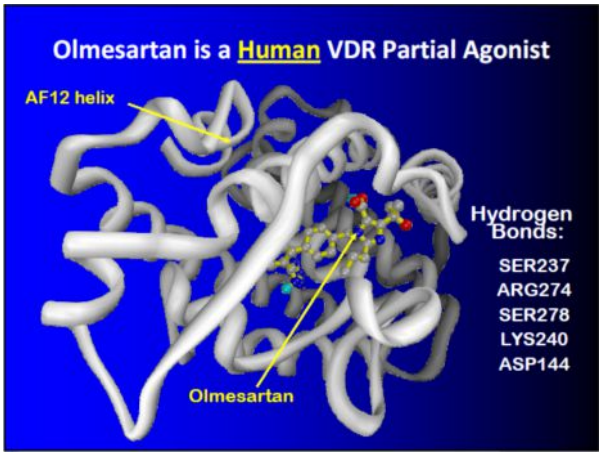
00:20:27
Computer Microscope Video: VDR

But you know, the microscope can do other things as well.

When I do highlight individual atoms within the VDR—this is the VDR—when I do highlight individual atoms you can actually see how the receptor activates.

Because these two entities [highlight lower center left] have to bind to the DRIP205, which is across here to form the heterodimer—well, the RXR heterodimer—and therefore, transcribe genes. And when we place the ligand in the binding pocket, it forces these atoms apart so they do not bind and so they can then take the co-activator. With no ligand, or with an antagonist in the binding pocket, it does not happen.

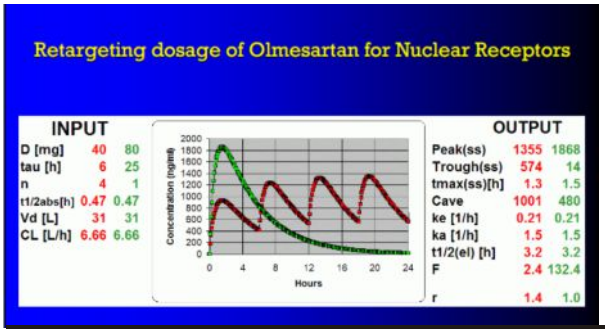
So we are able to study activation of receptors as well.



00:21:17
Olmesartan is a Human VDR Partial Agonist

And what we found was that there is a molecule which is effective—very high affinity for the binding pocket of the VDR—and which very effectively can overcome the effect of the microbes on the VDR.

That is a molecule called olmesartan, which is a Sartan, licensed for blood pressure/hypertensive indications.



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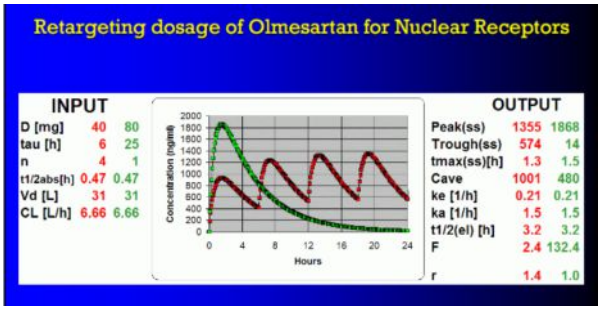
Retargeting dosage of Olmesartan for Nuclear Receptors

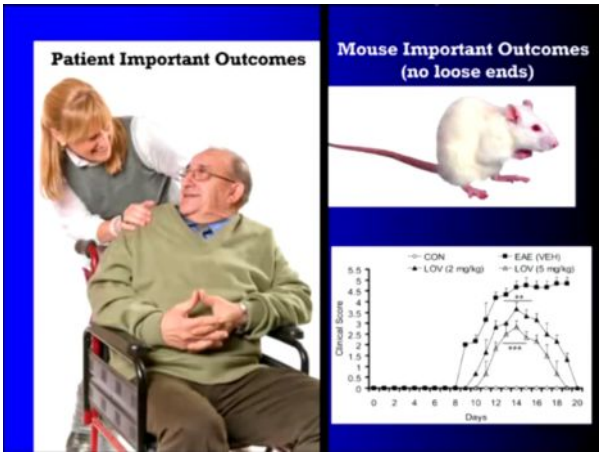
The main thing we have to do in retargeting it, is to make sure the concentration remains reasonably constant by giving a pulsatile dosing, rather than normal daily dosing of the olmesartan that you see in green [see chart].



By giving a more frequent dosing of olmesartan, we manage to keep a basal level in the blood stream.

We need to do that because the Nuclear Receptor lifespan is only hours—three to six hours for the VDR—and then they have all turned over. So you have got to keep the concentration in there. That is the secret of retargeting.

But look, that is really old technology. My (PhD) Thesis was full of that.

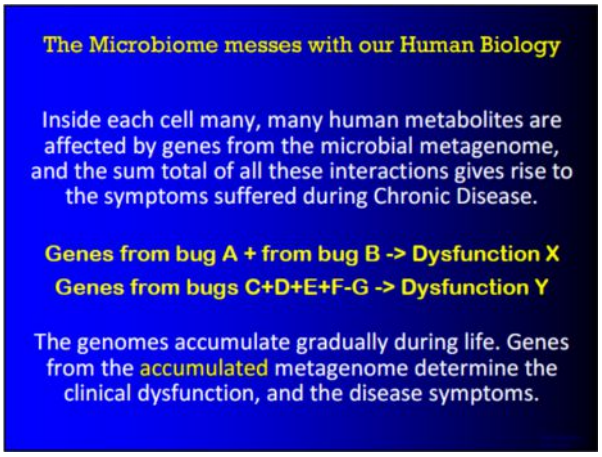


00:22:21
Patient Important Outcomes

Well, at this point, having evolved the fundamental understanding of what was going on between the microbes and mankind, we were able to start focusing on 'patient-important' outcomes. And we were able to skip the phase of 'mouse-important' outcomes.

So whereas, with mouse studies—I should not say 'no loose ends,' but you try to get no loose ends, certainly 'few loose ends'—in our case with patients, you have a lot of loose ends.

We have to ask whether those reports that I showed you at the start of the presentation are perhaps just placebo effect; are they perhaps, are they just outlyers—outlying responders—or are they indicative of something more definite, more important?



00:23:13
The Microbiome messes with our Human Biology

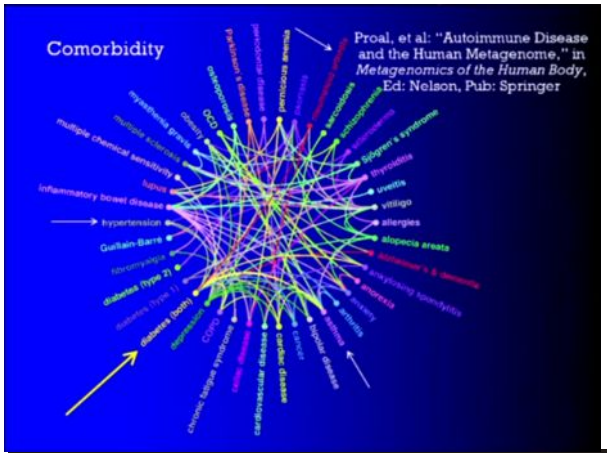
So, how does the Microbiome actually cause disease?

Well, it messes with our human body. Because inside each cell, many, many human metabolites are affected by the genes.

And you know, you might have some genes from bug A combining with the genes from bug B, which might lead to a body dysfunction X, which is just one syndrome that would add up to a disease diagnosis.

Or you might need genes from bugs C, D, E and F—with the absence of genes from another pathogen—because many microbes are pathogenic to each other. Strep and Staph are two examples.

The genomes accumulate gradually during life. And it is genes from the accumulated metagenome which determine the clinical dysfunction, and the disease symptoms.



00:24:04
Comorbidity

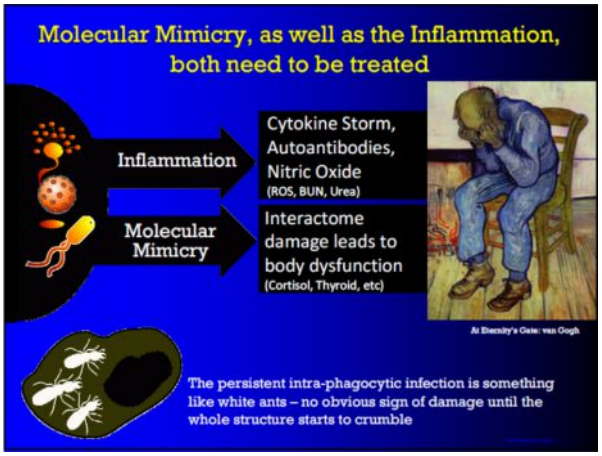
And that is why this comorbidity chart shows so much inter-relationship between the various autoimmune and other chronic diagnoses.

00:24:16
[Top 120 diagnoses in cohort]

If we plot a chart of the top 120 diagnoses in the cohort we are following, starting at CFS, Sarcoidosis, Fibromyalgia, Hypothyroidism, RA—we have many, many cases in that—going through to PTSD, epilepsy, sciatica, seizures, etc. That is a pretty big cross section. And in many cases, you will see that those are the types of diseases that you see [in your clinical practice].

[technical: momentary lost presentation]

Yes! Excellent.



00:25:25
Molecular Mimicry, as well as the Inflammation, both need to be treated

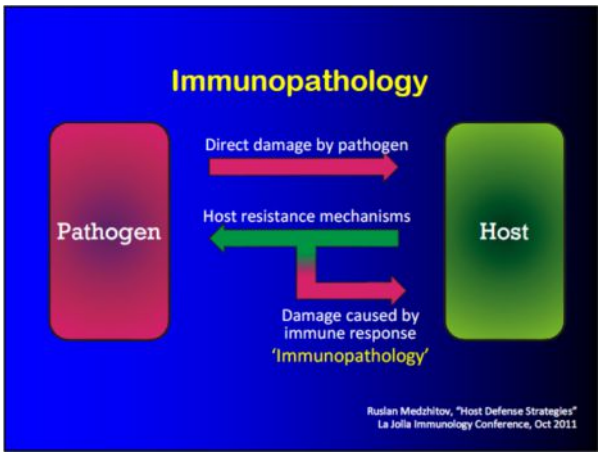
OK, so the key thing is that when you are dealing with microbes they have two effects.

The first effect is Inflammation—that is the body's response. But the second effect is Molecular Mimicry. And they create different symptoms in the patients.

From the Inflammation you are going to get the cytokine storm, antibodies, Nitric Oxide, Reactive Oxygen Species (ROS), etc.

From Molecular Mimicry, you are going to get the Interactome damage—the interactions—which leads to body dysfunction; Cortisol axis dysfunction, Thyroid axis dysfunction, etc.

In a way, you can look at these persistent anti-phagocytic infections as something like white ants. There is no obvious sign of damage while they accumulate—until the whole structure starts to crumble.



00:26:10
Immunopathology

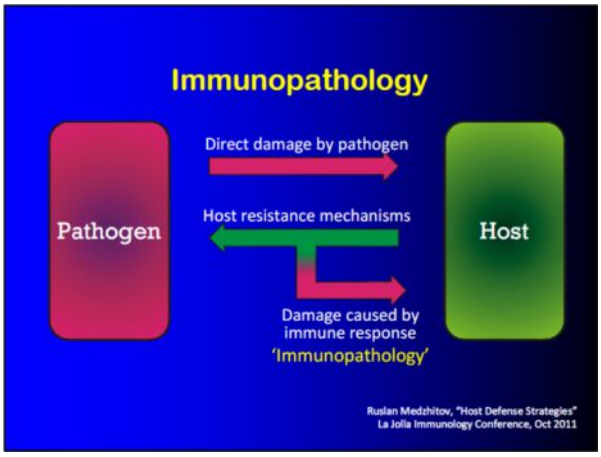
OK, there is a problem. The problem is Immunopathology.

(Chair: Summarize.)

(I am summarizing now, this is my last slide.)

There is a problem: Immunopathology. And what you have with immunopathology, you have direct damage by the pathogen on the host, then you have the host resistance mechanisms feeding inflammation back on the pathogen, but some of those mechanisms also cause damage to the host itself: Immunopathology.

That is a big problem because once we re-enable the innate immune system so that it can see these intracellular microbes and



starts to kill them, then the level of inflammation rises, the level of symptoms rise. And that is a problem when we are dealing with people that are right on the edge in terms of being able to manage from day to day in the first instance.

We recently published a paper "Immunostimmulation in the Era of the Metagenome" which specifically deals with Immunopathology for those of you that are interested.

So, thank you Mr Chairman.

[applause]

00:27:29
Question

Chairman: Dr Marshall, a new talk and a stimmulating lecture. Time for one question. Yes, [pointing] please.

Q: So, just very quickly, with the VDR you suggest olmesartan as a partial agonist. Vitamin D is a very good agonist. Why use olmesartan?

A: Because vitamin D has to be used in such a high concentration that it wipes out other receptors as well. We published a paper on that a couple of years ago, in one of Yehuda's [publications].

So it [vitamin D] knocks out Thyroid, it knocks out Cortisol—mineral corticoid receptor—at the levels that would be needed to get the VDR working again.

The 1,25D [concentration] has to go too high.

Chairman: OK, well, thank you very much. We thank the audience for their attention.