

**MURDOCH UNIVERSITY**  
PERTH, WESTERN AUSTRALIA

Autoimmunity Research Foundation

**What can microbial genomes tell us about human health?**

**Prof. Trevor G. Marshall**  
Faculty of Health Sciences, Murdoch University, West Australia  
Autoimmunity Research Foundation, California

# What can microbial genomes tell us about human health?

**PRESENTED BY PROFESSOR TREVOR G MARSHALL**  
Autoimmunity Research Foundation, CA, USA  
Murdoch University, Western Australia



Molecular Basis of Clinical Medicine, St Petersburg, June 2012.  
Transcript of [http://youtu.be/\\_rFmAMDdbjs](http://youtu.be/_rFmAMDdbjs) video.

## Transcript

**00:00:02**

Dr Marshall: Thank you very much Professor Paltsev and Professor Suchkov for inviting me here to see what we have been doing and for the opportunity to see this wonderful city here that you have—St Petersburg.

Well, I am going to talk a little bit about results, because I have been noticing the lack of the clinical focus so far today, and yet the conference is called “Molecular Basis of Clinical Medicine.”

So let us talk a little bit about the clinical side of things.

**00:00:47**

Well, we started a world-wide collaborative study back in about 2004. By about 2008 some of our international collaborators were starting to publish their results.

At the International Congress on Autoimmunity in Porto, in Portugal in 2008, Dr Greg Blaney from Canada reported on a few very interesting case studies.

The first one was a patient with progressive Multiple Sclerosis, an EDSS around 8, and in that case the disease had reversed and the patient was back to about 7 and was mobile again.

A patient with a diagnoses of Chronic Fatigue Syndrome/Myalgic Encephalomyelitis (CFS/ME), with also positive ANA, who was positive *Borellia*, doing well.

A patient with Fibromyalgia and Chronic Fatigue Syndrome who is also doing well.

**00:01:50**

But also, the very first of our study analyses was done. We did a retrospective on about 100 or so of the thousand totally enrolled at that particular point. And Capt. Tom Perez, recently retired from 25 years at the US FDA, summarized those results.

The interesting thing is that this particular therapy that these collaborators were using, diseases or diagnoses, ranging from Rheumatoid Arthritis, through Hashimoto's, Psoriasis, and somewhere in the middle is Multiple Sclerosis—of course, which we



**MURDOCH UNIVERSITY**  
PERTH, WESTERN AUSTRALIA

Autoimmunity Research Foundation

**What can microbial genomes tell us about human health?**

**Prof. Trevor G. Marshall**  
Faculty of Health Sciences, Murdoch University, West Australia  
Autoimmunity Research Foundation, California

June 11, 2012



**Intl Congress on Autoimmunity, Porto, 2008**

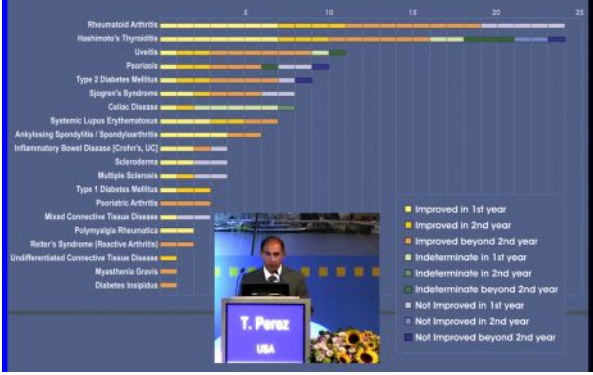
Dr Greg Blaney, Canada, presented 3 case histories:

1. Patient with progressive MS, disease reversed
2. Patient with CFS, pos ANA, pos *Borellia*
3. Patient with FM, CFS

**G. Blaney**  
Canada

**Waterhouse JC, Perez T, Intl Congress on Autoimmunity 2008, Porto**

**Number of Patients Reporting Symptom Improvement by Diagnosis**



Diagnosis	Improved in 1st year	Improved in 2nd year	Improved beyond 2nd year	Indeterminate in 1st year	Indeterminate in 2nd year	Indeterminate beyond 2nd year	Not Improved in 1st year	Not Improved in 2nd year	Not Improved beyond 2nd year
Rheumatoid Arthritis	5	10	15	20	25				
Hashimoto's Thyroiditis	5	10	15	20	25				
Uveitis	5	10	15	20	25				
Psoriasis	5	10	15	20	25				
Type 2 Diabetes Mellitus	5	10	15	20	25				
Sjogren's Syndrome	5	10	15	20	25				
Celiac Disease	5	10	15	20	25				
Systemic Lupus Erythematosus	5	10	15	20	25				
Ankylosing Spondylitis / Spondyloarthritis	5	10	15	20	25				
Inflammatory Bowel Disease [Crohn's, UC]	5	10	15	20	25				
Scleroderma	5	10	15	20	25				
Multiple Sclerosis	5	10	15	20	25				
Type 1 Diabetes Mellitus	5	10	15	20	25				
Psoriasis	5	10	15	20	25				
Mixed Connective Tissue Disease	5	10	15	20	25				
Polymyalgia Rheumatica	5	10	15	20	25				
Behr's Syndrome [Reactive Arthritis]	5	10	15	20	25				
Undifferentiated Connective Tissue Disease	5	10	15	20	25				
Myasthenia Gravis	5	10	15	20	25				
Diabetes Insipidus	5	10	15	20	25				

**T. Perez**  
USA

### Intl Congress on Autoimmunity, Ljubljana, 2010

Dr Greg Blaney, Canada,  
"Olmesartan medoxomil and treatment of autoimmune disease"



1. Ankylosing Spondylitis
2. Autoimmune Arthritis, Reynauds
3. Rheumatoid Arthritis (aggressive)

Endpoint achieved – Return to work

mentioned earlier—right to Myasthenia Gravis and Diabetes Insipidus, were all responding in some favorable fashion to the therapy that was being used: An immuno-stimulative therapy, not immuno-suppressive, but immuno-stimulative.

#### 00:02:50

Two years later, at the International Congress on Autoimmunity in Ljubljana, Dr Greg Blaney chronicled three cases: Ankylosing Spondylitis, which responded very quickly, Autoimmune Arthritis with Reynaud's comorbidity, Rheumatoid Arthritis—an aggressive Rheumatoid Arthritis.

The thing that is very interesting in all of these cases is that by this time, the end point which the clinical collaborators were looking to achieve was return to work. Return to the family, return to work.

**In other words, the disease reversal rather than just disease containment.**

### Autoimmunity Congress Asia, Singapore, 2011



Roswitha Goetze-Pelka, (Germany) demonstrated causative correlation between Psychiatric disease and Autoimmune Inflammation with two case histories, Sarcoidosis and MS, both involving paresthesia, cognitive and memory difficulties.

Endpoint: Return to work

#### 00:03:35

By last year, at the Autoimmunity Congress in Asia, Dr Goetze-Pelka from Berlin gave some results of patients with Sarcoidosis and Multiple Sclerosis who had recovered well, but in particular was focusing on the Psychiatric comorbidities that had also recovered along with the inflammatory disease, pointing out that the Psychiatric disease and Neurological disease appear to be very, very tightly coupled to the underlying systemic inflammatory processes. She gave two case histories, once again with the end point of return to work.

### Intl Congress on Autoimmunity, Granada, 2012



Last month, Norway's Inge Lindseth gave a 64 subject case-series demonstrating resolution of CFS/ME with Olmesartan

Endpoint: Return to work

#### 00:04:16

And just a few weeks ago at the Autoimmunity Congress in Granada, in Spain, Inge Lindseth from Norway gave a case series demonstrating resolution of CFS/ME using this drug that they had been using, olmesartan, once again with the end point of return to work.

He had people with two to six years—if I recall—of therapy, and around 20 out of 60 were able to return to work. At the start of the study, only two were in gainful employment.

### Molecular Mimicry – the Interactome

It is not altogether unusual that one therapy might address so many diagnoses – immunosuppressives such as prednisone have been used for decades – but these physicians were using a diametrically opposite approach – immunostimulation.

The clues lie in molecular biology: in molecular mimicry.

Just a decade ago the Human Genome was decoded. The burst of discovery which resulted has made this last decade unique in scientific history. But the biggest discoveries are not the ones you read about most often.

#### 00:04:57

So, it is not all that unusual for such a wide range of diseases, such a wide range of diagnoses, to be affected by one therapy. For example, TNF therapy and Prednisone, the immuno-suppressive therapies, have been used for decades.

But in this case, it was a diametrically opposite therapy that was in use. **It is an immuno-stimulative therapy.**

So how could this possibly work? At the molecular level, what is happening here?



## Molecular Mimicry – the Interactome

It is not altogether unusual that one therapy might address so many diagnoses – immunosuppressives such as prednisone have been used for decades – but these physicians were using a diametrically opposite approach – immunostimulation.

The clues lie in molecular biology: in molecular mimicry.

Just a decade ago the Human Genome was decoded. The burst of discovery which resulted has made this last decade unique in scientific history. But the biggest discoveries are not the ones you read about most often.

## The clue lies in Molecular Mimicry.

You know, just a decade ago, the Human Genome was decoded, fully decoded, and there has been a burst of discovery since that time. But the biggest discoveries are not the ones you have been reading about in your newspapers or even, in fact, hearing about at the major conferences. Because the biggest discovery is the extension of the Human Genome to the understanding of the microbiota that is *Homo sapiens*.

The NIH **Human Microbiome Project**, 2007-2011

### Man is a Superorganism

The HMP identified millions of genes belonging to the thousands of species of microbes which live in, and on, the human body

25,000 human genes,  
> 1,000,000 bacterial genes  
Plus the viral genes...  
Plus the fungal genes...



### 00:06:06

*Homo sapiens* is a super organism. Each one of us is carrying around thousands of species and subspecies of other organisms. It is complex how to describe it, let me just call it “other” organisms in the Human Microbiome.

They are in all our tissues, they are in our blood. Any sample of blood from a live human being, run that through a sampler—through a sequencer, through PCR—and you will find the organisms. It is absolutely clear.

Several years ago, 2007, the National Institutes of Health had started the Human Microbiome Project (HMP) to try and find out exactly where these microbes, what these microbes are doing and where they were in our body.

Those of you that read the *Los Angeles Times* or *New York Times* of yesterday would have seen front page stories on the results of the HMP because it is now finished. Late last year it finished.

The HMP identified millions of genes belonging to thousands of species of microbes which live in and on the human body.

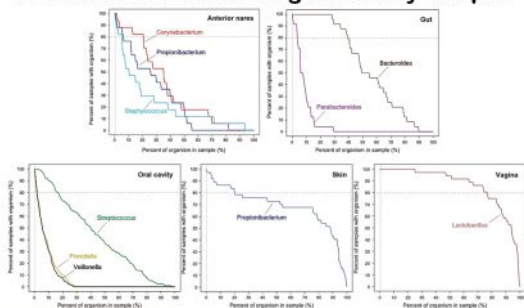
25,000 human genes, **millions of microbial genes**. Over three million was the estimate given by NIH yesterday. Plus, all the viral genes and the fungal genes as well.

### 00:07:38

And in fact, our microbes make us genetically unique. Everybody has a different, slightly different, family of microbes. Our genes might be fairly similar, we may have some SNP differences, I will talk about them later, but basically our microbes are very different. No matter which part of the body we sample, the microbes in each of us are different.

And more importantly, those microbes change from week to week and month to month. Our microbes change. The balance is continuously changing. And at some point in our life, some of us will get a balance that causes dysfunction of the human genome and we become ill.

### Our Microbes make us genetically unique



**Metagenomics** is the genetic study of the Microbes

Slide author: Bruce Birren, Human Microbiome Conference, May 2011

Methe, Yooseph, Li, Bihan

**Metagenomics – Opening the Window on Chronic Disease**



The human body contains an ecosystem of microbes. Thousands of species live in the cells and tissues of the human body, their genomes and proteomes interacting with those of the host (the Interactome).

Subclinical chronic infection is now confirmed, not just a concept...

**00:08:29**

Metagenomics is the study of those microbes. This textbook was published last year, "Metagenomics of the Human Body". The human body contains an ecosystem of microbes. We wrote the chapter in there on Autoimmunity.

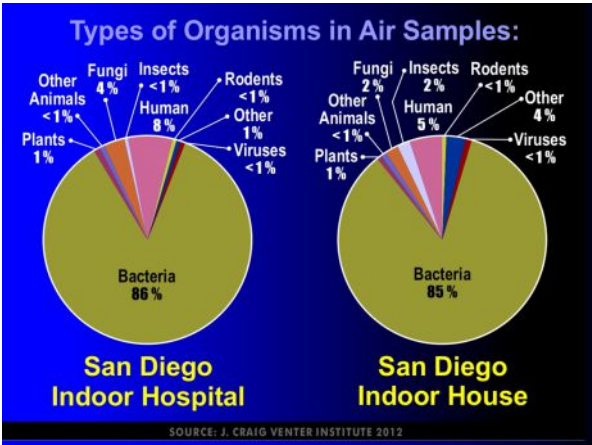
The important thing is to note that subclinical chronic infection is now confirmed and not just a concept. It has been debated for decades, "Can microbes persist in the human body?" Yes, they do persist. Thousands of species persist.

**00:09:03**

And in fact, babies are born dirty. When you are born, you have a good complement of microbes. I was going to say a "healthy complement," not necessarily, some babies are born ill, but a good complement of microbes when you are born.

**00:09:22**

And from that point on, you cannot avoid accreting or gathering more microbes as you go through life.



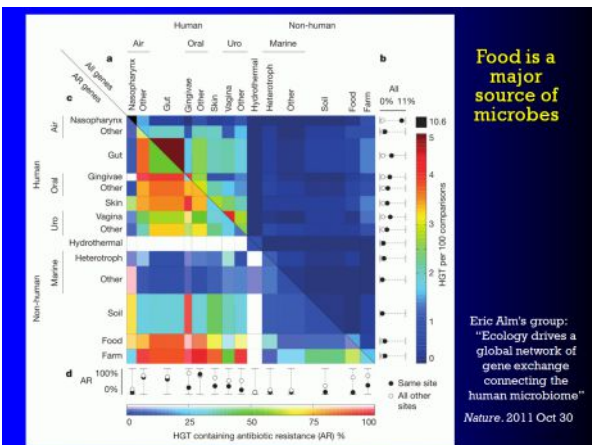
This is data from the Craig Venter Institute and it shows organisms in air samples, DNA in air. And you can see in a hospital, of course, at San Diego indoor hospital—a hospital indoors—but there is a large percentage of bacterial DNA in the air. That is probably no surprise in a hospital.

But you go to a house and you find about the same percentage, that 85% of the the air floating around in the house—about 85% of the DNA floating around in the air of the house—is bacterial.

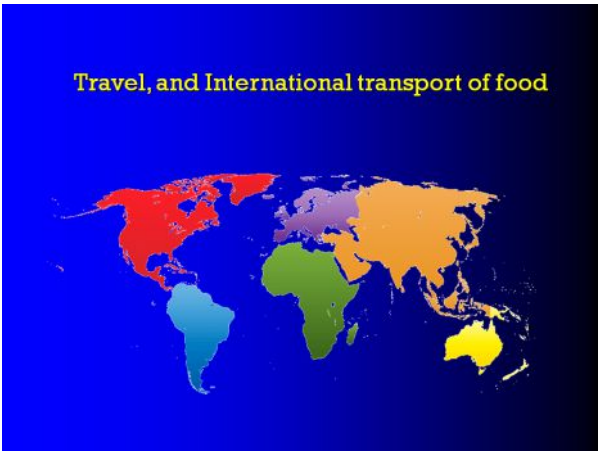
And then there is some human content, some fungi, insects, rodents, etc. If you take an outdoor sample, then the insects tend to be a very large relative proportion.

**00:10:15**

And you cannot avoid it in food, either. This is a wonderful study that was done at MIT by Eric Alm's group, and what they did was a heat map showing the locations on the human body where antimicrobial-resistant genes were present, where bacterial genes were present that generally were regarded as conferring antimicrobial resistance. And you can see a mirroring between the human genes down from the genes that were found in the human body and those that were found in food from farm, agrarian foods, soil, and then it goes quiet from that point on.



**Food is a major source of microbes**  
Eric Alm's group: "Ecology drives a global network of gene exchange connecting the human microbiome" Nature, 2011 Oct 30

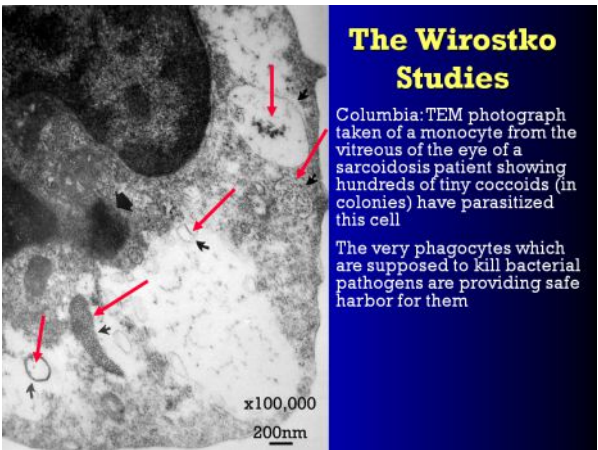


But there is a huge correlation between the bacterial genes we get from our food and the bacterial genes that collect in our body.

**00:11:07**

And of course, in these last few decades of travel, international travel, to which we are all contributing, and the international transport of food, particularly. We are eating food, in many cases—US food comes from South America—from all over the world.

It makes it much easier for the microbes which exist in the human body, and indeed, in the body of the farm animals, to spread.



**The Wiostko Studies**

Columbia: TEM photograph taken of a monocyte from the vitreous of the eye of a sarcoidosis patient showing hundreds of tiny coccoids (in colonies) have parasitized this cell  
The very phagocytes which are supposed to kill bacterial pathogens are providing safe harbor for them

**00:11:35**

But look, the really important microbes, the were not the ones you read about in the *New York Times* yesterday, were not the microbes that are in our gut.

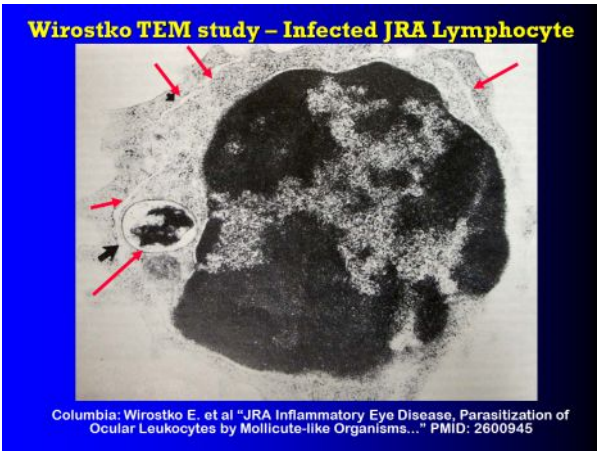
It is really easy to study microbes in our gut, and most of the early studies that have been done on the microbiome have focused on the GI tract.

But the really important microbes are the ones that manage to get inside the nucleated cells and live within the cells, as the last speaker pointed out, the key is to get inside the cells. **If you are a pathogen and you can get inside the nucleated cell and persist inside a cell, then you can do whatever you like.**

That has been noticed before.

There was a study done at Columbia University back some time ago by Wiostko’s group. What they did was stain for nuclear DNA in phagocytes, that is white cells, from patients with a number of chronic diseases. And they found in the cytoplasm of these phagocytes there were colonies of microbes; different types of colonies, of course, different types of microbes.

So the very phagocytes that were supposed to kill the bacterial pathogens were providing safe harbor for them.



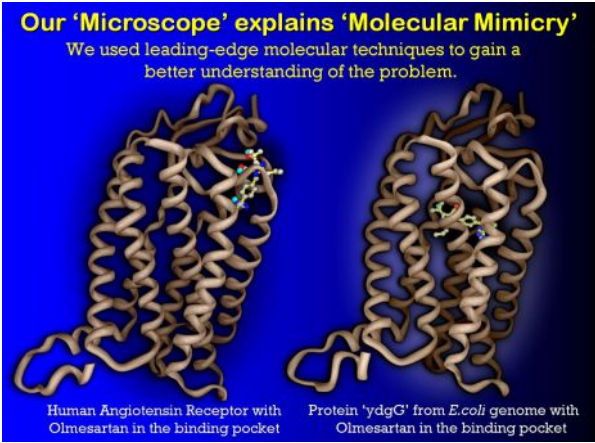
**00:12:56**

This is a similar case, this one is from an infected lymphocyte from a Juvenile Rheumatoid Arthritis patient.

You can see some cluster like a vacuole, which contains infected DNA or infected organisms in a vacuole-type environment, and also long slivers as well.



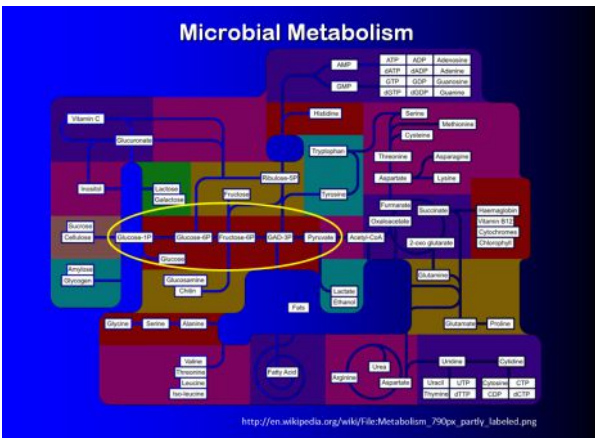




When these are mixed in the cytoplasm, they will cause all sorts of problems for the Golgi mechanisms and the other mechanisms that drive human metabolism.

And it is interesting. There is some main difference in the specific amino acids and the olmesartan drug docks in a slightly different position inside the receptor.

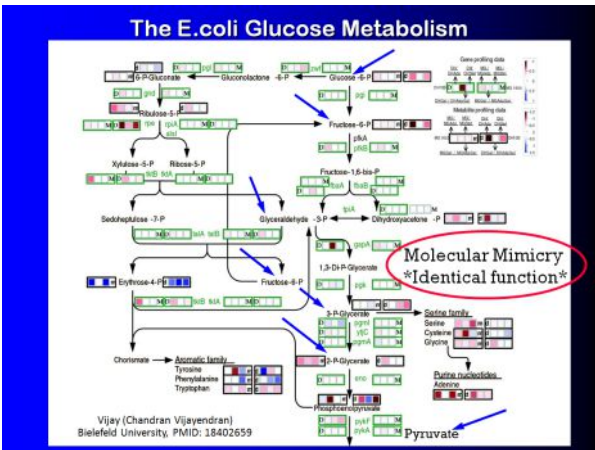
Wait a minute! The drug docks inside a bacterial protein? Our drugs, the drugs we are taking directly affect bacteria? That is an interesting observation in and of itself!



**00:16:50**

But you know, bacteria also have metabolisms that are very similar to man.

Now the eukaryotes are special, but even the prokaryotes are somewhat similar because there is an evolutionary process that is linking them together.



**00:17:10**

And if we take microbial metabolism and look in particular at the glucose metabolism, we find that *E. coli* gets its energy from glucose-6-phosphate and it generates pyruvate (at the bottom). But each of these arrows—seven arrows if I remember—are specific intermediates in the glucose cycle for *E. coli* that are identical with the substrate metabolites in man. In other words, there are genes which are listed here, there are genes in the *E. coli* genome that are identical in function—but slightly different in molecular composition but identical in function to those of the man, that are in man, in *Homo sapien's* genome.

Very, very similar, but slight differences.

We have been hearing that so much from the laboratory people. They are saying, "Look, we are seeing dysfunction, we are seeing SNPs, we are seeing longer substitutions."

**Maybe we are seeing the bacteria.**

So in this particular case Molecular Mimicry is not any specific attempt by the microbe to mimic a function, it is the microbe needing to live. It needs to turn glucose into energy and it does it the same way that we do it.



**Key innate immune functions are unique to *Homo sapiens***

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, all of which are essential to intra-cellular innate immune defenses.

In order to survive inside cells, the microbes would clearly have to evolve to knock out the VDR, so that they don't have to deal with the cell's innate defenses

**00:18:33**

But you know, two speakers ago, we heard that the mouse is not a very good model. We now have a complete genome of the mouse and in fact, it is amazing how different the mouse is to man in terms of the way it actually works.

So what we were able to tell from our molecular microscope working with *Homo sapiens*'s genes was that in *Homo sapiens*, and only *Homo sapiens*—not in the mice, not even in the higher primates—there is a key immune function that is weak.

In *Homo sapiens*, there is one nuclear receptor which expresses the genes for a key toll-like receptor, TLR2, as well as Cathelicidin and beta-Defensin anti-microbial peptides [AMPs]. And all of those, of course, are essential to intracellular innate immune defenses.

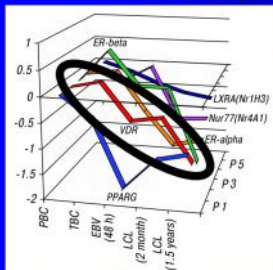
So in order to survive within cells, microbes clearly need to evolve to knock out that receptor.

**00:19:38**

And that is exactly what they do. The microbes that are specialized to knock out that receptor are the typical ones that you have seen in chronic disease.

EBV knocks it out about to a factor of 15 times in the lymphoblastoid cell lines [LCL] at about one and a half years, but also *Mycobacterium tuberculosis*, *Borrelia burgdorferi*, *Chlamydia trachomatis*, *Aspergillus*, HCV and CMV, all specifically manage to suppress the innate immune system and survive by knocking down expression of and by the VDR nuclear receptor.

**EBV – Epstein Barr Virus**



Persistent EBV down-regulates VDR more than 10 fold.

Note that the most pronounced effect is in the immature lymphoblastoid cell lines (LCL)

(Venamandra SP et al: *Exp Oncol* 2009,31,2)

**Other species knocking VDR:**

*Mycobacterium tuberculosis*,  
*Borrelia burgdorferi*,  
*Chlamydia trachomatis*,  
*Aspergillus fumigatus*,  
HCV and CMV

**The Microbiome messes with our Human Biology**

Inside each cell many, many human metabolites are affected by genes from the microbial metagenome, and the sum total of all these interactions gives rise to the symptoms suffered during Chronic Disease.

**Genes from bug A + from bug B -> Dysfunction X**  
**Genes from bugs C+D+E+F-G -> Dysfunction Y**

The genomes accumulate gradually during life. Genes from the accumulated metagenome determine the clinical dysfunction, and the disease symptoms.

**00:20:15**

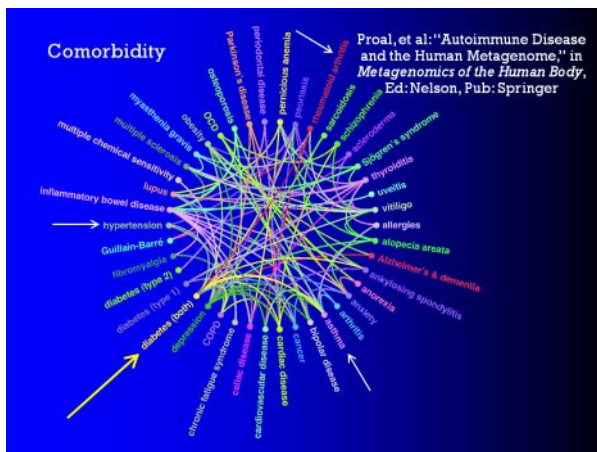
But you know the microbiome also messes with our human biology. They are not just knocking down the VDR—which definitely affects our human biology—VDR is a very important receptor—but they knock it down in millions and imponderable quantity of other ways as well.

For example, a dysfunction X, which may be a symptom, can come from genes from bug A combining with genes from bug B and causing a dysfunction X.

And also, you can have multiple bugs with one bug or one microbe missing, for example, some microbes are antagonistic to each other. For example, *Strep* and *Staph* seem to get at each other's throats all the time.

So you have both combinations of microbial components that can cause human dysfunction and then you also have missing components as well.



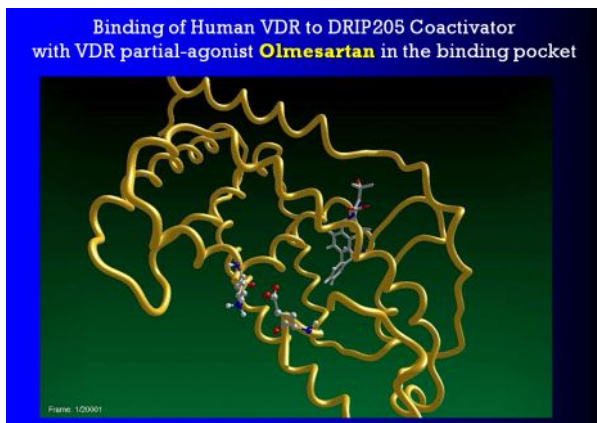


00:21:25

And the genomes of the microbes accumulate gradually during life. It is genes from the accumulated metagenome at any point in life which determine the clinical dysfunction and the disease symptoms. And that is why you see the comorbidities.

When I was first studying diabetes in 1978, many years ago, one of the things I noticed was that the patients that we were treating for diabetes also tended to have Asthma, Rheumatoid arthritis, Hypertension—a number of comorbidities.

This particular chart came from Dr Proal's paper in *Metagenomics...*, actually, came from our chapter in the book. Look up my colleague Dr Proal. But the chart was obtained from some studies on comorbidities that are published in PubMed. They are very interesting all these links in diagnoses. We like to think of disease is separate but we can not.



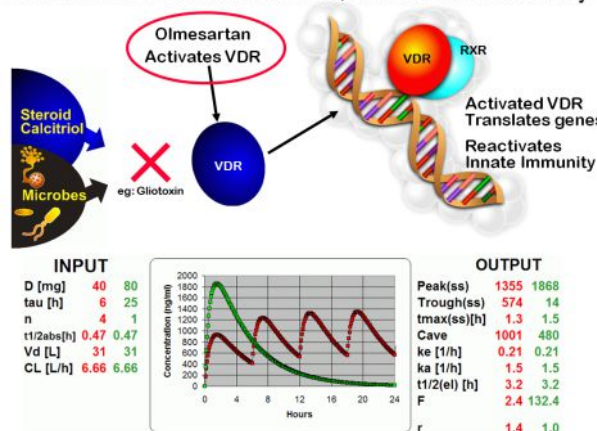
00:22:17

There was something else we were able to do with our molecular microscope—see how the molecules interact.

This is actually the VDR receptor, and across this region of the receptor is the co-activator, which needs to be bound in order to produce the heterodimer and transcribe genes.

We were able to show with the drug that we were using in the human receptor that the active atoms here were ready to bind to the co-activator. And we were also able to show that in the rat that was not the case. **In the rat, the drug does not work.**

Olmesartan re-activates VDR, and Innate Immunity

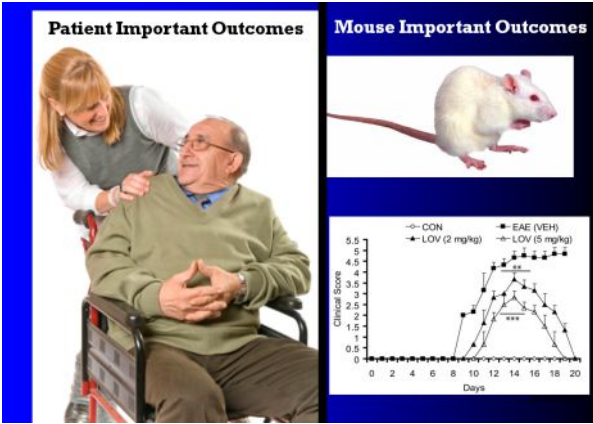


00:22:54

What happens is this: You have got the VDR nuclear receptor which is very important. It transcribes at least a thousand genes, probably as much as a quarter of the total genome is affected. Expression is affected by the VDR.

And normally there is a steroid, calcitriol, that activates the VDR, but when the microbes come along, the microbes have toxins—for example gliotoxin is one of the toxins—there are many ways they can knock out the functions of the VDR. The microbes knock out the activation of the VDR and stop the VDR from producing antimicrobials that would otherwise kill the intracellular microbes.

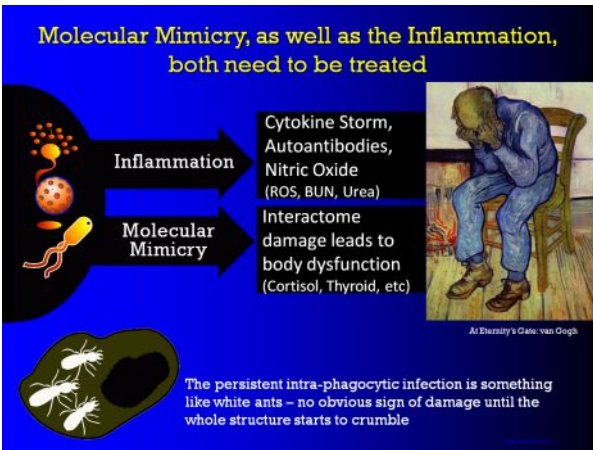
This drug that we found, olmesartan, is capable of re-activating the VDR, provided the dosing is changed. You see a normal dosing of olmesartan gives a single peak and it trails away through the rest of the day. When we re-target the drug for multiple dosing during the day you can see we keep a basal level in the blood stream and then that allows the drug to have totally different effects from when it is given as a blood pressure drug, because it was developed as a blood pressure drug.



00:24:09

Because we had a drug that was already approved by the FDA that could be prescribed with this different dosing off-label, we were able to go straight to patient-important outcomes.

We were not distracted by worrying about the mouse and how the mouse behaved and the differences between mouse and man. We were able to go straight to the patient-important outcomes with our clinical collaborators.

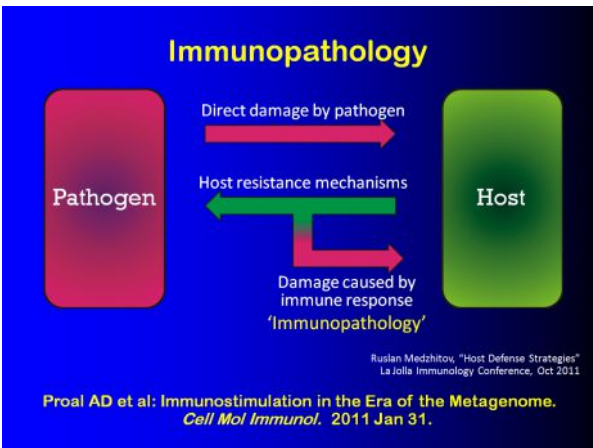


00:24:39

And what we found was this: That in order to help people with chronic disease—huge wide ranges of chronic diseases, about 120 diagnoses in our cohort—you not only have to not only deal with the inflammation, you also have to deal with the molecular mimicry.

The inflammation gives rise to cytokine storm, auto-antibodies, nitric oxide, ROS, BUN, urea, etc. But then the Molecular Mimicry provides the dysfunction—the interactome damage which leads to the body dysfunction—the cortisol dysfunction, the thyroid dysfunction. You have to deal with both of those. Just attacking the inflammation does not prevent the spread of the disease nor does [attacking inflammation remove] all of the disease symptoms.

In a way, you can think if these persistent intraphagocytic, intracellular infections, as something like white ants. You know with white ants in a building, there is no obvious kind of damage until the whole structure starts to crumble. And that is the stage we are in at this moment in medicine and we have got to move toward a stage of predictive medicine, where we can say, “Look, there seems to be white ants in this structure.” The Microbiome gives us the ability to do that.



00:25:56

Of course, there is always a problem. And in this case, the problem is immunopathology, which means that when the immune system starts recognizing the microbes within the cells and killing the microbes within the cells, many of the cells die as well.

Because the immune system is more active, you have a surge in the cytokine storm, you have a surge in the immune functions and that is what is termed immunopathology.

So the pathogen is exerting direct damage to the host, the host resistance mechanisms exert damage back onto the pathogen. But there is some of this damage caused by the immune response that gets back and hurts the host a bit, too.



---

And this is immunopathology.

We covered it in detail in our paper, our recent paper, I will not spend any more time because we are tight on time, but immunopathology has to be dealt with and it has taken us a decade to figure out to manage immunopathology in order to induce recovery.

**00:27:00**

**So—predictive and preventative medicine.**

Now that we have a working model for autoimmune disease processes, it becomes practical and sensible to start treating patients early. For example, as soon as we see autoantibodies, and typically, with the new tests that are available, autoantibodies can be found years before any symptomatic dysfunction occurs in an individual.

Most of us are carrying around autoantibodies. The Red Cross blood supply has autoantibodies in it, so when people first start to present with autoantibodies, maybe that is the point at which we need to say, “Look, you should not have those, there is a dysfunction. Let us start looking at your immune system.”

Measure the markers of the immune system. VDR effectiveness is one of the markers we can use. There are some—CYP24, for example, which is characteristic of VDR—that have been measured clinically as a VDR indicator. But at that point we can initiate therapy, because olmesartan, the drug that overcomes the effects of the microbes, is one of the safest drugs in the US formulary.

I hate saying a drug is safe, especially when I am espousing its use, however, it is a very, very safe drug. The FDA has set no unsafe dosage for this drug, there is no known unsafe dosage for it, and it is FDA-approved to be taken for a lifelong dysfunction, which is high blood pressure.

Our off-label re-targeting does change the safety profile a little because of immunopathology, but the earlier that a patient begins therapy, the less is the amount of immunopathology that they exhibit.

In fact, healthy people exhibit no immunopathology at all. And that is very important because the drug would never have been approved if the cohort were exhibiting immunopathology at the same time as the lower blood pressure.

**So the earlier we get the patients, the much easier it is to stop the disease progressing in the long term. So maybe we have an opportunity here for predictive and preventive medicine.**

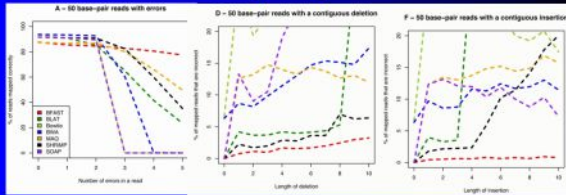
### Predictive and Preventative Medicine

Now that a working model of Autoimmune Disease processes has been defined, it becomes practical to start treating patients early – for example, as soon as they start to exhibit ‘autoantibodies’.

Olmesartan is one of the safest drugs in the US formulary, and is FDA-approved to be taken for a lifelong dysfunction. Our off-label re-targeting does change the safety profile a little, because of Immunopathology, but the earlier that a patient commences therapy, the less is the Immunopathology they generate.

**Indeed, a person with no inflammatory dysfunction suffers no Immunopathology at all.**

## Non-Mendelian Genetic Predispositions – SNPs can be caused by computer software error



US Dept Of Energy: Argonne National Labs: <http://TheSeed.org>

Nikkari S, McLaughlin IJ, Bi W, Dodge DE, Reiman DA.: Does blood of healthy subjects contain bacterial ribosomal DNA? J Clin Microbiol. 2001 May;39(5)

Homer N, Merriman B, Nelson SF: BFAST: an alignment tool for large scale genome resequencing. PLoS One. 2009 Nov 11. <http://bfast.sourceforge.net>

The Edwards Lab Blog, SDSU. <http://Edwards.SDSU.edu/research>

00:29:20

Now I want to talk about something else as well. There is a huge impact of the microbiome on the work that each one of us does. Because when you take a sample from a human being, whether it be blood, or whether it be tissue, or whether it be cells, that sample contains microbes.

It contains microbial DNA which you can find with PCR, or with sequencing approach, and you have to be very careful when you are doing your study of SNPs or mutations that the DNA that you are actually looking at is human DNA—that all of the microbial DNA has been stripped out.

This was realized some time ago by the US government, of course, after the anthrax attacks in America, and they put a lot of money into searching through DNA databases to find which are the microbial genes and which are the human genes.

And if you look at that particular URL from the Argonne National Labs, you will find that any studies that you have done of the human body where you have not sorted out the microbes, they will help you do it, either by providing the software or providing the computing power.

There are a number of other very important papers here that I have listed for those of you that are doing genetic testing and genetic analysis, to help you better analyze your sample and make sure that what you are thinking is a SNP is actually a SNP and not just a piece of microbial receptor that looks awfully the same as the human receptor.

Because what happens is the software that has been provided you for your genome sequencing is very faulty. It only needs one or two base-pair errors and it goes totally wrong, as it forces the RNA up into the Human Genome that it has in its databank.

Because when you feed in an RNA sample into one of the sequencing machines, it is trying to put that RNA up into the Human Genome that it has remembered in its memory—the standardized Human Genomes we have had for a decade.

And as we can see from these graphs here, which came from Barry Merriman's paper. He checked the six major pieces of software that are used by all the commercial manufacturers, and he found that just one position deletion was enough to cause 10-15 percent errors in the mapped reads—and the same with insertion—a one position insertion was in some software enough to cause more than 10 percent poor assembly of that RNA.

This is very, very important. We have to remember that every sample you are working from a human being has got microbes in it. And if you look at the DNA and the RNA, you will find them.



## Take-Home Points

1. *Homo sapiens* is a super-organism, a symbiotic metagenome
2. When the metagenome becomes unbalanced, disease ensues
3. Intraphagocytic microbiota persist by suppressing innate immunity -- knocking down the VDR Nuclear Receptor
4. Olmesartan can reactivate the VDR, reactivate immune function
5. Immunostimulation causes Immunopathology, a temporary worsening of disease, before healing becomes dominant
6. Before commencing a Genetic study, remove the microbial RNA
7. Disease is complex, not simple, the Interactome is semi-infinite

00:32:25

So here we are, take-home points. There are a lot of them. I am sorry.

[1] First take-home point is "***Homo sapiens* is a super-organism**". It is a symbiotic metagenome.

[2] **When the metagenome becomes unbalanced then disease ensues.**

[3] **Intraphagocytic microbiota persist by suppressing innate immunity, by knocking down the VDR nuclear receptor.** And all the antibodies that we see are, of course, a cascade. Once the innate immune system is compromised, then the cytokine release causes the adaptive immune system to say, "Wow, we better try to start working extra hard and try and deal with this problem that the innate immunity has!"

So it is the innate immune system that is key.

[4] **Olmesartan can reactivate the VDR and reactivate immune function, if it is used properly.**

[5] **But you have to be very careful because many people will get very, very ill.** There is nothing like having a call from a hospital—it was in the middle of the night I think about ten years ago—one of the subjects had a heart rate of 10 with occasionally missing beats. You do not want that sort of thing. That is too much immunopathology. You have to control the immunopathology.

The temporary worsening of disease is measurable, usually the patients feel better right away but you can measure the temporary worsening of disease—read our paper for that—before the healing becomes dominant.

[6] **Before commencing a genetic study, always remove the microbial RNA.** For those of you who have already done a study, you can take your raw data and feed it back through Argonne Labs and they will take the microbial RNA out of that for you, so you will have a cleaner sample to look at again.

[7] **Disease is complex, and not simple.** This is pretty obvious, I think. Pretty much everyone has said that here, but the reason for that is because the Interactome, the potential for interactions between the millions of microbial genes and the 25,000 human genes, is semi-infinite. Semi-infinite is really the only way we can quantify it at this point.

So, thank you very much Mr Chairman, and once again thank you to the organizing committee.

[applause]