Dr Marshall

Thank you Mr. Chairman, and thank you to the organizing committee, it is great to be here.

You know, sometimes one gets ideas from the strangest places. The other day, I was watching an old movie, an old Mel Brooks movie, in fact. And the strangest thing happened...

Excerpt cited from:
History of the World... Part 1
(c) 1981, 20th Century Fox. Produced: Mel Brooks

[Moses and 3 stone tablet "laws", one breaks, and the error is glossed over.]

00:01:18
DATA RECOVERY

11. Thou shalt not ignore the Microbiome lest it cause a 'Mosaic of Autoimmunity'
12. Thou shalt not ignore the Interactome lest it make disease seem complex
13. Vitamin D ... Scourge

So my new computer has apparently reconstructed some of the lost data. Let us have a look and see if it gives us any ideas.

00:01:32
Well, Vitamin D, unfortunately, we did not get very much on vitamin D. Maybe we can get some clues on the first couple of items.
Let us see. The first one is saying the Microbiome causes a "Mosaic of Autoimmunity," and something called an Interactome makes disease seem complex.

Goodness. Let us have a closer look at those.

00:01:54
NIH Human Microbiome Project

Well the Microbiome, of course, is all of the microbes that exist in and around the human body.

The NIH Human Microbiome Project was kicked off four years ago now, and has just grown exponentially in the last few years.

When it started, it was understood there were about 25,000 human genes and about a million bacterial genes were expected, plus all the viral genes, plus fungal genes.

00:02:27
Cores Across Body Sites. Our Metagenome makes is genetically unique.

Now the results are in. This is a summary from the human microbiome conference earlier this year in Vancouver by Bruce Birren.

And basically for all the body sites, the anterior nares, the gut, the oral cavity, the skin—all of the body sites, showed that there was vast difference in flora, vast difference in microbiota, vast difference in the microbes that live at those body sites—between individuals.

Now, metagenome makes us unique. And in particular, our metagenome makes us genetically unique.

Because, when the genes from the metagenome—the genes from the thousands of microbes that live in and around our human body—interfere with the way that our human genome is working then we get disease or dysfunction or groups of dysfunction being categorized as disease.

The microbes that I am particularly interested in are those microbes that are within the cell itself—within the cytoplasm of the cell itself.

00:03:36
Wirostko TEM study—Infected JRA Lymphocyte

This is a study, electron microscopy, from Wirostko's group at Columbia University, and this is an infected lymphocyte from an infected juvenile rheumatic arthritis patient.
There is a large colony here [see slide left arrow], and some smaller colonies up along the top [just above colony, dark spots]. And with the Uranyl acetate stain they used.

Now this is very important, because when the persistent pathogen can survive in the phagocyte, can survive in the nucleated cells, then it directly effects the way that the genes are changed into proteins... and its own genes and its own proteins and its own RNA can contribute to the overall meta-metabolome, or all of the metabolites that the human body produces.

00:04:21

On Mouse and Man

Well, that gives us a problem.

How do we study microbes, thousands of microbes in the human body? How do we study a genome, when it is really not a human genome, it is a human-metagenome—it is a microbiome, many, many genomes, a metagenome—and the normal methods of studying in mice really do not work very well?

Firstly, mice have vastly different genomes from man. They have different innate immune systems. They have different adaptive immune systems and, they react differently to pathogens. Mice succumb to different microbes. They have a different microbiome.

So why do we have to rely on murine-centered research?

00:05:11

Bruce Beutler, Nobel Laureate in Medicine, 2011

Bruce Beutler, the Nobel Laureate in Medicine this year, summarized at the conference I was at:

"... Humans have Toll Like Receptor (TLR) 10, whereas mice have TLRs 11, 12, and 13 and the functions of those receptors are not really well defined, although TLR11 is believed to be involved in sensing profilin and is also believed to be involved in sensing neurotrophic E.coli."

"... Those [TLRs] that do exist [in common], mostly have the same specificity, the only example to the contrary is that TLR8 seems to be an active detector of SS RNA in the human, and has no known ligand (so far) in the mouse."

Not only are they different, but we do not really know what the differences are very well just yet.

00:06:00

Murine vs Human Adaptive Immunity

In the adaptive immune system, between animal and man, is similarly different.
For a start, what we have here is the immunoglobulin axis. This is the actual part of the genome where the immunoglobulins, the actual antibodies, are produced by the human genome.

We have it for mouse pro-B cells, and also for human pre-B cells.

The first thing to notice is that on the mouse, it sits in chromosome 2. And in man, it is in chromosome 11.

But let us look further, also, the length is different, and in particular these long-distance interactions that you see on man are very uncommon in the mouse.

As you sink down to more and more detail, you find there are more and more differences between a mouse adaptive immune system and a human adaptive immune system.

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**00:06:57**

**Albert Einstein**

And as the great Albert Einstein noted: "We can't solve problems by using the same type of thinking we used when we created them."

So, I was looking about a decade ago for other ways of analyzing the microbiome, analyzing microbes—microbial genes working in conjunction with human genes...

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**00:07:18**

**On our Approach—and ‘molecular mimicry’**

...I started to use in silico analyses. What I did was go through the genomes of bacteria.

This is a protein, called 'ydgG' from *E.coli* genome [right figure highlighted], and as a matter of fact, it has got a drug up there in the binding pocket, a human drug.

And [left figure highlighted], here is a very similar receptor, 'GPCR' receptor from *Homo sapiens*, it is actually the angiotensin II receptor.

And you know, if you look very, very carefully, maybe you can see a difference between these two proteins, but you have to look very, very carefully. The clue is that the amino acids are slightly different in the binding pocket region, therefore the drug binds at a slightly different position.

But it still binds, it still binds into the microbe.

In other words, the human drug binds into the microbe, the microbial protein is the same as the human protein! It is not a mimic, it is nothing that the microbes set out to do necessarily just to cause problems. It is needed by the microbe to work.
**The E.coli Glucose Metabolism**

For example, if you look at *E.coli* Glucose Metabolism, you start up here with Glucose-6 Phosphate, you end up down here with Pyruvate, you have some nucleic acids produced along the way, but at every step you will find that the intermediate metabolites are essentially the same as the human glucose metabolism.

And the genes that produce it are almost the same as the human's glucose metabolism.

There are only a few ways to skin a cat. You know, when you have got a good thing, then you stick with it, and the bacteria have very, very similar metabolisms, in some cases, in some species, to man. So it is natural that they are going to interfere.

**Comorbidity**

Another piece of data that we brought in, in the early days, was the fact that there were so many comorbidities.

I was studying diabetes back in the 1970's and what I was really surprised about, was that there so much asthma, rheumatoid arthritis, and hypertensions amongst the patients that I was working with.

These might seem to be totally different diseases, yet people that we were working with had multiple manifestations, multiple dysfunctions, which were gathered into these diagnoses buckets.

**Suboptimal Health**

The second thing that I felt was important, was that even in individuals who have no diagnosable disease often suffer from 'suboptimal health'.

'Health' is a continuum, not a binary state.

**Innate immune functions are unique to Homo sapiens VDR**

And the key thing was to note that the Innate immune functions are unique to *Homo sapiens*.

There is no animal that has an innate immune system substantially similar to Homo sapiens.

In *Homo sapiens*, and only in *Homo sapiens*, there is 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.
intracellular microbes, so it is partially responsible for detecting intracellular microbes.

And also, the VDR is responsible for the Cathelicidin and beta-Defensin anti-microbial peptides—well they are proteins that get broken into peptides.

And all of those are essential to intracellular innate immune defenses.

In other words, if there is an intracellular pathogen that manages to knock out the VDR, then its the likelihood of persisting is very much greater.

And there is no animal—no animal—that produces Cathelicidin out of the VDR. So, therefore, the pathogens and the animals have to knock out different receptors to what they knock out in man.

And naturally, it is not surprising that the human pathogens, or the ones that we know as being most common, very effectively knock out the VDR.

EBV — Epstein Barr Virus

Here is Epstein Barr Virus (EBV), it is shown to knock out the VDR in lymphoblastoid cell lines after one and a half years by fifteen times. Very effectively knocks out the VDR where expression by the VDR is as well. And those antimicrobials that the human innate is trying to make are just not available.

Other species knocking down the VDR include the usual suspects:
- *Mycobacterium tuberculosis*
- *Borrelia burgdorferi*
- *Chlamydia trachomatis*
- *Aspergillus fumigatus*
- *HCV* and *CMV*

And they are just the ones that we have already confirmed, not our group but other groups that science has already confirmed by studies, absolutely knock out the VDR as one of their survival mechanisms.

Translation to Homo sapiens, 2002 to date

1. We had a pretty solid understanding—we thought we did—of chronic disease, that pathogens suppressing immunity via the VDR.
2. We found a safe, already-approved drug to reverse the VDR damage being done by the pathogens (frequently-dosed Olmesartan/pix wean all other drugs) (No MTX, No Steroids, occasional NSAIDS p.r.n.)

We shared our insights with clinical collaborators and patients, worldwide, and started an observational study.

We now have a total of 2878 progress reports (as of January 2011), more than 718 discrete inflammatory diagnoses, of which 119 are shared by 5 or more subjects.
olmesartan, but we changed the dosage so that it addresses the nuclear receptors.

And the other thing to note is that patients undergoing this therapy wean off of all other drugs; No MTX, no steroids, occasional NSAIDs when they need them to deal with immunopathology.

We shared our insights with clinical collaborators all over the world and [with] patients, and we started an observational study.

We now have a total of about 2578 progress reports (as of January 2011), with more than 719 discrete inflammatory diagnoses, of which 119 are shared by five or more subjects.

And you know, much to our surprise, it worked!

00:13:12

Number of Patients Reporting Symptom Improvement by Diagnosis

When patients, who are seriously ill with autoimmune and other chronic inflammatory conditions, were subjected to the monotherapy of the VDR agonist olmesartan, we were able to reverse rheumatoid arthritis, Hashimoto’s, uveitis, psoriasis, multiple sclerosis, a couple of cases of diabetes type I, Psoriatic Arthritis (and incidentally, the type I [diabetes] was fully reversed at the time this was published).

These are some intermediate results which were published back in 2008 at the Porto [Autoimmunity] Conference in Portugal by one of my colleagues, Captain Tom Perez, just recently retired from the FDA. And he and some his colleagues did a study of our results, the reports that we had gotten in, and we took about one hundred autoimmune diagnoses and this is what they came up with.

And just about everybody, these non-responders here, up to the second year, but just about everybody has started to respond by the second year.

00:14:16

And when we list all of those diagnoses by the number of patients in the cohort, it goes from vitiligo, vasculitis, seizures, all the way up to chronic fatigue syndrome being the most common–575 diagnoses, sarcoidosis–552 (historically high because of selection in the enrollment), 376 with whatever Lyme is, 354 with fibromyalgia, RA at 111, IBS–102, and going down through just about every inflammatory diagnosis.
Comorbidity

And the thing that was really strange, was that as the primary inflammatory disease receded in the patients, that the comorbidities changed as well.

And in particular, things such as Bipolar disorder and even Schizophrenia started to reverse along with the primary autoimmune inflammatory condition.

So let us see. The tablet that came out of the mountain says something about the Interactome and making disease complex.

Well, what is that all about?

The Interactome

The Interactome is all of the molecular interactions in a cell.

It is these millions of interactions which lead to disease, or the dysfunction in some of the millions of interactions that lead to disease.

The 100,000 human proteins in the human body can interact with proteins from the metagenome, the microbial genomes, in a semi-infinite number of ways.

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

For example: Genes from microbe A plus genes from bug B might lead to dysfunction X,

whereas,

Genes from other microbes C plus D plus E plus F, MINUS G—because many microbes displace each other from the microbiota, from the community—are antagonistic to each other, that might lead to dysfunction Y.
And as you accumulate more of these dysfunctions as your immune system gets more compromised by the microbiome during life, then eventually you will get enough dysfunctions to put into a little bucket, which is a diagnosis; Rheumatoid Arthritis, Fibromyalgia, whatever it happens to be.

The Genomes accumulate gradually during life.

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00:16:44

**Why vitamin-D is more effective in Early Stage disease than in Late Stage disease.**

So, Vitamin D. Let us get back to the topic I was given.

Why is Vitamin D more effective in the Early Stage disease?

Well, this VDR [NR1I1] I have been talking about, I think most of you recognize as the Vitamin D Receptor. And it is called the Vitamin D Receptor because for a long time, people thought that Vitamin D was the only thing that activated the Vitamin D Receptor. But in fact, there are a number of other lipids and other metabolites in the body that can activate the VDR to express genes. And in fact, VDR even self-activates to express some genes.

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00:17:20

**Only 1,25-dihydroxyvitamin-D can activate VDR**

But what I want to point out, this is back to the molecular emulation again, and I put all of the key Vitamin D metabolites in the position that they mount into the binding pocket on the VDR.

Only one of them [highlight mid-lower right] this alpha-hydroxylation that can activate the VDR, but all of them fit into the VDR in approximately the same place and the others just get in the way. They stop the activation of the receptor.

Here are the Steroid Rings [mid-left highlight], characteristic of the steroid. Vitamin D is not really a vitamin, it is a secosteroid the body can make all it needs.

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00:00:00

**'Vitamin-D' is a steroid, and it acts like a steroid**

Vitamin D is a steroid, and it acts like a steroid.

Low plasma vitamin D (25-D) is a marker of chronic disease. It is not causal.

Association is not causation.

Low vitamin D is a marker of chronic disease.

When you give people vitamin D, it suppresses their innate immune system, it behaves like an immunosuppressant.
The microbiota knock-out expression by the VDR, but supplements cannot be used to restore that expression.

And the reason you cannot force Vitamin D to be high by giving people the active form 1,25-D or any of the inactive forms that you can buy at a shop...

00:18:42

**Vitamin D affects other receptors**

... Is because Vitamin D affects other receptors. It affects the Androgen receptor, it affects the Progesterone receptor, and it definitely effects the Thyroid receptor.

Most of you know that thyroiditis is a very early and very common marker amongst many of these disease states.

What happens is that high levels of 1,25-D, which is what you need to re-activate the VDR after the microbes have knocked it out, the high levels of 1,25-D displace T3 from the receptor.

00:19:16

**Human studies show Innate Immune Suppression by exogenous Vitamin-D**

Skip that, because we are running short.

00:19:19

**Question posted**

Yehouda posted a question on the 'Autoimmunity-Network':

"How many autoantibodies are in SLE?? Can they explain the diversity of clinical manifestations??"

We’ve just discussed this down at the Salk Institute a week or two before, and I will quickly go over that particular issue.
When you look at the genome, the VH axis of the immunoglobulins that form the genes that form the antibodies, you find there are about one hundred and twenty-three to a hundred and twenty-nine genes when you lay them all out side by side.

So the initial feeling was that when you are talking about the human body may be able to produce about one hundred and twenty-nine genes plus different length codons in the variable areas, and you know, maybe a couple of hundred of genes are possible, but in fact...

Those genes are shaped like a rosette in 3D space. They are shaped like a rosette, rather than being linear as we laid them out, years ago.

And when the rosette moves in 3D space, then different parts of the genes can interact with different parts of each other.

And the question, therefore, arises, what are we talking about, are we talking about a hundred twenty-nine factorial or a semi-infinite number of antibodies?

And the answer is a strange one. We sat down with some of the experts to try and figure out how many antibodies are we talking about, and how many auto-antibodies are we talking about?

And we came up with a consensus...

...And the consensus is: A Gazillion.

Not a thousand, not a hundred thousand, certainly more than a million, and certainly less than ten to the sixteenth. Somewhere in that range; a very large number.

And a significant proprotion of those will be auto-antibodies because the hundred thousand proteins in the human body will necessarily interact statistically affect the proportion of all antibodies produced.

So, how do we cope with numbers, big numbers, like this?

The Human body has evolved to be very complex. We all try and look at it in little small portions hoping that everything will fit together, the mosaic will fit together.
But you have really got to start with the mosaic as it was, the complete mosaic, and say 'Wow! That bit fits with that bit and that bit fits with this bit.' It is much easier to do a puzzle if you have once you have the whole puzzle [picture] there, sitting in front of you.

The only way to do that is to step back and 'look at the forest'. Do not get fussy about the trees, look at the forest.

Once again, Hollywood has helped us with some aids on how to deal with some very large numbers and I will end with their suggestions...

00:22:07

Hollywood—on Complexity

Excerpt from 'The Meaning of Life' (c) 1983 Celandine films.

We embraced the complexity, decoded the 'Mosaic of Autoimmunity,' and made thousands of patients very happy indeed.

[Planetary/galaxy complexity ditty.]

2011 Nobel Prize in Physics: The universe itself keeps on expanding and expanding in all of the directions it can whizz.

Thank you all.