

# Sarcoidosis and Tuberculosis—insights from an immunostimulative therapy

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

1st Congress of the National TB Association, St Petersburg, Russia. October 18-20, 2012. Transcript of <u>http://youtu.be/E2QzPVKtEr0</u> video.







Sarcoidosis and Tuberculosis – Insights from Immunostimulative Therapy Trevor G. Marshall Faculty of Health Sciences, Murdoch University, West Australia Autoimmusity Research Foundation, California

#### Transcript Moderator:

Professor Trevor Marshall, you are welcome for the first presentation. You have 20 minutes.

#### 00:20 Dr March

Dr Marshall:

Thank you Leonid. OK, this is going to be interesting.

I am going to talk about Sarcoidosis and *Mycobacterium tuberculosis* and what we have discovered from a number of years now, nearly a decade of use of an immunostimmulary therapy, in Sarcoidosis.

00:49

I start with a quotation from Professors Yehuda Shoenfeld and Noel Rose, both very eminent and well known immunologists, who in 2004 made the observation in a book called "Infection and Autoimmunity," that everything is infectious until proven otherwise.

That is a pretty bold statement. Now I am going to extend that in 2012. We can say that "The human microbiome causes human chronic disease, and predisposes for much infectious disease." That is what I am going to be talking about today.

"Everything is infectious until proven otherwise" Infection and Autoimmunity, Ed: Yehuda Shoenfeld and Noel Rose, 2004



Dogma: "The human microbiome causes human chronic disease"



#### 01:28

I will offer no less than the eminent medical journal, *The Economist*, as part of the hypothesis here <humor>. *The Economist* carried in August [2012] the very, very good article "Microbes maketh man," talking specifically about the microbiome and the various diseases which are already being linked causally to the microbiome.

I wanted to point out to you what a wonderful illustration has been used here by the artist that drew the front cover image. You will see that a few times in my presentation.

 $\ensuremath{\textcircled{C}}$  2012 Autoimmunity Research Foundation. All rights reserved.

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

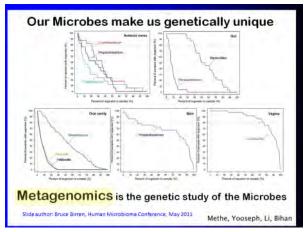
Man is a Superorganism

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

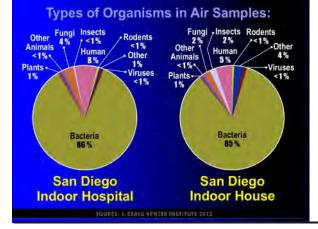
25,000 human genes, > 1,000,000 bacterial genes Plus the viral genes...

Plus the fungal genes...









#### 02:15

But if you want something a little more austere, we will talk about the National Institute of Health (NIH) Human Microbiome Project (HMP), which ran from 2007 to 2011. It is finished now, and what they found was that man is a super-organism.

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

So, for the 25,000 human genes that fundamentally control our existence, there are greater than a million bacterial genes, plus a whole stack of viral genes, and also the fungal genes, and of course, the phage genes.

#### 02:57

#### That is, it is our microbes that make us genetically unique.

There is more difference between the microbiome of everybody sitting in this audience than there is between the genome of everybody sitting in this audience. Our microbes make us genetically unique.

Metagenomics—meta-genomics—is the study of those microbes.

#### 03:20

So where do you get the microbes from? Well, firstly, you get them from your mother. And at birth, babies are born "dirty" with a gut full of bacteria.

Then very quickly pick more up within the first couple of weeks of life. They will pick up most of the microbes of the extended family. Some very good work has been done by Dave Relman's group at Stanford on that era of life.

#### 03:48

Then you really cannot avoid them from that point onward. If you test the air, which we all breathe—we cannot avoid it—and you sequence the organisms, the DNA which you find in air, well, in a hospital you would expect, maybe in a hospital, that you would find a large percentage of bacteria, about 86%, in addition to human DNA, fungal, and animal DNA, plants and viral DNA. But a huge contribution from bacteria, much greater than the human DNA that is floating in and around a hospital.

It is surprising that you find a very similar thing in the house. Inside a standard house in San Diego, Craig Venter from the Craig

© 2012 Autoimmunity Research Foundation. All rights reserved.

#### Travel, and International transport of food



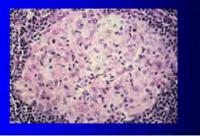
Venter Institute found that once again the air was... about 85% of the DNA in the air was due to bacterial DNA.

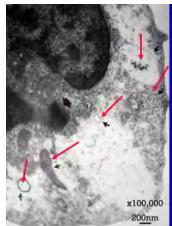
#### 04:46

You also pick up bacteria from travel, and particularly from international transport of foods. Over the last five decades our food has become an internationalized commodity. And with it, it brings the microbiome of the region from where the food comes.

#### Granuloma Histology

What struck me about Sarcoid granuloma was that 90% of the cells were monocytes. Lymphocytes are expelled to the periphery. That seemed to point at activity due to innate immunity, rather than adaptive immunity





#### The Wirostko 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

Wirostko TEM study - Infected JRA Lymphocyte

umbia: Wirostko E. et al "JRA Inflammatory Eye Disease, Parasitization of Ocular Leukocytes by Mollicute-like Organisms..." PMID: 2600945

#### 05:04

So a decade ago, I was looking at Sarcoidosis and looking at Sarcoid granuloma, and something hit me about this Sarcoid granuloma.

I noticed that most of the lymphocytes—which are the black stained cells, of course—most of the lymphocytes were around the periphery of the granuloma as if they were expelled to the periphery and not a key part of the process that formed the granuloma.

About 90% of the cells in the granuloma are monocytes and macrophages, but particularly monocytes.

And you know that seemed to point, to me, that the innate activity, the protection within the cells, was likely to be key in a granuloma formation, rather than adaptive immunity—the T cells and the B cells as everybody was suspecting at that time.

#### 06:02

Well, there was a very elegant study done by Wirostko, Emil Wirostko's group, at Columbia University in the 1980's. They used electron microscopy to look at the cyptoplasm of cells—the cells of the immune system.

This [slide] is a monocyte taken from the eye of a Sarcoidosis patient. And you can see hundreds of tiny coccoids which stained—they used a urinal acitate stain for nuclear material—and found that there were many colonies of microbes in the cytoplasm of the cells of the immune system.

The very cells, the monocytes that are supposed to kill pathogens, were being parasitized and providing safe harbor for pathogens.

#### 06:54

The same thing happens to Lymphocytes. This is from a Juvenile Rhumatoid Arthritis patient, with a large cluster there, looking something like ... a linear cluster there, with linear DNA segments as well.

© 2012 Autoimmunity Research Foundation. All rights reserved.

#### Mycobacterium tuberculosis (≈ 4.4 mbp ≈ 4000 genes)

12,788 cDNA Microarray was used to profile gene expression in U937 macrophages infected with *M.tuberculosis* 

"463 differentially expressed genes .. 97 unknown .. intracellular signaling, cytoskeletal rearrangement, apoptosis, transcriptional regulation, cell surface receptors, cell-mediated immunity as well as a variety of cellular metabolic pathways"

25 genes up-regulated

341 genes down-regulated

→VDR nuclear receptor down-regulated 3.3 fold

Yongzhong, et al: Using a cDNA microarray to study cellular gene expression altered by Mycobacterium tuberculosis. Chin Med J 2003

#### 07:35

What happens when pathogens manage to get inside a cell? Well, that has been studied.

In China, in 2003, a DNA microarray was used to study an infection of a macrophage by *Mycobacterium tuberculosis*. What was found was a tremendous change in the operation of the cell.

There were 25 genes up-regulated; 341 genes down-regulated; and the VDR [NR1I1] nuclear receptor, which is the one that we have found is the focus of disease activity in Sarcoidosis, was down-regulated 3.3 fold by the infection of *Mycobacterium tuberculosis*.

In other words, once a pathogen gets inside the cells of the immune system, it changes the way that the immune system works.

#### 08:36

The Microbiome messes with our own human biology.

Inside the cell many, many metabolites are affected by genes from the microbial metagenome and the sum total of all these interactions gives rise to the symptoms which are suffered during chronic disease.

The sum total: we are not talking about one or two symptoms, we are talking about thousands of symptoms.

The genes from microbe A and genes from microbe B might lead to a particular dysfunction which I will call X.

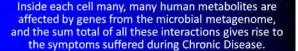
Wheras genes from a number of other microbes [C+D+E+F] combine their dysfunction which I will call Y.

The genomes in the microbiome accumulate gradually during life. We are born with some but we pick them up gradually, based on our environment. Genes from the accumulated metagenome determine the clinical dysfunction, and therefore, the disease symptoms.

#### 09:34

The Interactome is huge. The Interactome is a name given for all the interactions within a cell. When we look at those that are involved in disease, it is absolutely huge.

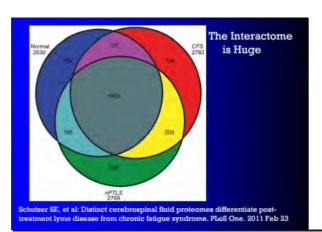
This is a study which was just performed on CSF from Chronic Fatigue Syndrome patients here [next to red], Chronic Lyme Disease, TPLDS, patients here [next to green], and healthy controls up there [next to dark blue].



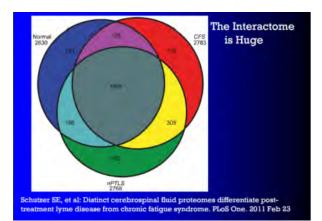
The Microbiome messes with our Human Biology

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

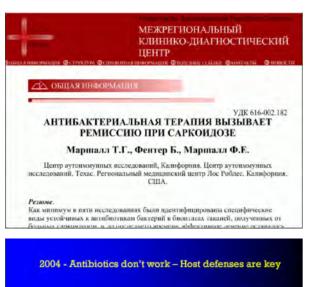
The genomes accumulate gradually during life. Genes from the accumulated metagenome determine the clinical dysfunction, and the disease symptoms.



© 2012 Autoimmunity Research Foundation. All rights reserved.



Genetic science not yet grasping this complexity UCSF Oregon 20-gene signature TCR+JS+CCR genes 20-gene signature TCR+JS+CCR genes 0.65 0.70 0.75 0.80 0.85 0.7 0,5 0.5 0.6 0,0 Acountcy u T. Zhang W. Swe iss NJ, Chen ES, Moller DR, et al. (2012) Peripheral Blood Gene is PLOS ONE 7(9) d Sa e44818. doi:10.1371/journal.pone.0044818



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 human cells, microbes clearly have to evolve to knock out the VDR, so that they don't have to deal with the cell's innate defenses Out of about 2783 total proteins measured in CSF, a similar number for healthy controls and PTLDS, these were absolutely unique to each of the disease processes:

For healthy human beings, there were 724 proteins [dark blue] that were not found in either CFS or PTLDS. In PTLDS, there were 692 found that were unique [green] and in CFS found, there were 738 that were unique [red].

We are not talking about a single [intracellular interaction] pathway here. We are not talking about something simple like JAK-STAT that we can knock out. We are talking about an incredibly complex set of interactions which lead to these incredibly complex diseases we call chronic disease.

#### 10:54

And of course, genetic science is still struggling with that complexity. This is a paper published just last week trying to differentiate healthy controls from Sarcoidosis patients, with about an 80% accuracy, which—they used 20 genes—which clearly is not nearly enough. We are talking about hundreds of thousands of total genes.

#### 11:19

So back in 2004, professor Alexandre Vizel translated one of our papers for the Kazan medical journal. As you can see, it says that antibiotics were effective in causing remission in Sarcoidosis.

Well, that is not exactly true. We were wrong.

#### 11:49

The patients were, in fact, being given antibiotics but they were also being given a drug for palliation—a drug called olmesartan for palliation. And in the years which have followed 2004, in the last decade almost, we found that the thing that did all the work—the key thing to knocking down the microbiome, the key element or strengthening the innate immune activity so the innate immunity of the idividual can attack the microbiome.

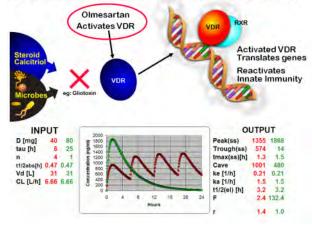
The antibiotics are relatively ineffective and we dropped them about four years ago.

2004 - Antibiotics don't work - Host defenses are key

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 human cells, microbes clearly have to evolve to knock out the VDR, so that they don't have to deal with the cell's innate defenses

#### Olmesartan re-activates VDR, and Innate Immunity



#### 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

The other thing that is very interesting that this is unique. The way that the innate immune system works is unique to *Homo sapiens*.

Because only in *Homo sapiens* is one nuclear receptor which expresses genes for TLR2, which is a key defense for the cytoplasmic region if the cell—as well as Cathelicidin and beta Defensin anti-miocrobials—all of which are associated and essential to intra-cellular innate immune defenses.

In order to survive within human cells, microbes clearly have to knock out the VDR. Microbes cannot exist in the presence of Cathelicidins. They are very, very effective anti-microbials. The beta Defensins are also very effective and TLR2 is a key signalling receptor. So in order to survive, they [microbes] have to knock out the VDR. That understanding is key to the next step.

#### 13:31

The next step is to find a drug which actually activates the VDR.

What we found was the drug that we were using back when we published the article from Kazan, we were using this drug as a palliative, because it was designed to knock out the angiotensin-II, type 1 receptor, the angiotensin receptor. It is a sartan and it was designed to reduce blood pressure.

But if you change the dosing a little — if you change the dosing so that instead of giving one dose a day—shown by the green pulse on the graph—we give multiple pulses at four to six hour intervals, you can see we build up a basal level of concentration in the bloodstream.

## And that changes the operation of the drug. At that point, it starts to activate the VDR.

That is important because in normal, healthy idividuals, the endogenous steroid calcitriol will activate the VDR and that will then cause gene transcription of at least a thousand genes that the VDR is totally responsible for.

But when the microbes come along, they produce toxins. I mentioned gliotoxin and we will see that in a later slide. Gliotoxin is one of the identified toxins that knocks out the VDR, stops it from being activated, stops the endogenous steroid from activating it.

#### 15:07

Once we applied that to a human cohort, we got some amazing results.

This is one of my colleagues from the Autoimmunity Congress in Asia last year [2011], and the key that was really interesting was the coupling of cognitive and memory difficulties with the primary condition, the Sarcoidosis. As the Sarcoidosis disappeared, so did the cognitive and memory difficulties.

### Case History – AF (Male)

1982, Jan: Diagnosis – Pulmonary Sarcoidosis – Rx: Prednisone 1982, July: Committed to mental hospital – Dx: prednisone induced 1985, Feb: Kidney stone surgically removed 1987: Cataract removed, left eye 1993, May: Squamous Cell Carcinoma removed from rt ear 1995: Pacemaker – first degree, bundle branch blockage

1999, Aug: Histiocytoma removed 2000 Nov: Ejection fraction 15%; Surgery for inability to swallow 2001, March: Heart Attack – Quadruple Bypass Surgery

2003, Jan: Cardioversion

2003, Nov: Quad Bypass repeated (and calcification reported) 2004, Jan: Pleural effusion drained - 2400ml

2005, May: - began Olmesartan

ept: Calcified Lymph Nodes noted

011 July: 72yo, no Sarcoidosis remains, residual fibrosis & scarring, cataracts gone, eyes stable, cardiac auscultation normal, colitis in remission, no oxygen, tinnitus gone, enjoying life

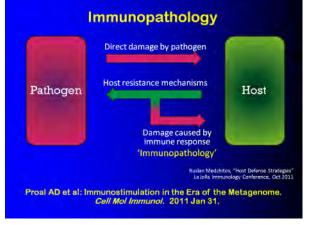
#### Case History - AC (Female)

As a child—kidney inflammation, tonsillectomy 1975: Post-partum Breast Lumpectomy, peripheral neuropathy 1979: sacroiliac arthritis 1982: goiter, nodules on thyroid, pituitary adenoma removed 1985: Enlarged spleen, Mononucleosis, Palpitations. Foot edema. 1989: Levothyroxine prescribed 1995: Decreased mobility, positive ANA (Dx: Lupus) 2002: Prescribed Beta Blocker (Atenolol) 2003, Sept: Hip fracture – DXA scan osteoporosis – T4 now 125ug 2003, Dec: CT Thorax - Hilar Adenopathy - chest rash 2004, Feb: Mediastenoscopy, Non-caseating granuloma -> Sarcoidosis 2004, July: ---- Began Olmesartan -2005, Oct: Levothyroxine reduced to 75ug 2011, May: DXA scan- Osteoporosis remits to Osteopenia, 25-D=30nmol 2011, Dec: Chest Xray negative for Sarcoidosis, no fibrosis, infiltrates or lymphadenopathy, 61yo, enjoying life, active in community affairs

#### Case History - AS (Female)

- 2003: Unable to walk >10 steps w/o rest.
- 2004, Feb: Rx: Oxygen, 4L/min
- 2004, March: Oxygen 3L/min at rest, 6L/min walking (stop every 200ft) 2004, May: DLCO=25%, Dx: Sarcoidosis, Rx:Prednisone 40mg qd
- 2005, May Weaned prednisone
- 2006, April: In pulmonary distress, prescribed End Of Life Care
- 2006, May: Commenced Olmesartan -2007: Oxygen 5L/min 24/7, but surviving

- 2008: Oxygen 3L/min 24/7 2009: Oxygen 2L/min, intermittent (p.r.n.)
- 2011: Felt much better, resumed some personal routine, light exercise, was able to fly w/o Oxygen (12hr flight, sat=85-90%) 2012: 62yo, Snowshoeing added to daily exercise, in May hiked to view Mt St Helens at 5000ft without stopping, went on Sea-kayaking trip, in June walked half-marathon, DLCO=38%



#### 15:36

Now I have three quick case histories here. All three of them were pretty sick individuals.

This is a male, born in 1939 with Ricketts. He is now 72 years old. [He] had two various problems and then ended up with two quadruple bypass surgeries, plus echocardiogram version and also had a pacemaker implanted. Then [he] was really sick and about 2004 had a 2400ml pleural effusion. But after beginning olmesartan you will see that there are very few entries. The patient is doing very well. No oxygen anymore. No Sarcoidosis remains clinically at this point. The patient is enjoying life.

#### 16:34

This is a female, AC, who as a child had various problems; tonsillectomy, kidney inflammation, breast lumpectomy post partum, goiter, enlarged spleen and once she began olmesartan in about 2005, over the six years intervening, has basically cleared and is now active in community affairs and enjoying life again at 61 years old.

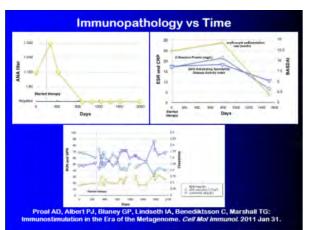
#### 17:06

This is a case history of somebody who lives in Alaska. Was on oxygen-very seriously on oxygen-was prescribed end of life care in 2006 but went instead to the experimental therapy of olmesartan and now does not need oxygen, is very active, just did a sea kyaking trip without any oxygen at all and walked a halfmarathon without any oxygen at all.

So this is the sort of improvement we are seeing when the innate immune system is reactivated to help people recover.

#### 17:58

The problem, of course, is immunopathology. Because when the host resistance kicks in here, then there is always some damage caused by the immune response-the Immunopathology. That has to be managed.



#### 18:11

Immunopathology changes with time. Unfortunately, the symptoms get worse before they get better. You can see here there is a peak in the ANA of this arthritis patient, and this ankylosing spondelitis patient also gets worse before they start to resolve.

#### 18:28

Let us go back to the start again:

"Everything is infectious until proven otherwise."

The question is, why do we treat infection with immunospression? Palliation of symptoms usually makes the underlying condition worse over time.

We now can routinely find microbial DNA in "healthy" human blood using modern sequencing techniques. So, surely, we have to adapt the clinical paradigm. We have to understand we are dealing with microbes inside people and not just symptoms that have to be palliated.

Ultimately, this may come down to a choice for mankind, between the current paradigm of feeling good, palliation of symptoms, and a choice for health, the absence of disease long term.

#### 19:18

I am going to talk more about TB in my next presentation which is on Saturday [October 20, 2012], mid-day, so those of you that can get to that presentation I just want you to know I will talk more about TB in that particular one. Particularly, drug resistance and how it relates to the microbiome.

Thank you.

#### **Moderator:**

One minute for quick questions.

#### Question:

What is the name of the drug you used?

#### Marshall:

We used a drug which was approved by the FDA in America for high blood pressure called a Sartan [spelled], a drug called olmesartan medoxomil. We found that olmesartan medoxomil was unique in that when we changed the dosing, it targeted other

"Everything is infectious until proven otherwise" Infection and Autoimmunity, Ed: Yehuda Shoenfeld and Noel Rose, 2004

### So why do we treat infection with immunosuppression?

Palliation of symptoms usually makes an underlying infectious condition worse over time.



Since we can routinely find microbial DNA in 'healthy' human blood, surely the clinical paradigm must adapt? Mankind faces a choice between 'feeling good' (palliation of symptoms) and 'health' (the absence of disease).

World TB cases fall, but drug-resistance a worry : WHO

By set test test houses DHEADD Pleases: New Addectation relation trapped 13 period workshop and task test and new y 5 million hav intentions, the Rode Input Organization and 18 entries a manual problem that sould ensure 1 increment annual have solving to legit to

It to ensure assessment research thereavers, 4HO pais, and integrate the property with they revealed a result of 18 a barry dampined end enter, year manage functionals of thesametic of annual ensure an automotally tritecting others with the particularity deadly tori of the devices (barrant the significantly deadly tori of the devices). Concern the significant facility of 3.1 million parage 50.8 with Sectionary in the significant in the significant of the devices.



based on tips for this contrary and antistics points a result phase of program + Ne Aply agains (B comp for 1) remains been based and and all of other two free free free table that 1000. Receiptor the channel program is developing and disputition time for the patients is to publy based to they excitate disputition of H and H is subset for they excitate disputition.

But the sease cand allow progress in standard planes of drig-resonant future. Second result officials called a proving

receptors beside the angiotensin-II receptor that it was designed to target. So it was olmesartan medoxomil is what we found was useful.

#### Question:

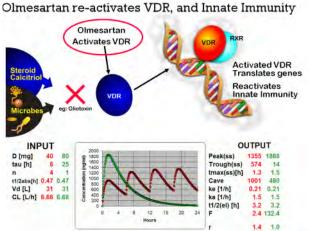
What are the doses and what is the duration of treatment?

#### Marshall:

The duration of treatment is very long. Hardly anybody responds within the first two years. They start to respond clinically, typically, at beyond the two-year point. The disease resolution typically takes three to six years. The patients often get better beyond that, particularly in terms of cognitive and memory issues.

So effectively, we are talking about a disease that takes a lifetime to gradually come on and which takes about a decade to fully get rid of.

It is not a quick fix at all.



The dosing is 40mg, every four to six hours, of olmesartan medoxomil. It is what we use. So that keeps the blood levels—if you remember the slide—you can see the blood levels stay below what is known to be safe for that drug.