

Microbial Metagenomics – Predicting and Preventing human chronic disease

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Abstract

For decades, researchers have observed that obligate intracellular pathogens, such as EBV and *Mycobacteria*, seemed to be associated with chronic autoimmune and neurologic disease - yet no definitive causal relationship could be established. Lacking an understanding of the disease process, Science was unable to develop predictive or preventative therapies. Antibiotics had limited therapeutic effectiveness, neither were antivirals capable of reliably reversing the chronic inflammation. However, the new science of Metagenomics has allowed us to clarify the mechanism whereby microbial pathogens drive human chronic disease processes - thousands of persistent viral and bacterial genomes, accumulating in the nucleated cells of a human body over the course of a lifetime - forming the 'Human Microbiome'. Persistent intra-phagocytic genomes interfere with human gene transcription (and the Human Interactome) so that bodily dysfunction incrementally increases with the passage of years. In order to persist, these intra-cellular microbial communities must suppress human innate immunity, suppress the defenses of the nucleated cells in which they have made their homes. As they incrementally act upon the human genome they additionally cause disease dysfunction due both to molecular mimicry and persistent inflammation¹. So, how is it possible to delay or reverse these disease processes? We utilized a Translational approach, concentrating on a better understanding of a key pathway which the microbes have to knock down, the VDR Nuclear Receptor. A decade ago we retargeted a common drug to reverse the microbes' effect on this pathway, and our clinical collaborators have subsequently treated hundreds of patients with chronic diseases ranging from Sarcoidosis, Arthritis and Spondylitis to manic depression (bipolar affective disorder)². Since humans accrete organisms into their microbiota progressively throughout life, this new knowledge allows us to predict who will become ill, and choose the best therapy to prevent subclinical disease progression. At least one biomarker of innate dysfunction is already in common use, and our ongoing research has identified other biomarkers which change during the recovery process, opening the door to population-

wide interventions to predict and prevent the autoimmune, neurologic, and psychiatric manifestations of chronic inflammatory disease.

What is this ‘VDR Nuclear Receptor’?

The VDR Nuclear Receptor is a Type 1 Nuclear Receptor, similar to the Glucocorticoid, Thyroid and Androgen receptor families. It was initially thought to be only activated by a steroid which is part of the body’s ‘vitamin D’ metabolism, but retinoids and lipids are now also recognized as just some of the many ligands which can activate the VDR. Actually, the use of the word ‘vitamin’ is a misnomer, as all the D metabolites are steroidal in nature, and are synthesized in the nucleated cells from cholesterol, or, to be precise, 7-dehydrocholesterol. Decades of folklore have built a misleading narrative as to how these steroids act on the human body, our several papers on this issue elucidate their precise molecular activity^{3,4}.

Nowadays, it is understood that the activities of the human VDR extend well beyond the confines of PTH and Calcium metabolisms. It has been confirmed that over 913 genes are transcribed by the VDR, including some which are key to the body’s response to Cancers (eg MTSS1 -- Metastasis Suppressing Protein #1) as well as key components of the innate immune system (eg Cathelicidin, Beta-defensins, and TLR2)⁵.

It should be noted that the importance of the VDR to innate immune function is unique to *Homo sapiens* -- no other animal models have evolved the same functionality for this receptor. This is a primary reason for the mistakes embodied in current Vitamin D pragma, as mouse models have misled researchers from understanding the unique activity of this receptor in *Homo sapiens*. Indeed, the innate immune system of the mouse is substantially different from that of man. Nobel Laureate Bruce Beutler summarized some of these differences at the 2009 Mammalian Genomes Conference as:

"Well, humans have TLR10, and mice have TLRs 11, 12 and 13 and the functions of these 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 neuropathogenic E.coli .. Those [TLR] 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"

The differences between the actions of SIV and HIV are another example of this immune dichotomy.

VDR and Estrogen Receptors – ‘Yin and Yang’

Although it has been confirmed that VDR activity definitely affects the expression of at least 913 genes, the advent of Fluorescence in situ hybridization (FISH) has shown that the presence of VDR promoters is ubiquitous throughout the human genome. In this respect it is useful to note that the Estrogen Receptor (ER) precursor protein is expressed by the VDR, and the VDR precursor protein is expressed by ER-beta, not unlike ‘Yin and Yang’. The known ligands for ER do not activate the VDR, and vice versa. This is unusual amongst the Nuclear Receptors, as the Type 1 receptor ligands often have significant activity in receptors other than their primary target. This is especially true for 1,25-dihydroxyvitamin-D, which has a high affinity for both the adrenal and thyroid axes⁶.

Nasun Hah has shown that the Estrogen Receptor is directly and indirectly responsible for expression of 6003 genes, or 26% of the entire genome⁷, and FISH indicates that it is likely the VDR will ultimately be found to be closely involved with the expression of a similarly significant proportion of the entire human genome.

The VDR is at the heart of Human Chronic Disease

Those components of the Human Microbiota which have evolved to parasitize phagocytes, and live within them, are able to modify the Interactome of those phagocytes so as to suppress the expression of the endogenous antimicrobials which would otherwise be able to kill these intracellular microbial colonies. A significant, perhaps critical, human innate defense is provided by Cathelicidin and TLR2. Unless the antimicrobial activities of cathelicidin, and the cytoplasmic signaling of TLR2, can be disabled there is little possibility of microbial survival (in this context it should be noted that Cathelicidin is capable of anti-bacterial, anti-viral and anti-fungal activity).

Since the VDR is a key element in the expression of the human Cathelicidin and TLR2 genes, it is no surprise that some microbial species have evolved methods for down-regulating expression of and by the VDR. Species confirmed to suppress VDR expression include Epstein-Barr Virus (EBV)¹⁹, Cytomegalovirus (CMV)²⁰, Hepatitis C Virus (HCV)²¹, *Mycobacteria leprae*⁹, *Mycobacteria tuberculosis*²², *Borrelia burgdorferi*²³, *Chlamydia trachomatis*²⁴ and *Aspergillus fumigatus*²⁵.

It is therefore not surprising that the vitamin-D metabolism is dysregulated in so many chronic human diseases.

VDR dysfunction is an Predictive marker for chronic inflammatory disease

We have previously reported⁸, based on data from patients with a variety of chronic diagnoses, that the active metabolite 1,25-dihydroxyvitamin-D, which is rarely measured, is elevated in a majority of patients with chronic inflammatory disease. Further, the precursor, 25-hydroxyvitamin-D, is most

often expressed well below the 50 nanomolar level at which it starts to exert its immunosuppressive activity.

Measurement of these two metabolites can give early prediction that an individual is accumulating a microbiota which is likely to lead to immune dysfunction, and chronic inflammatory disease.

Other readily-available Predictive Markers

VDR dysfunction can also be measured by looking for those metabolites most sensitively expressed by the VDR. Suppression of cathelicidin has already been identified in patients with Sarcoidosis, and the degree of cathelicidin suppression is associated with the severity of the disease progression¹³.

Assay of the enzyme CYP24 may well provide an even more sensitive marker of VDR dysfunction, as CYP24 is very highly dependent on the VDR for its expression.

Comorbidities can be Predictive

As one practices medicine, it is hard not to be struck by the number of patients who present with more than one disease. Indeed, the art of diagnosis is in discerning each specific diagnosis from the multitude of often very similar signs and symptoms.

But study of these comorbidities, as a collective entity, can reveal a lot to us about the chronic disease process itself. Indeed, the PRIME study showed that in healthy European men, depressive symptoms were a time-dependent risk factor for increased coronary heart disease in the short-term, and for stroke in the long-term¹⁵.

It is critical for a practitioner to recognize the wide-ranging nature of comorbidities arising from chronic disease, and to understand the underlying common mechanisms which lead to these comorbidities. It is so easy to conclude “the patient is depressed because he/she is feeling ill,” yet it might well be much more important to observe that many comorbidities which are currently believed to be neurological or psychiatric in nature, seem to have an underlying inflammatory pathogenesis.

Bacteria in the Bloodstream

Humans are born with elemental components of the microbiota they will accumulate throughout life¹⁰. If a mother has accumulated a heavy microbial load it is likely that her children will succumb to one or other chronic disease more rapidly and more profoundly than the median. This familial aggregation down the maternal line is easily observed, even in the general-practice clinic.

Although it has long been thought that the human systemic compartment was essentially sterile, it is now clear that the human microbiota is accumulated gradually during life. Microbial RNA is easily detectable in healthy human blood by PCR, as well as Sequencing technologies^{1,11,12}

It has already been shown that the nature of the microbiota circulating in blood is *predictive* of the onset of *Diabetes* type II, and associated with the onset of Obesity¹³. However, despite the efforts of many research groups, no definitive correlations have been identified between species or phyla of bacteria in blood, and the resulting symptomology or disease presentation. Indeed, the same is true of the changes observable in the human GI tract – a dysregulation is associated with disease onset, but not in any way which would be useful as a predictive marker.

Nevertheless, the recognition that the human systemic compartment is not sterile is an essential element of any understanding which might lead to a therapeutic intervention effective against chronic inflammatory disease.

Immunosuppression vs. Immunostimulation

Various forms of immunosuppression comprise the ‘standard of care’ for chronic inflammatory disease. However, immunosuppressive therapies focus on suppression of the inflammatory symptoms, allowing the microbiome to continue to accumulate species, most probably at an increasing rate. Additionally, those elements of Interactome disorder which result from Molecular Mimicry are not reduced by immunosuppressive therapies.

We have argued that immunostimulation is the logical therapy for chronic disease. Surely the aim of any preventative therapy must be to restore those immune functions which have been perverted by the accumulating microbiota? It is fortunate that identifying the VDR Pathway, as we have done, allowed a therapeutic intervention to restore that pathway, and restore ‘health.’

Potential Preventative and Curative Therapies – Vitamin D supplementation

Even though exogenous Vitamin D supplementation might seem to be an obvious remedial therapy, study after study is confirming our own early observations -- that it just doesn’t work^{3,4}. In fact, the J-shaped curves resulting from many of the new studies are confirming that, as we earlier noted^{3,4}, Vitamin-D supplementation is providing short-term palliation, but accelerating disease progression by suppressing activity of the innate immune system, particularly by suppressing PMBC activity¹⁸. The homeostasis of both 25-hydroxyvitamin-D and 1,25-dihydroxyvitamin-D *inside each cell*, at a level of about 2 nanomolar, drives gene transcription, not the level of the metabolites in the plasma.

Potential Preventative and Curative Therapies – Antibiotics

Neither do antibiotics provide an effective cure for chronic autoimmune and inflammatory disease. Most antibiotics exert some biochemical actions on the human body, limiting the dose which one can safely use. Because the key persistent infections are intra-cellular, dilution of the blood concentration

through the cell wall means that the concentration of antibiotic where it is needed, in the cytoplasm of nucleated cells, is so low as to be only minimally efficient at killing the microbiota. It is interesting to note that many of the commonly used antibiotics do in fact molecularly modify the activity of the innate immune system, and this may well account for some of the short-term efficacy previously reported.

Olmesartan

A decade ago, we identified that the drug Olmesartan, developed to treat hypertension and having as its primary target the Angiotensin II Receptor, was capable of being retargeted to reactivate the VDR. We set up an International network of clinical collaborators, and started collecting a stream of observational data from individuals with diagnoses ranging from Sarcoidosis to CFS/ME, from Multiple Sclerosis to Bipolar Affective Disorder, from Rheumatoid Arthritis to Amyotrophic Lateral Sclerosis. In all, patients attempting the therapy presented with over 119 comorbid diagnoses. Many have been lost to followup, over the years, but many have reported objective and subjective reversal of the disease processes. The body of data we have collected allows retrospective data mining which will cut years off any future efforts to translate our study methodologies into widespread clinical use.

Immunopathology – a problem when implementing an Immunostimulative Therapy

When the stimulated immune system recognizes the intracellular microbiota, it attacks -- causing a cytokine cascade which can result in Immunopathology, a worsening of symptoms before the disease begins to fade. I will not dwell on this problem here, as a focus on predictive medicine will allow individuals to receive preventative therapy before their bodies become so ill that management of immunopathology becomes a major problem. In this context, I will note that *healthy* individuals can ingest the retargeted dosage of Olmesartan without generating any immunopathology at all.

‘Prevention’ will be much easier than it has been to implement a ‘cure’.

Treatment must be guided by the Disease Model

Perhaps the biggest problem Medicine will have in assimilating the new Science is in retiring the concept that drugs only affect one subsystem, that you give an NSAID for rheumatic disease, an ARB for cardiovascular disease, and an SSRI for depressive disorders. Sadly, drugs don’t work that way. Both SSRI and NSAIDS heavily suppress the immune system, inhibiting the immunostimulative activity of the Olmesartan, and terminating recovery. Extremely judicious use of drugs is required, and prescription needs to be guided by science, by disease model, and not by piecemeal therapy of individual comorbidities.

The disease model is also needed, for example, to understand the surge in creatinine some patients experience as they recover. We have seen creatinine surge to five times normal without any harm accruing to the individual. Medicine has adopted a model of metabolic health, characterized by a relatively small number of key metabolites, and is not yet equipped to deal with idiosyncratic departures from the normal range it expects for those metabolites^{2,16,17}.

Looking forward...

For just over a decade, our clinical collaboration has been working with (mostly) seriously ill patients. The follow-up periods, typically exceeding 5 years, have made it a struggle to maintain the integrity of the data we were collecting. Yet, looking back, it has been successful in ways that we never thought possible. Hindsight has given us 20:20 vision. For example, we saw Bipolar Disorder disappear as a patient's Fibromyalgia resolved. We saw arthritis auto-antibodies fall away to zero as patients recovered their mobility. We saw Sarcoidosis Xrays and CT scans become clear, we saw patients rejoining family life, patients going back to work. Yet the true value of all that work could well be the quarter of a million discrete progress reports, candid Patient Reported Outcomes, documenting the tribulations and joys of an immunostimulative therapy in chronic disease. The next decade must be clearly focused on the goal of reducing Predictive and Preventative Medicine to clinical practice, so that future generations do not have to endure the suffering that our first cohort has sustained.

Citations:

1. Proal AD, Albert PJ, Marshall TG: **Autoimmune Disease and the Human Metagenome.** in *Metagenomics of the Human Body*; Ed. Nelson, K; pub Springer, 2011. ISBN: 978-1-4419-7088-6
2. Proal AD, Albert PJ, Blaney GP, Lindseth IA, Benediktsson C, Marshall TG: **Immunostimulation in the Era of the Metagenome.** *Cell Mol Immunol.* 2011 Jan 31.
3. Marshall TG: **Vitamin D discovery outpaces FDA decision making.** *BioEssays.* 2008 Feb;30(2):173-82
4. Albert PJ, Proal AD, Marshall TG: **Vitamin D: the alternative hypothesis.** *Autoimmunity Reviews.* 2009 Jul;8(8):677-81
5. Wang TT, et al: **Large-scale in silico and microarray-based identification of direct 1,25-dihydroxyvitamin D3 target genes.** *Mol Endocrinol.* 19(11):2685-95.
6. Proal AD, Albert PJ, Marshall TG: **Dysregulation of the Vitamin D Nuclear Receptor may contribute to the higher prevalence of some autoimmune diseases in women.** *Ann N Y Acad Sci.* 2009 Sep;1173.
7. Hah N, et al: **A rapid, extensive, and transient transcriptional response to estrogen signaling in breast cancer cells.** *Cell.* 2011 May 13;145(4):622-34.

8. Blaney GP, Albert PJ, Proal AD: **Vitamin D metabolites as clinical markers in autoimmune and chronic disease.** *Ann N Y Acad Sci.* 2009 Sep;1173:384-90.
9. Liu PT, et al: **MicroRNA-21 targets the vitamin D-dependent antimicrobial pathway in leprosy.** *Nat Med.* 2012 Jan 29;18(2):267-73. doi: 10.1038/nm.2584
10. DiGiulio DB, et al: **Microbial prevalence, diversity and abundance in amniotic fluid during preterm labor: a molecular and culture-based investigation.** *PLoS One.* 2008 Aug 26;3(8):e3056.
11. Nikkari S, et al: **Does blood of healthy subjects contain bacterial ribosomal DNA?** *J Clin Microbiol* 39: 1956–9
12. Moriyama K, et al: **Polymerase chain reaction detection of bacterial 16S rRNA gene in human blood.** *Microbiol Immunol.* 2008 Jul;52(7):375-82.
13. Barna BP, et al: **Alveolar Macrophage Cathelicidin Deficiency in Severe Sarcoidosis.** *J Innate Immun.* 2012 Jul 2:569-578
14. Lantieri O, et al: **Involvement of tissue bacteria in the onset of diabetes in humans: evidence for a concept.** *Diabetologia.* 2011 Dec;54(12):3055-61.
15. Majed B, et al: **Depressive Symptoms, a Time-Dependent Risk Factor for Coronary Heart Disease and Stroke in Middle-Aged Men: The PRIME Study.** *Stroke.* 2012 Jul;43(7):1761-1767. Epub 2012 Apr 19.
16. Ryan MJ, Tuttle KR: **Elevations in serum creatinine with RAAS blockade: why isn't it a sign of kidney injury?** *Curr Opin Nephrol Hypertens.* 2008 Sep;17(5):443-9.
17. Palmer BF: **Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers: what to do if the serum creatinine and/or serum potassium concentration rises.** *Nephrol Dial Transplant.* 2003 Oct;18(10):1973-5.
18. Kimball S, et al: **Cholecalciferol plus calcium suppresses abnormal PBMC reactivity in patients with multiple sclerosis.** *J Clin Endocrinol Metab.* 2011 Sep;96(9):2826-34.
19. Yenamandra SP, et al: **Expression profile of nuclear receptors upon Epstein -- Barr virus induced B cell transformation.** *Exp Oncol.* 2009 Jun;31(2):92-6.
20. Chan G, et al: **Transcriptome analysis reveals human cytomegalovirus reprograms monocyte differentiation toward an M1 macrophage.** *J Immunol.* 2008 Jul 1;181(1):698-711.
21. Gal-Tanamy M, et al: **Vitamin D: an innate antiviral agent suppressing hepatitis C virus in human hepatocytes.** *Hepatology.* 2011 Nov;54(5):1570-9.
22. Xu Y, et al: **Using a cDNA microarray to study cellular gene expression altered by Mycobacterium tuberculosis.** *Chin Med J (Engl).* 2003 Jul;116(7):1070-3.
23. Salazar JC, et al: **Activation of human monocytes by live Borrelia burgdorferi generates TLR2-dependent and -independent responses which include induction of IFN-beta.** *PLoS Pathog.* 2009 May;5(5):e1000444.
24. Dlugosz, et al: **Infection of human enteroendocrine cells with Chlamydia trachomatis: a possible model for pathogenesis in irritable bowel syndrome.** *Neurogastroenterol Motil* (2011)

25. Coughlon, et al: **Elevated Gliotoxin Production By Aspergillus Fumigatus Downregulates Vitamin D Receptor Expression in Cystic Fibrosis Airways.** *Am J Respir Crit Care Med* 183;2011:A5733