ME/CFS in the Era of the Human Microbiome: Persistent Pathogens Drive Chronic Symptoms by Interfering With Host Metabolism, Gene Expression, and Immunity

with Amy Proal, Ph.D.

November 14, 2019 | 1:00 PM Eastern
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ME/CFS in the Era of the Human Microbiome: Persistent Pathogens Drive Chronic Symptoms by Interfering With Host Metabolism, Gene Expression, and Immunity

with Amy Proal, Ph.D.

November 14, 2019 | 1:00 PM Eastern
Myalgic Encephalomyelitis/Chronic Fatigue Syndrome in the Era of the Human Microbiome: Persistent Pathogens Drive Chronic Symptoms by Interfering With Host Metabolism, Gene Expression, and Immunity

Amy Proal, Autoimmunity Research Foundation/PolyBio
Millions of patients across the globe are suffering with myalgic encephalomyelitis (ME/CFS).

Currently there is no one disease-specific biomarker and severely ill patients are often wheelchair dependent, bedridden and unable to perform basic tasks of work or daily living.

#millionsmissing
Myalgic Encephalomyelitis/Chronic Fatigue Syndrome

Myalgic Encephalomyelitis (ME) = swelling of the brain

- Unrelenting fatigue that does not improve with rest
- Post-exertional malaise (cannot recover for several days after exercise)
- Cognitive impairment
- Orthostatic intolerance
- Muscle pain
- Pain in the joints without swelling or redness
- Headaches of a new type, pattern, or severity
- Swollen or tender lymph nodes in the neck or armpit
- A sore throat that is frequent or recurring
- Chills and night sweats
- Visual disturbances
- Sensitivity to light and sound
- Nausea
- Allergies or sensitivities to foods, odors, chemicals, or medications
ME/CFS may be a **neuroinflammatory** disease.

Nakatomi et al. (2014) found increased microglial activation in the brainstem of ME/CFS patients relative to controls.
ME/CFS may be a **neuroinflammatory** disease

Evidence of widespread metabolite abnormalities in Myalgic encephalomyelitis/chronic fatigue syndrome: assessment with whole-brain magnetic resonance spectroscopy

Christina Mueller¹ · Joanne C. Lin¹ · Sulaiman Sheriff² · Andrew A. Maudsley² · Jarred W. Younger¹

Choline and lactate are markers of rapid brain cell turnover and neuroinflammation. **Levels of choline/lactate were elevated in the ME/CFS brain** (left) but not in healthy brains (right).

There are also early signs of **heat buildup** (indicating neuroinflammation) in the ME/CFS brain. Five brain regions involved in regulating **fatigue and flu-like symptoms** were most impacted.
Our recent paper explains how persistent infection + microbiome + virome imbalance can contribute to ME/CFS.
Why study persistent infection in ME/CFS?

When you study ME/CFS through the lens of persistent infection the disease is not a “mystery”
Why study persistent infection in ME/CFS?

The Outbreaks

## A New Clinical Entity?

In 1917 von Economo reported a small outbreak of an illness in which the main features were fever, stupor, and ophthalmoplegia: 2 of his 13 patients died and at necropsy there was evidence of inflammation of the brain substance. During the next two years a great many similar outbreaks were recorded and by 1921 the disease had reached epidemic proportions in almost every country in Europe. In spite of perplexing variations in the clinical picture from case to case, locality to locality, and even from season to season, it soon became clear that for practical purposes a new clinical entity had appeared. In 1924, 5089 cases of encephalitis lethargica were notified in England and Wales alone, but by the beginning of the next decade confirmed cases of this dangerous disease had become sporadic and by 1939 they were extremely rare. By the end of the late war, the centre of interest had shifted to poliomyelitis as by far the most prevalent and disabling infection.
ME/CFS is repeatedly tied to signs and symptoms of persistent infection

- Most ME/CFS patients present with symptoms after suffering from a severe bacterial or viral infection

- These infections often correlate with travel to a foreign country or exposure to pollutants or molds, suggesting that such pathogens take advantage of factors that compromise the host immune system

- At different points in history, ME/CFS has been called “Post-Polio Syndrome,” “Chronic Mononucleosis Syndrome” and “Post-Viral Syndrome” due to the fact that chronic symptoms are often noted after acute infection with Polio Virus, Epstein Barr Virus, enteroviruses, influenza or a range of other pathogens

- Severe flu-like symptoms (sore throat, swollen lymph nodes etc)

- The relapsing-remitting nature of ME/CFS symptoms

- Cytokine activation/immune cell activation in ME/CFS clarifies that the disease is associated with an inflammatory response
Trend #1: There are growing number of “post”-infectious chronic conditions

In most cases the infecting organism can still be detected months + years after initial infection in certain tissues

- Post-Zika
- Post-Measles
- Post-Ebola
- Post-Influenza
- Post-Dengue
- Post-Polio
- Post Treatment Lyme disease (*Borrelia*)
“After acute infections, enteroviruses can persist in patients resulting in manifestation of ME/CFS. Chronic enterovirus infection in an immunocompetent host may be an example of a stalemate between attenuated, intracellular viruses and an ineffective immune response”
Trend #2: A growing number of neuroinflammatory conditions are now increasingly tied to persistent infection in the blood + brain: Alzheimers, Parkinson’s, ALS

**Neuron**

*Multiscale Analysis of Independent Alzheimer’s Cohorts Finds Disruption of Molecular, Genetic, and Clinical Networks by Human Herpesvirus*

**Highlights**
- Common viral species frequently detected in normal, aging brain
- Increased HHV-6A and HHV-7 in brains of subjects with Alzheimer’s disease (AD)
- Findings were replicated in two additional, independent cohorts
- Multiscale networks reveal viral regulation of AD risk, and APP processing genes

**Authors**
- Ben Readhead, Jean-Marie Haase-Miranda, Cory C. Funk, ..., Michelle E. Bricht, Sam Gandy, Joel T. Dudley

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**In Brief**
Readhead et al. construct multiscale networks of the late-onset Alzheimer’s disease (AD)-associated viruses and observe pathological regulation of molecular, clinical, and neuropathological networks by several common viruses, particularly human herpesvirus 6A and human herpesvirus 7.

**Original Research Article**
*Neuron*, 30 February 2019; https://doi.org/10.1016/j.neuron.2019.02.017

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**Porphyromonas gingivalis** in Alzheimer’s disease brains: Evidence for disease causation and treatment with small-molecule inhibitors

Stephen S. Dominy, Casey Lynch, Florian Ermini, Małgorzata Benedyk, Agata Marczyk, Andrei Konradi, Mai Ngu...

See all authors and affiliations

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**Searching for Bacteria in Neural Tissue From Amyotrophic Lateral Sclerosis**

Ruth Almoro, Diana Pera and Luis Carreño

Centro de Biología Molecular Severo Ochoa (CIBIO-UCM, Universidad Autónoma de Madrid, Madrid, Spain)

Despite great efforts in the investigation, the exact etiology of amyotrophic lateral sclerosis (ALS) is a matter of intensive research. We recently advanced the idea that ALS might be caused by fungal infection. Indeed, fungal and bacterial structures can be directly visualized in neural tissues of ALS patients, and a number of fungal species have been identified in the central nervous system (CNS). To date, the possibility that bacterial infections can accompany these scenarios remains elusive. Our findings establish the presence of bacterial DNA in different regions of the CNS from all ALS patients examined. Specifically, we used PCR and next generation sequencing (NGS) to precisely determine the bacterial species present in ALS tissues. Consistent with these findings, immunohistochemistry analysis of CNS sections using specific anti-bacterial antibodies identified prokaryotic cells in neural tissue. Finally, we analyzed the repeat expansions of the hexanucleotide repeat GGGGCC in SOD1, which is considered the most common genetic cause of ALS in patients, using DNA extracted from ALS CNS tissues. We failed to find this repeated sequence in any of the seven patients analyzed. Our results indicate that bacterial DNA and prokaryotic cells are present in CNS tissues, leading to the concept that both fungal and bacterial infections exist in patients with ALS. These observations lay the groundwork for the use of appropriate therapies to eradicate the polymicrobial infections in ALS.

**Virus-like particles and enterovirus antigen found in the brainstem neurons of Parkinson’s disease**

Robert R. Dourmashkin, Sherman A McCall, Steven Patterson

*Published in F1000Research 2018* •
DOI: 10.12688/f1000research.13626.2

Background: In a previous study on encephalitis lethargica, we identified a virus related to enterovirus in autopsy brain material. Transmission electron microscopy (TEM), immunohistochemistry (IHC) and molecular analyses were employed. Our present objective was to investigate, using a similar approach, as to whether virus-like particles (VLP) and enterovirus antigen are present in Parkinson’s disease (PD) brainstem neurons. Methods: Fixed tissue from autopsy specimens of late onset PD and... CONTINUE READING
Amyloid beta also exhibited antimicrobial activity against a range of common microorganisms with a potency equivalent to, and in some cases greater than, cathelicidin (LL-37). These pathogens included *Salmonella. typhimurium, Candida albicans*

Rudy Tanzi + Robert Moir at Harvard show that amyloid beta: Alzheimer’s “plaque” may be a potent antimicrobial peptide (an important part of the human immune response towards infectious agents in brain and other tissues)
Amyloid traps and neutralizes pathogens in brain tissue

*R Tanzi, R. Moir, Harvard University: amyloid beta plaques form to “fight off” microbes in the brain
Takeaway
The presence of amyloid in a sample could indicate the presence of specific pathogens

*Snare that Virus.* Aβ42 rapidly coconos viral particles, forming fibrils after 15 minutes (left), nets after 30 (middle), and impenetrable clumps after two hours (right). [Courtesy of Neuron, Eimer et al.]

*This image shows amyloid beta “entrapping” a herpesvirus*
Can infections trigger alpha-synucleinopathies?

Christopher T. Tulisiak, Gabriela Mercado, Wouter Peelaerts, Lena Brundin, Patrik Brundin

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Can infections trigger alpha-synucleinopathies?
Cognitive impairment in ME/CFS can be extremely debilitating.

“I went from being a company executive to barely reading over the 4th grade level.” --Alayne, age 45

“...and all of a sudden I couldn’t understand how to find anything. I couldn’t write an email, because I couldn’t construct a sentence.” --Natalie, age 21

“Sometimes when people are talking, it is as if some of the words are in a foreign language. I hear the words. But they don’t make sense.” --Claire, age 52
One research team has identified amyloid deposits ("plaques") in the ME/CFS brain

What are the new findings?

- Neuropathological autopsy findings from a patient who died with a prior diagnosis of CFS report focal areas of white matter loss, neurite beading, and neuritic pathology of axons in the white matter with axonal spheroids.

- Atypical neurons displaying aberrant sprouting processes in response to injury are observed throughout cortical gray and white matter. Abundant amyloid deposits identical to AD plaques with accompanying intracellular granular structures are observed as well.

- Neurofibrillary tangles are also present in the white matter of the frontal cortex, thalamus and basal ganglia.
A chronic fatigue syndrome – related proteome in human cerebrospinal fluid

James N Baraniuk*1, Begona Casado1,2, Hilda Maibach1, Daniel J Clauw3, Lewis K Pannell4,5 and Sonja Hess S5

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**Conclusion:** This pilot study detected an identical set of central nervous system, innate immune and amyloidogenic proteins in cerebrospinal fluids from two independent cohorts of subjects with overlapping CFS, PGI and fibromyalgia. Although syndrome names and definitions were different, the proteome and presumed pathological mechanism(s) may be shared.
Virchow-Robin perivascular spaces: common in Alzheimer’s and ME/CFS (early data)

Perivascular spaces (Virchow-Robin)

1. Basal ganglia
2. Parietal cortex
3. Red nucleus of midbrain

*unpublished data from VanElzakker et al.*
Perivascular spaces + activated immune cells in the autism brain

Research Article

T lymphocytes and cytotoxic astrocyte blebs correlate across autism brains

Marcello M. DiStasio MD, PhD, Ikue Nagakura PhD, Monica J. Nadler PhD, Matthew P. Anderson MD, PhD

“Either the T-cells are reacting normally to a pathogen such as a virus, or they are reacting abnormally to normal tissue – the definition of an autoimmune disorder.”

- Matthew P. Anderson, MD, PhD (Beth Israel Deaconess Medical Center)
Trend #3: The human microbiome
There are vast microbial + viral ecosystems in the human body

These communities extend far beyond the gut and into nearly all tissue and blood

Microbiome body sites:

Skin
Oral (mouth)
Gut
Lung
Nasal (nose)
Ocular (eye)
Bladder
Vaginal/Penile
Pancreas
Liver
Circulatory (blood)
Atherosclerotic plaque
Brain (central nervous system)
The human microbiome harbors many different kinds of interacting organisms.
Microbiome communities are not always “good” and can also drive disease

**Cell**

**Tumor Microbiome Diversity and Composition Influence Pancreatic Cancer Outcomes**

**Graphical Abstract**

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**In Brief**
The distinct tumor microbiome from pancreatic cancer long-term survivors can be used to predict PDAC survival in humans, and transfer of long-term survivor gut microbiomes can alter the tumor microbiome and tumor growth in mouse models.
Vastly more organisms persist in blood than previously believed

Stanford study indicates that more than 99 percent of the microbes inside us are unknown to science

A survey of DNA fragments circulating in the blood suggests the microbes living within us are vastly more diverse than previously known. In fact, 99 percent of that DNA has never been seen before.

Numerous uncharacterized and highly divergent microbes which colonize humans are revealed by circulating cell-free DNA


PNAS September 5, 2017 114 (36) 9609-9614; first published August 22, 2017 https://doi.org/10.1073/pnas.1703939114
Contributed by Stephen R. Quake, July 13, 2017; revised April 28, 2017; reviewed by Serena Bruce and Enr Sega

Significance

Through massive shotgun sequencing of circulating cell-free DNA from the blood of more than 1,000 independent samples, we identified hundreds of new bacteria and viruses which represent previously unidentified members of the human microbiome. Previous studies targeted specific niches such as feces, skin, or the oral cavity, whereas our approach of using blood effectively enables sampling of the entire body and reveals the colonization of niches which have been previously inaccessible. We were thus able to discover that the human body contains a vast and unexpected diversity of microbes, many of which have highly divergent relationships to the known tree of life.

Searching for rejection

The survey was inspired by a curious observation Quake’s lab made while searching for non-invasive ways to predict whether an organ transplant patient’s immune system would recognize the new organ as foreign and attack it, an event known as rejection. Ordinarily, it takes a tissue biopsy — meaning a large needle jabbed into one’s side and at least an afternoon in a hospital bed for observation — to detect rejection.

The lab members figured there was a better way. In theory, they might be able to detect rejection by taking blood samples and looking at the cell-free DNA — bits and pieces of DNA circulating freely in blood plasma – contained therein. Apart from fragments of a patient’s DNA, those samples would contain fragments of the organ donor’s DNA as well as a comprehensive view of the collection of bacteria, viruses and other microbes living inside human beings. (Image credit: L.A. Cicero)
“…these novel microbes have potential consequences for human health. They may prove to be the cause of acute or chronic diseases that, to date, have unknown etiology…”

-Stephen Quake, Stanford University
“Seek and ye shall find”

Extensive phyla (families) of bacteria persist in healthy human blood.

The composition of these blood microbiome communities changed in patients with ALS, bipolar disorder and schizophrenia.
"Following maximal exercise challenge, there was an **increase in relative abundance of 6 of the 9 major bacterial phyla/genera in ME/CFS patients** from baseline to 72 hours post-exercise compared to only 2 of the 9 phyla/genera in controls."
Major paradigm shift:

The Harvard University Brain Microbiome Project

Mapping The Brain's Microbiome: Can Studying Germs In The Brain Lead To A Cure For Alzheimer's?

Robin Seaton Jefferson Contributor

Could it really all come down to infection? Two scientists and a team of researchers are trying to find out.

Harvard researchers, Dr. Rudolph Tanzi and Robert D. Maloi, PhD, are heading up a team, funded by the Cure Alzheimer's Fund and the Good Ventures Foundation, that has taken on mapping the microbiome, the population of microorganisms, some helpful and some pathological, that exists inside the brain.

The monumental task, dubbed The Brain Microbiome Project, will, they hope, tell them if amyloid beta plaques—known to initiate the pathological cascade of Alzheimer's disease—are being made to protect the brain and if so, from what? In
“We saw that bacteria like to be inside astrocyte cells around the blood-brain barrier. They also like to be in axons, which are projections between brain regions that conduct information.”

-Rosalinda Roberts, University of Alabama Birmingham
The human virome:

Vast communities of viruses in the human body

Viruses are the most abundant life form on the planet and in the human body.

Bacteriophages (phages) are viruses that infect bacteria and modulate their activity.

Trillions of phages persist in human tissue and blood.

We have identified and characterized ~.001% of these viruses in the human body.
An *e.coli* bacteriophage (computer generated image)
Phages interact with the human immune system

Phages **directly interact** with human cells + impact/modulate the human immune response

Provides examples of how phages can modulate innate immunity via phagocytosis and cytokine responses

Phages can impact adaptive immunity via effects on antibody production

Computational modeling predicts that phages **may play important roles** in shaping mammalian-bacterial interactions
“We’ve long known that you’ve got up to 10 quadrillion phages in your body, but we just figured whatever they were doing was strictly between them and your commensal bacteria. Now we know that phages can get inside your cells too, and make you sick”

- Paul Bollyky (Stanford University)
Nikos Kyrpides + David Paez-Espino at Berkeley’s Joint Genome Institute have transformed our ability to identify a broad range of known + novel environmental + human viruses thanks to their “Uncovering the Earth’s Virome Project”

The first paper published on the Project in 2016 increased the number of known viruses on Earth by 16-fold

**Abstract**

Viruses are the most abundant biological entities on Earth, but challenges in detecting, isolating, and classifying unknown viruses have prevented exhaustive surveys of the global virome. Here we analysed over 5 Tb of metagenomic sequence data from 3,042 geographically diverse samples to assess the global distribution, phylogenetic diversity, and host specificity of viruses. We discovered over 125,000 partial DNA viral genomes, including the largest phage yet identified, and increased the number of known viral genes by 16-fold. Half of the predicted partial viral genomes were clustered into genetically distinct groups, most of which included genes unrelated to those in known viruses. Using CRISPR spacers and transfer RNA matches to link viral groups to microbial host(s), we doubled the number of microbial phyla known to be infected by viruses, and identified viruses that can infect organisms from different phyla. Analysis of viral distribution across diverse ecosystems revealed strong habitat-type specificity for the vast majority of viruses, but also identified some cosmopolitan groups. Our results highlight an extensive global viral diversity and provide detailed insight into viral habitat distribution and host–virus interactions.

**Figure 1** Overview of the computational workflow. General pipeline of the protocol, showing the different steps required for the detection of abundant and low-abundant metagenomic viral contigs, as well as their classification into viral groups. Viral protein family models (VPPs) and all metagenomic viral contigs from IMG/VR are available through the aforementioned FTP site.
2018: The IMG/VR database **triples in size**

The JGI IMG/VR database catalogs viruses in Earth’s ecosystems including the human body. Viral diversity in IMG/VR has more than tripled since August 2016.
Meanwhile: A typical ME/CFS blood test at the doctor’s office:

Includes antibody testing for only 5-10 well-known viruses, 2-3 bacterial species

Epstein Barr Virus
Cytomegalovirus
Herpes Virus 6
Parvovirus 19
Coxsakie viruses
*M. tuberculosis*
Polymicrobial disease

ME/CFS-associated pathogens are members of complex microbiome communities

ME/CFS-associated pathogens can now be studied as interacting members of these microbiome ecosystems.
The microbiome community a pathogen enters plays a role in determining if it will survive/persist in its host.

Vaginal microbiota and susceptibility to HIV

McKenna C. Eastment\textsuperscript{a} and R. Scott McClelland\textsuperscript{a,b,c}

\textit{Image: Diego Spitaleri, SEM}
Microbiome/virome dysbiosis

Entire communities of organisms in ME/CFS tissue + blood may act + signal together to drive symptoms (microbiome/virome dysbiosis)

- Almost every well-studied human inflammatory disease is now tied to microbiome/virome dysbiosis
- Microbiome dysbiosis also happens in microbial + viral communities outside the gut
How does dysbiosis happen?

Microbiome dysbiosis is often **driven by dominant pathogens** whose signaling and activity negatively influences the activity of other nearby organisms.

Single criminal vs. a gang of criminals

- Induce biofilm formation
- Virulence factor expression
- Persist inside the cells of the immune system (intracellular)
How does dysbiosis happen?

Immunosuppression

A robust immune response is often capable of controlling pathogen virulence. However, if pathogens overcome the immune response, or the immune system is suppressed by medications, chemicals, or other environmental factors, dominant pathogens are more likely to alter their gene expression in a manner that promotes disease.
How does dysbiosis happen?

Pathobiont behavior:
Most human “commensal” microbes can change their gene expression (turn genes on/off) to act as pathogens under conditions of imbalance and/or immunosuppression.

Example: *S. aureus* causes a range of illnesses, from skin infections to life-threatening diseases such as endocarditis and meningitis. However, ~30% of the healthy human population harbors *S. aureus* as a member of the normal nasal microbiome. *S. aureus* virulence in these communities is determined by a number of factors, including the signaling and competitive strategies employed by neighboring microbes.
Dysbiosis in driven by changes in organism/pathogen ACTIVITY

Oral microbiome example: different organisms act together to drive periodontitis (a complex oral polymicrobial disease)

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**Genome Medicine**

Research | Open Access | Published: 27 April 2015

**Functional signatures of oral dysbiosis during periodontitis progression revealed by microbial metatranscriptome analysis**

Susan Yost, Ana E Duran-Pinedo, Ricardo Teles, Keerthana Krishnan & Jorge Frias-Lopez
*P. gingivalis*: the bacterial pathogen often comprises just .01% of periodontal biofilms, yet impairs innate immune activity so profoundly that it becomes a central player in biofilm growth and development.

Community gene expression changes included:
- pH changes (acidic environment)
- peptidoglycan biosynthesis
- potassium transport
- ciliary motility
- iron transport
- response to oxidative stress
The team concluded that periodontitis progression “is driven by the whole oral microbial community and not just a few select pathogens. In effect, under conditions of increasing inflammation and imbalance, the entire oral community appeared to act together as a pathogen.”
Trend #2: A growing number of neuroinflammatory conditions are now increasingly tied to persistent infection in the blood + brain: Alzheimers, Parkinson’s, ALS
Our work "serves as a preliminary study showing a role of *P. gingivalis* LPS and gingipain protease in abnormal blood clotting observed in our Parkinson's samples"
Takeaways

1. A healthy person and a patient with ME/CFS could harbor the same pathogen and/or communities of pathogens...**but the organisms may be ACTING differently** in the ME/CFS patient.

2. It is hard to fully understand the symptoms of a disease without factoring in how a dominant pathogen **influences the activity of other organisms** in its microbiome/virome community.
Human organisms/pathogens create a broad range of proteins and metabolites
Dominant pathogens are almost always capable of persisting inside the nucleus of human cells where they:

1. Slow immune response

2. Create proteins/metabolites that Interfere with human transcription/translation/DNA repair processes and the epigenetic environment
Human signaling pathways are controlled by proteins + metabolites (ligands) binding into receptors

*Image Khan Academy
Molecular mimicry

Microbial proteins and metabolites are often identical or similar in structure to those created by their human hosts.

It follows that proteins/metabolites created by pathogens can dysregulate the activity of human receptors + signaling pathways.
Viral, bacterial and fungal proteins + metabolites can directly alter the activity of human signaling pathways

Viral insulin-like peptides activate human insulin and IGF-1 receptor signaling: A paradigm shift for host–microbe interactions

Emrah Altindis\textsuperscript{a}, Weikang Cai\textsuperscript{a}, Masaji Sakaguchi\textsuperscript{a,b}, Fa Zhang\textsuperscript{c}, Wang GuoXiao\textsuperscript{a}, Fa Liu\textsuperscript{c}, Pierre De Meyts\textsuperscript{d,e}, Vasily Gelfanov\textsuperscript{c}, Hui Pan\textsuperscript{a}, Richard DiMarchi\textsuperscript{c}, and C. Ronald Kahn\textsuperscript{a,1}

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Viral/bacteria/fungal proteins + metabolites have also been shown to dysregulate human signaling pathways that control:

- Blood pressure, circulatory issues and autonomic function
- Natural killer cell activity
- Lactate accumulation in blood/brain
- Glutamate metabolism
- Pain signaling + neuropathy
- Mast cell activation
- Mitochondrial activity
Pathogen/organism activity is characterized by a high level of functional redundancy


Review
Roles of Mitochondrial Respiratory Complexes during Infection

Pedro Escoll $^{1,2,*}$, Lucien Platon $^{1,2,3}$, Carmen Buchrieser $^{1,2,*}$
Most pathogens associated with the respiratory, gastrointestinal, or urogenital tracts, as well as with the central nervous system or the skin, have the capacity to bind and degrade collagen(s) in order to adhere to and invade host tissues. The major pathogens discussed are:

- *Streptococcus*
- *Staphylococcus*
- *Pseudomonas*,
- *Salmonella*,
- *Yersinia*,
- *Treponema*,
- *Mycobacterium*,
- *Clostridium*,
- *Listeria*,
- *Porphyromonas* and *Haemophilus*;
- *Candida*,
- *Aspergillus*,
- *Pneumocystis*,
- *Cryptococcus* and *Coccidioides*; *Acanthamoeba*,
- *Trypanosoma* and *Trichomonas*;
- retrovirus and papilloma virus.
ME/CFS research goal: better characterize the signaling pathways + tissues + cell types that redundant ME/CFS dominant pathogens can dysregulate
Area postrema lies between pons and medulla, and is a window in the blood-brain barrier, allowing immune cells and molecules into the brain.

The vagus nerve that control the “sickness behavior” response enters at the exits at the brainstem.

ME/CFS is a spectrum disorder

C. Immune, gastro-intestinal and genitourinary Impairments
At least one symptom from three of the following five symptom categories

1. Flu-like symptoms may be recurrent or chronic and typically activate or worsen with exertion. e.g. sore throat, sinusitis, cervical and/or axillary lymph nodes may enlarge or be tender on palpitation
2. Susceptibility to viral infections with prolonged recovery periods
3. Gastro-intestinal tract: e.g. nausea, abdominal pain, bloating, irritable bowel syndrome
4. Genitourinary: e.g. urinary urgency or frequency, nocturia
5. Sensitivities to food, medications, odours or chemicals

Notes: Sore throat, tender lymph nodes, and flu-like symptoms obviously are not specific to ME but their activation in reaction to exertion is abnormal. The throat may feel sore, dry and scratchy. Fauclial injection and crimson crescents may be seen in the tonsillar fossae, which are an indication of immune activation.

D. Energy production/transportation impairments: At least one symptom

1. Cardiovascular: e.g. inability to tolerate an upright position - orthostatic intolerance, neurally mediated hypotension, postural orthostatic tachycardia syndrome, palpitations with or without cardiac arrhythmias, light-headedness/dizziness
2. Respiratory: e.g. air hunger, laboured breathing, fatigue of chest wall muscles
3. Loss of thermostatic stability: e.g. subnormal body temperature, marked diurnal fluctuations; sweating episodes, recurrent feelings of feverishness with or without low grade fever, cold extremities
4. Intolerance of extremes of temperature

Notes: Orthostatic intolerance may be delayed by several minutes. Patients who have orthostatic intolerance may exhibit mottling of extremities, extreme pallor or Raynaud's Phenomenon. In the chronic phase, moons of finger nails may recede.

Paediatric considerations
Symptoms may progress more slowly in children than in teenagers or adults. In addition to postexertional neuroimmune exhaustion, the most prominent symptoms tend to be neurological: headaches, cognitive impairments, and sleep disturbances.

1. Headaches: Severe or chronic headaches are often debilitating. Migraine may be accompanied by a rapid drop in temperature, shaking, vomiting, diarrhoea and severe weakness.
2. Neurocognitive impairments: Difficulty focusing eyes and reading are common. Children may become dyslexic, which may only be evident when fatigued. Slow processing of information makes it difficult to follow auditory instructions or take notes. All cognitive impairments worsen with physical or mental exertion. Young people will not...
Every patient’s chronic inflammatory symptoms are unique

Even in HIV/AIDS, where an easily detected virus dysregulates immunity, disease symptoms reflect a mix of those driven by HIV, and those driven by “co-infectious” agents able to take advantage of the immunocompromised host. No two patients with HIV/AIDS are expected to harbor the same mix of these additional persistent bacteria, fungi, and viruses.

- Bacterial infections, including tuberculosis and a serious related disease, Mycobacterium avium complex (MAC)
- Viral infections, such as cytomegalovirus (CMV) and hepatitis C
- Fungal infections, like yeast infections, cryptococcal meningitis, pneumocystis carinii pneumonia (PCP) and histoplasmosis
- Parasitic infections, such as crypto (cryptosporidiosis) and toxo (toxoplasmosis)
Other environmental factors that can contribute to ME/CFS

- mold exposure
- chemical exposures
- injuries (especially CNS injuries)
- high levels of stress
- immunosuppressive drugs/supplements
- rare human genome variants
Successive infection as a model for ME/CFS disease development

A successive infection “snowball” could drive many forms of disease
Successive infection as a model for ME/CFS disease development

1. An acquired persistent pathogen, an inherited pathogen and/or environmental exposure dysregulates the host immune system. This makes it easier for microbes + viruses to subvert the immune response by acting as polymicrobial entities.

3. Pathobionts alter their gene expression to better promote community-wide virulence. The proteins/metabolites created by these (intracellular) organisms begin to **dysregulate human signaling pathways**. Dysfunction driven by **molecular mimicry** increases. Certain pathogens may **infect mitochondria, central nervous system tissue** etc.

5. Intracellular pathogens **slow the human immune response, causing the host to more easily acquire other infectious agents** or become more sensitive to additional environmental exposures. This creates a snowball effect in which the microbiome + virome in various body cites become increasingly dysbiotic as the strength of the immune response weakens over time.
Successive infection: the importance of inherited organisms

Bacterial, viral and fungal communities are passed in families, especially down the maternal line

- Breast milk microbiome
- Organisms passed in the womb (placenta, amniotic fluid)
- Vaginal microbiome
Directions for treatment

Treatment is already changing in Alzheimer’s to include/develop antiviral + antimicrobial medications.

**Detailed Description**

Valacyclovir is a drug approved by the U.S. Food & Drug Administration to treat herpes and shingles. HSV-1 (oral herpes) and HSV-2 (genital herpes) are known to trigger amyloid aggregation and their DNA is commonly found in beta-amyloid plaques, which are a hallmark of Alzheimer's disease. In studies in mice, anti-HSV drugs have reduced accumulation of beta-amyloid and p-tau, another protein found in the brains of people with Alzheimer’s disease. This study will test valacyclovir as a possible treatment in slowing or preventing Alzheimer’s disease.

Participants will take four to eight coated tablets of either 500 mg of valacyclovir or placebo daily for 78 weeks. Researchers will measure amyloid accumulation on PET scans in multiple regions of the brain, as well as changes in cognitive function and activities of daily living using assessments and tests. Investigators also will obtain measures of beta-amyloid and tau in cerebrospinal fluid from participants who agree to lumbar puncture.

**Locations**

<table>
<thead>
<tr>
<th>Name</th>
<th>City</th>
<th>State</th>
<th>Zip</th>
<th>Status</th>
<th>Primary Contact</th>
</tr>
</thead>
</table>
| New York University School  | New York    | New York | 10016  | Not yet recruiting | Shannon Chen, BA  
| of Medicine                 |             |       |        | 212-263-5495    | Shannon.Chen@nyumc.org                  |
| New York State Psychiatric  | New York    | New York | 10032  | Recruiting     | Julianna Pollina, BS  
| Institute                    |             |       |        | 564-774-6038    | julianna.pollina@nyspi.columbia.edu     |

**Lead Sponsor Agency**

New York State Psychiatric Institute

**Collaborator Sponsor**

- National Institutes of Health (NIH)

**COULD ALZHEIMER’S BE TREATED WITH AN ANTI-VIRAL DRUG?**

March 21, 2016  | Penny Dacks, PhD
Paper highlight: “For decades, it was accepted that the CNS is an “immune-privileged site”… This view ascribed the inflammation in chronic neurodegenerative disease to autoimmunity. As a consequence, attempts were made to treat such conditions with immunosuppressive drugs, all of which failed”
“In conclusion, the development of a therapy that boosts the immune system in a well-controlled way, and thereby restores and/or activates brain–immune communication, is an outcome of a general shift toward the perception of the CNS as a tissue that engages in a constant dialog with peripheral immunity. Such an approach is expected to provide novel treatment modalities in order to harness common immune repair mechanisms to combat Alzheimer’s disease and perhaps other neurodegenerative diseases.”
Treatment: support the immune system to better target persistent pathogens/manage toxic exposures

Abstract

Chronic fatigue syndrome (CFS)/myalgic encephalomyelitis (ME) has long been associated with the presence of infectious agents, but no single pathogen has been reliably identified in all patients with the disease. Recent studies using metagenomic techniques have demonstrated the presence of thousands of microbes in the human body that were previously undetected and unknown to science. More importantly, such species interact together by sharing genes and genetic function within communities. It follows that searching for a singular pathogen may greatly underestimate the microbial complexity potentially driving a complex disease like CFS/ME. Intracellular microbes alter the expression of human genes in order to facilitate their survival. We have put forth a model describing how multiple species—bacterial, viral, and fungal—can cumulatively dysregulate expression by the VDR nuclear receptor in order to survive and thus drive a disease process. Based on this model, we have developed an immunostimulatory therapy that is showing promise inducing both subjective and objective improvement in patients suffering from CFS/ME.
Our Organism Identification Project:

The Organism Identification Pipeline: A collaboration between top global research teams to standardize the process of using novel, cutting-edge technologies and algorithms to search for viruses + bacteria + other organisms in the blood/cerebrospinal fluid of ME/CFS subjects.
The Organism Identification Pipeline Team

Nikos Kyrpides (Joint Genome Institute Berkeley)

Kris Fobes, GeneSavvy

Robert Moir, Harvard University

David Paez-Espino, Joint Genome Institute Berkeley

Amy Proal, Autoimmunity Research Foundation

Rudy Tanzi, Harvard University
Organism Identification Pipeline: Part 3

Nikos + David at will use their “Uncovering the Earth’s Virome” technologies to characterize the microbiome + virome communities in ME/CFS blood.

They will use their existing pipelines that identify general ss and ds RNA and DNA viruses + phages + specific viral groups. These include phages, mycoviruses, giant viruses, virophages and even retroviruses.

The analysis will also identify known and novel bacteria + fungi and use algorithms to predict their relationship to phages. These relationships can then be used to infer organism activity and microbial ecosystem dynamics.
The Organism Identification Pipeline: Further Benefits for ME/CFS

- Further establishes ME/CFS as a **serious, biomedical condition**
- Connects researchers all focused on **developing actual treatment** for patients
- Allows us to immediately **apply the latest discoveries** in Alzheimer’s, Parkinson’s etc to ME/CFS (and vice versa)
- Establishes a pipeline for further analyses **at the lowest possible cost**
- Can serve as a **basis for the development** of accurate microbiome/virome commercial testing
- Sets the stage for collaboration with yet other **cutting-edge** research teams (eg: George Tetz, HMI)
- Could lead to **increased funding** from private groups like Facebook…or even the NIH
- Will allow samples from **other ME/CFS body sites** to be additionally analyzed for organisms…and/or samples from patients with **related diagnoses such as EDS/fibromyalgia** to be analyzed
“It is unwise to dismiss the pathogenic capacities of any microbe in a patient with a mysterious disease. The so-called “autoimmune” conditions, in which no pathogen can be identified by routine testing are particularly suspect”

-Gerald Domingue, Professor Emeritus Tulane University
A personalized approach to ME/CFS diagnosis and treatment

1. Look for rare human genome variants that predispose towards persistent infection and/or environmental exposure risk

2. Characterize the maternal/inherited microbiome for the presence of inherited pathogens/dysbiotic microbiome + virome communities

3. Document and test for acquired pathogens

4. Document and test for environmental exposures

5. Document and test for microbiome/virome dysbiosis in various body sites

6. Factor in injuries, stress, pregnancies and other complications that can impact patient health
Dysbiosis in driven by changes in organism/pathogen ACTIVITY

Evolution of the gut microbiome following acute HIV-1 infection

Muntsa Rocafort, Marc Noguera-Julian, Javier Rivera, Lucía Pastor, Yolanda Guillén, Jost Langhorst, Mariona Parera, Inacio Mandomando, Jorge Carrillo, Víctor Urrea, Cristina Rodríguez, Maria Casadellà, Maria Luz Calle, Bonaventura Clotet, Julià Blanco, Denise Naniche & Roger Paredes

Prevalence of fecal virus shedding

<table>
<thead>
<tr>
<th>Virus</th>
<th>Recent HIV-1 infection</th>
<th>Chronic HIV-1 infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>26/49 (53.2%)**</td>
<td>36/71 (50.7%)**</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>3/49 (6.1%)</td>
<td>4/71 (5.6%)*</td>
</tr>
<tr>
<td>Enterovirus</td>
<td>1/43 (2.4%)</td>
<td>4/19 (21.1%)*</td>
</tr>
<tr>
<td>Human herpes virus 6A, 6B, and 8</td>
<td>0/49 (0%)</td>
<td>0/71 (0%)</td>
</tr>
</tbody>
</table>

- loss of bacterial taxonomic richness
- long-term reductions in microbial gene richness
We discovered that during colitis the intestinal phage population is altered and transitions from an ordered state to a stochastic dysbiosis…. Our findings indicate that intestinal phage communities are altered during inflammatory disease, establishing a platform for investigating phage involvement in irritable bowel disorder.
Researchers at Cornell University found that ME/CFS patients had significantly higher levels of LPS in their blood than healthy individuals.

LPS are large toxic molecules found in the membranes of bacteria.
The team found that tryptophan created by the gut microbiome interacted with the AHR receptor on microglia/astrocytes. Subsequent changes in gene expression regulated communication between the two cell types.
Harvard University pilot data indicates dyregulated **blood perfusion** between the ME/CFS brain/body.
E. coli glucose metabolism

PMID: 18402659
How do organisms get into the brain?

Structural and functional features of central nervous system lymphatic vessels

Antoine Louveau, Igor Smirnov, Timothy J. Keyes, Jacob D. Eccles, Sherin J. Rouhani, J. David Peske, Noel C. Derecki, David Castle, James W. Mandell, Kevin S. Lee, Tadie H. Harris & Jonathan Kipnis

Maps of the lymphatic system: old (left) and updated to reflect the new discovery.
Pathogen/organism activity is characterized by a high level of functional redundancy

The VDR Nuclear Receptor

- Regulates expression of thousands of human genes, many of which regulate inflammatory and cancerous processes

  - Controls multiple families of important antimicrobial peptides including cathelicidin (LL-37)

- Controls signaling of TLR2 + TLR4 (proteins that recognize foreign substances + pathogens and alert other parts of the immune response)
Pathogens that dysregulate/slow VDR activity:

- Epstein Barr Virus
- HIV
- *Mycobacterium tuberculosis*
- Cytomegalovirus
- *Borrelia burgdorferi*
- *Mycobacterium leprae*
- Aspergillus fumigatus
- *Chlamydia trachomatis*

- Because disabling the innate immune system via the VDR pathway is such a logical survival mechanism, other uncharacterized bacteria, viruses or fungi may have also evolved to dysregulate receptor activity.

*Figure 2. Nuclear receptors mRNA expression is downregulated upon infection of B-cells with EBV*
Taxonomic composition based on relative abundance plotted for each tumor (0%–100%).
Invasion of the Central Nervous System by Intracellular Bacteria

Douglas A. Drevets,1,* Pieter J. M. Leenen,2 and Ronald A. Greenfield1

ABSTRACT

Infection of the central nervous system (CNS) is a severe and frequently fatal event during the course of many diseases caused by microbes with predominantly intracellular life cycles. Examples of these include the facultative intracellular bacteria Listeria monocytogenes, Mycobacterium tuberculosis, and Brucella and Salmonella spp. and obligate intracellular microbes of the Rickettsiaceae family and Tropheryma whippeli. Unfortunately, the mechanisms used by intracellular bacterial pathogens to enter the CNS are less well known than those used by bacterial pathogens with an extracellular life cycle. The goal of this review is to elaborate on the means by which intracellular bacterial pathogens establish infection within the CNS. This review encompasses the clinical and pathological findings that pertain to the CNS infection in humans and includes experimental data from animal models that illuminate how these microbes enter the CNS. Recent experimental data showing that L. monocytogenes can invade the CNS by more than one mechanism make it a useful model for discussing the various routes for neuroinvasion used by intracellular bacterial pathogens.
Pathobiont behavior could be compared to children misbehaving when the teacher leaves the classroom.
Upcoming Webinars: Advances in ME/CFS Research and Clinical Series

Estimating Prevalence, Demographics, and Costs of ME/CFS Using Large Scale Medical Claims Data and Machine Learning. Presented by Charmian Proskauer

Thursday, December 12, 2019 at 10am PT // 1pm ET

www.MEAdvocacyWeek.com
The Solve M.E. $750,000 Double-Your-Impact Challenge

We’ve secured $750,000 in gifts from generous donors who wish to remain anonymous.

Your gift today will unlock an additional $750,000, making a real difference in the lives of people with ME/CFS. Act now and your gift will be doubled!

Your donation of any amount by December 31st will generate at least $1.5 million in funding to fight this terrible disease.

www.SolveME.org/donate