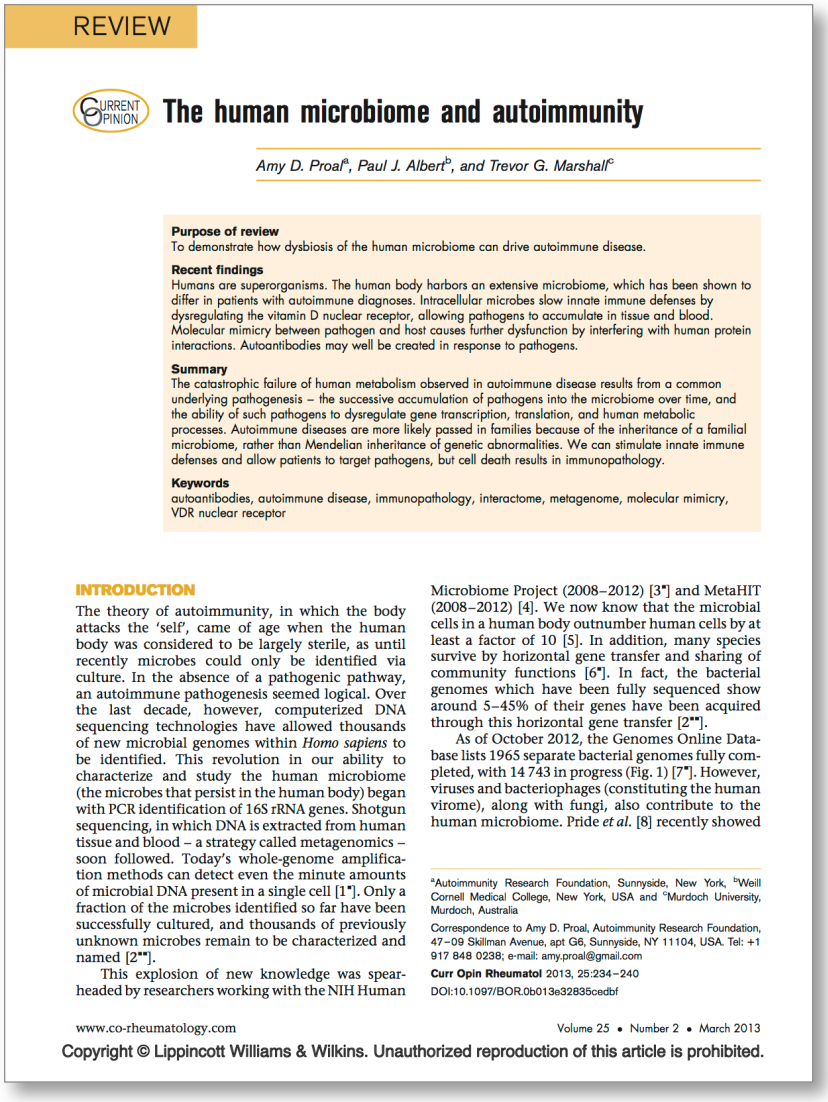

REVIEW

The human microbiome and autoimmunity

Amy D. Proal[a], *Paul J. Albert*[b], *and Trevor G. Marshall*[c]

Purpose of review
To demonstrate how dysbiosis of the human microbiome can drive autoimmune disease.

Recent findings
Humans are superorganisms. The human body harbors an extensive microbiome, which has been shown to differ in patients with autoimmune diagnoses. Intracellular microbes slow innate immune defenses by dysregulating the vitamin D nuclear receptor, allowing pathogens to accumulate in tissue and blood. Molecular mimicry between pathogen and host causes further dysfunction by interfering with human protein interactions. Autoantibodies may well be created in response to pathogens.

Summary
The catastrophic failure of human metabolism observed in autoimmune disease results from a common underlying pathogenesis – the successive accumulation of pathogens into the microbiome over time, and the ability of such pathogens to dysregulate gene transcription, translation, and human metabolic processes. Autoimmune diseases are more likely passed in families because of the inheritance of a familial microbiome, rather than Mendelian inheritance of genetic abnormalities. We can stimulate innate immune defenses and allow patients to target pathogens, but cell death results in immunopathology.

Keywords
autoantibodies, autoimmune disease, immunopathology, interactome, metagenome, molecular mimicry, VDR nuclear receptor

INTRODUCTION

The theory of autoimmunity, in which the body attacks the 'self', came of age when the human body was considered to be largely sterile, as until recently microbes could only be identified via culture. In the absence of a pathogenic pathway, an autoimmune pathogenesis seemed logical. Over the last decade, however, computerized DNA sequencing technologies have allowed thousands of new microbial genomes within *Homo sapiens* to be identified. This revolution in our ability to characterize and study the human microbiome (the microbes that persist in the human body) began with PCR identification of 16S rRNA genes. Shotgun sequencing, in which DNA is extracted from human tissue and blood – a strategy called metagenomics – soon followed. Today's whole-genome amplification methods can detect even the minute amounts of microbial DNA present in a single cell [1•]. Only a fraction of the microbes identified so far have been successfully cultured, and thousands of previously unknown microbes remain to be characterized and named [2••].

This explosion of new knowledge was spearheaded by researchers working with the NIH Human Microbiome Project (2008–2012) [3•] and MetaHIT (2008–2012) [4]. We now know that the microbial cells in a human body outnumber human cells by at least a factor of 10 [5]. In addition, many species survive by horizontal gene transfer and sharing of community functions [6•]. In fact, the bacterial genomes which have been fully sequenced show around 5–45% of their genes have been acquired through this horizontal gene transfer [2••].

As of October 2012, the Genomes Online Database lists 1965 separate bacterial genomes fully completed, with 14 743 in progress (Fig. 1) [7••]. However, viruses and bacteriophages (constituting the human virome), along with fungi, also contribute to the human microbiome. Pride *et al.* [8] recently showed

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The Human Microbiome and Autoimmunity

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Abstract

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Recent findings: Humans are superorganisms. The human body harbors an extensive microbiome, which has been shown to differ in patients with autoimmune diagnoses. Intracellular microbes slow innate immune defenses by dysregulating the VDR nuclear receptor, allowing pathogens to accumulate in tissue and blood. Molecular mimicry between pathogen and host causes further dysfunction by interfering with human protein interactions. Autoantibodies may well be created in response to pathogens.

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Introduction

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As of October 2012, the Genomes Online Database lists 1,965 separate bacterial genomes fully completed, with 14,743 in progress (Figure 1).[7] However, viruses and bacteriophages (constituting the human virome), along with fungi, also contribute to the human microbiome. Pride *et al.* recently showed that bacteriophages even dominate the oral cavity virome.[8]

These microbes persist not only on the body's mucosal surfaces but also in tissues such as the endometrium[9] and lungs[10], as well as in the blood [11]. Because infected monocytes and macrophages of the immune system traffic all tissues, they impact many body processes. For example, the analgesic acetaminophen is metabolized differently depending on the presence or absence of specific bacterial metabolites.[12]

Humans are superorganisms

The genes of the microbiota number in the millions, dwarfing the 20,500 that comprise the human genome [13]. The genes of these microbial inhabitants interact so

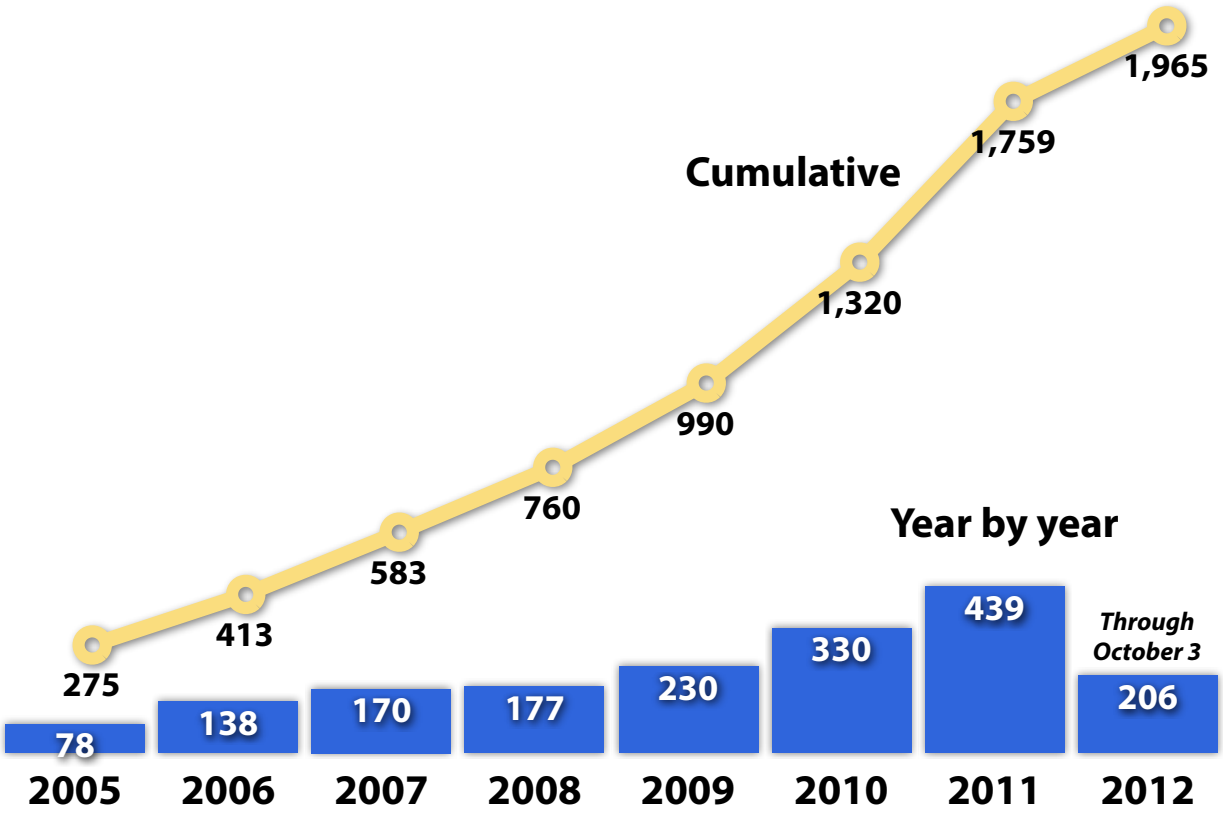

Figure 1. *Completed and published bacterial genomes in Genomes OnLine Database (GOLD) since 2005.*

often with our own that human beings are best described as "superorganisms."[14] One analysis demonstrated that the expression of 463 human genes is changed during a single infection with *Mycobacterium tuberculosis*.[15] The full extent of the interactome – these foreign and host protein interactions – is just beginning to be studied. Based on analysis of the human gene disease network, the human angiotensin converting enzyme (ACE) has been tied to myocardial infarction, Alzheimer's, diabetes mellitus, and sarcoidosis.[16] However, *Lactobacillus* and *Bifidobacteria* – microbes common in dairy products and considered to be innocuous – actually downregulate expression of ACE.[17] By altering the expression of ACE, such bacteria will also affect the progression of these inflammatory diseases in ways not yet fully understood.

Microbial composition in health and disease

Significant differences can be found in the microbiomes of patients with autoimmune diseases such as diabetes types I [18] and II [19], Crohn's [20], ulcerative colitis, and psoriasis [21]. Even the composition of the cerebrospinal proteome is significantly altered in autoimmune disease. Schutzer *et al.* found that while healthy subjects, and patients with chronic fatigue syndrome (CFS) [22], harbored many of the same proteins in the cerebrospinal fluid, 738 of 2,783 detected proteins were unique to patients with CFS [23]. Because so many of the proteins created in the body are microbial, these changes are a direct reflection of alterations to the human interactome.

In lieu of tracking the presence or absence of single pathogens, these studies show dysbiosis of entire communities of microbes in patients who are ill. Therefore, Koch's postulates, which dictate that a single microbe must cause a single disease, are no longer relevant in the era of the metagenome.

However, a plethora of other environmental factors besides illness can lead to significant changes in microbiome composition, making those shifts related to disease harder to pinpoint and study.[24] The vaginal microbiome varies with menses.[25] The microbial composition of the forearms and underarms are as

"ecologically dissimilar as rainforests are to deserts."[26] Thus, in addition to determining what species of pathogens an individual harbors, we must also examine what the genomes of these microbes *actually do* in order to cause dysfunction and disease.

We must focus on intracellular microbes

Many microbes successful in subverting the host immune system survive inside the cells, altering the expression of human genes by modifying transcription, translation and DNA repair processes.[27]

Pathogen-induced dysregulation of gene expression by the vitamin D nuclear receptor (VDR) provides an excellent example of how such interference can eventually lead to the systemic dysfunction characteristic of autoimmune disease. The VDR controls the expression of at least 1,000 genes [28]. It also regulates key components of the innate immune response including toll-like-receptor 2 (TLR2) along with the cathelicidin and beta-defensin antimicrobial peptides (AMPs), all of which play central roles in targeting intracellular pathogens.[29]

Given the VDR's importance to the host innate immune response, those microbes capable of dysregulating expression by this receptor greatly enhance their chances of survival. Epstein-Barr Virus, which has been associated with many autoimmune diagnoses, slows expression of the VDR in immature lymphoblastoid cell lines by a factor of greater than thirty.[30] *Borrelia burgdorferi,* [31] cytomegalovirus, [32] *Mycobacterium leprae*, [33] and *Mycobacterium tuberculosis,* [15] persist by decreasing receptor activity to varying degrees. *Aspergillus fumigatus* secretes a gliotoxin which significantly downregulates expression by the VDR.[34] Indeed, VDR dysregulation is such a logical survival mechanism that these well-characterized microbes likely represent only a handful of those that persist in the same or a similar fashion.

VDR dysregulation is signaled by rising levels of the active vitamin D metabolite 1,25-dihydroxyvitamin-D (1,25-D). [35] Our *in silico* data shows that 1,25-D can interfere with expression of other key nuclear receptors, including the glucocorticoid receptor, the androgen receptor and the thyroid receptor, causing an array of hormonal imbalances.[36, 37] In addition, these receptors become increasingly unable to express the other families of AMPs under their control, leading to a profound suppression of the innate immune system. In fact, sarcoidosis [38] and Crohn's disease [39] are characterized by decreased expression of cathelicidin over time.

Successive infection

Individuals suffering from dysregulation of the VDR or related receptors become increasingly immuno-compromised, acquiring more pathogens. As these microbes upregulate or downregulate human gene expression, the microbiome shifts further away from a homeostatic state, a process we call successive infection. The metabolites and proteins generated by these microbes interfere with host metabolism, leading to the accumulation of foreign proteins, enzymes, mRNA and waste metabolites in the cytoplasm. For example, humans and *E. coli* metabolize glucose-6-phosphate in similar fashion, producing almost identical metabolites.[40] Thus, as the genomes of the two species exchange nutrients and toxins, the complexity of transcription and translation increases for both species. Such interactions occur on a vast scale. Consider that the HIV genome codes for only nine genes, from which are transcribed 19 to 21 proteins. Yet 1,434 direct interactions have been identified between just these 19 proteins and the human metabolome.[41]

Eventually, as more pathogens are incorporated into the microbiome and levels of dysbiosis increase, people begin to present with symptoms characteristic of an autoimmune or inflammatory diagnosis. The particular disease symptoms an individual manifests will depend on the species, location, and virulence of the pathogens they accumulate over time, coupled with the semi-infinite ways in which the proteins of these microbes cause dysfunction by interacting with the proteins of the host [42].

Molecular mimicry

The sequence homology between the structures of many microbial proteins, and the genes that create them, make it difficult for the body to distinguish "foreign" from "self."[43] "Molecular mimicry" is extremely common.

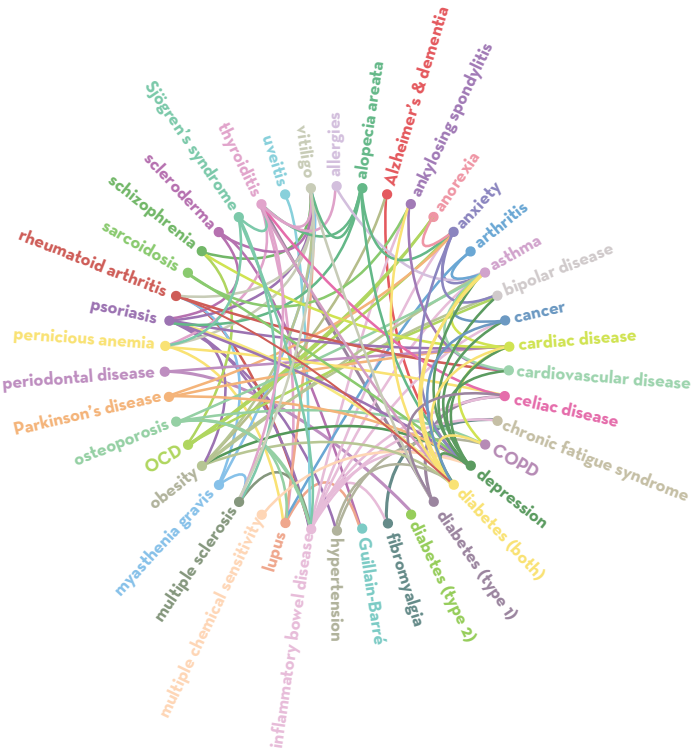

Figure 2. *Comorbidities among common inflammatory diseases. Each "spoke" of this wheel represents a published study appearing in MEDLINE, which shows a significant statistical relationship between one disease and another.*

Kusalik *et al.* found that 19,605 pentamers from the hepatitis C virus (HCV) polyprotein have a high level of similarity to the human proteome.[44] This remarkable similarity persisted even when the team used longer peptide motifs as probes for identity scanning. Krishnadev *et al.* reported tens of thousands of protein-protein interactions between the genomes of *E. coli*, *Salmonella, Yersinia* and the human genome [45]. Trost *et al.* discovered that proteins created by pathogens causing chronic infections had greater similarity to the human proteome than those created by microbes causing acute infections.[46] Indeed, those pathogens able to more effectively disable host metabolism should find it easier to persist.

Comorbidity

The high levels of multimorbidity [47] among patients with autoimmune disease emphasizes the importance of successive infection. No two people ever acquire exactly the same sequence of microbes and therefore never develop an identical disease presentation. Figure 2 shows the very high level of overlap between diagnoses. This overlap means that different inflammatory diagnoses would best be studied and treated in concert. For example, obesity is said to cause diabetes, but both conditions have now been tied to microbial dysbiosis [2]. It is more likely that both diseases arise from successive infection, a common underlying pathogenesis.

Autoantibodies

Molecular mimicry also contributes to autoantibody production. Since human antibodies are polyspecific, certain antibodies created to target pathogens often additionally target human proteins, causing "collateral damage".[48] Indeed, an increasing number of "autoantibodies" including rheumatoid factor, anti-phospholipid antibodies, anti-saccharomyces cerevisiae antibodies (ASCA), and anti-nuclear antibodies (ANA), are created in response to infectious agents.[49]

Familial aggregation

Clusters of disease often exist within families, suggesting a common etiology. In families who have a member with primary Sjögren's syndrome, 38% had at least one first-degree relative with an autoimmune disease, versus 22% in control families.[50] Spouses have much higher rates of sarcoidosis.[51] Explaining such associations would require high-risk gene variants.[52] Yet this is not viable from an evolutionary point of view, as such gene variants confer no known survival advantage and should have been weeded from the population. On the contrary, the prevalence of nearly every autoimmune disease is on the rise.

There is increasing evidence that autoimmune diseases run in families due to the sharing of common microbes [53]. For example, infected siblings, mothers, and fathers are all major sources for *Helicobacter pylori* acquisition among young children, with the infected mother serving as the main independent source.[54] The microbiome a child develops is a direct reflection of those harbored by the mother and close relatives. Microbes are introduced

by a multitude of sources including the placenta, sperm, egg, breast milk, and vaginal canal.[37]

Rethinking SNPs: there are not millions of separate diseases

Evidence for successive infection undermines the primacy of the human genome's role in disease. During the euphoria that resulted from the decoding of the human genome, scientists painted a future where disease would soon be predicted, diagnosed, and treated based solely upon analysis of a patient's genome. Many scientists expected to find a gene associated with each illness. However, ten years later, we have a catalog of *10 million* single nucleotide polymorphisms (SNPs) [55, 56]. Yet even this bewildering complexity has failed to deliver any significant understanding of, or therapies for, chronic disease. Recently, genetic focus has shifted to identifying groups of SNPs associated with each diagnosis, but even that has yet to bear fruit.

What are we actually measuring? Genetic science has not yet noticed the elephant in the room – the microbial DNA and RNA that the human microbiome exudes from infected cells**.** This contaminates the samples of "human DNA" being analyzed.

Merriman[57] wrote a genomic assembler that would not become confused by the myriad microbial RNA contaminating the human samples. What he found was startling – the commercial software that lies at the heart of today's genetic research is extremely sensitive to samples that do not quite fit exactly into the human gene map. Even the smallest amount of microbial RNA contamination – just one or two base pairs discrepancy – was enough to cause very significant errors. Thus, many of these 10 million SNPs, perhaps a majority of them, may be due to software error.

Edwards' group at Argonne National Labs [58] has developed sophisticated algorithms capable of identifying and removing non-human RNA from a GWAS sample prior to assembly. Unfortunately, few researchers use this tool, even though the algorithms also provide a list of microbial species detected in the sample.

Immunostimulation as a novel therapeutic avenue

If dysbiosis of the microbiome drives autoimmune disease, than treatments that target the root cause of these conditions must seek to stimulate rather than suppress immune defenses.[59] Over the past decade, we have been testing a therapy for autoimmune disease that appears to strongly activate the innate immune response. Treatment is based on the use of a putative VDR agonist olmesartan, which, by reactivating the receptor, appears to gradually restore expression of the innate immune defenses under its control.[59] Physicians have noted consequent symptomatic improvement within a clinical environment, however it remains to be seen whether this can be replicated in a randomized controlled trial.

Our clinical colleagues have noted that paramount to this treatment's success is the ability of patients to manage immunopathology the immune system's response to microbial death. As pathogens are targeted, the host immune system releases cytokines and chemokines in order to kill the infectious agents. In addition, the body must deal with the byproducts of dead microbes and the cells they once inhabited. As a result, patients' symptoms become worse before they eventually subside. Immunopathology is not unique to this therapy, rather it is a necessary result of the immune response to microbial death, one that has been well documented.[37] For example, Wang *et al.* showed that mice engineered to have a reduced innate immune response to the common cold actually showed less – not more – airway inflammation and bronchoconstriction following infection.[60] Thus, the cold-induced symptoms were caused by the body's immune response to the virus, rather than the virus itself.

The standard of care for autoimmune disease is immunosuppression. Immunosuppressive medications generate short-term symptom relief, often by slowing immunopathology. However, relapse and increased disease severity are common among patients administered these drugs, as over the long-term, they allow pathogens contributing to autoimmune processes to spread more easily.

The worldwide surge in autoimmune disease portends an impending health crisis. By using these immunosuppressive medications we are not targeting the root cause of these diseases and we are exacerbating the proliferation of the pathogens. Immunostimulative treatment could be administered at the first sign of illness, ensuring minimal immunopathology and minimal proliferation of pathogens. However, the ability of immunostimulatory therapies to succeed hinges on the willingness of institutional review boards to accept the phenomenon of immunopathology, the initial worsening of symptoms**,** in order to ensure a better long-term prognosis for the patient and for the community at large.

Conclusion

During the last decade, novel sequencing technologies that characterize microbes based on their DNA have revolutionized the field of microbiology. We now know that the human body harbors a vast microbiome, the composition of which contributes directly to autoimmune processes. Intracellular pathogens cause systemic dysregulation by directly altering gene transcription and translation mechanisms. Dysregulation of the VDR nuclear receptor by such pathogens successively decreases innate immune defenses, allowing individuals compromised in this fashion to accumulate a distinct pathogenic microbiota. Over time, this may lead to the development of a unique cluster of symptoms presenting as one or more autoimmune diagnoses. Molecular mimicry between pathogenic metabolites and those of the host further contributes to dysfunction via the interactome. Autoimmune diseases are more likely passed in families due to inheritance of the familial microbiome than inheritance of Mendelian genetic abnormalities. In lieu of immunosuppression, therapies that treat autoimmune conditions should aim to stimulate innate immunity in order to allow targeting of the pathogens underlying the disease process.

Key Points

- Every human being is a superorganism with a unique microbiome. Persistent intraphagocytic pathogens directly alter gene transcription, translation, and DNA repair processes.
- Throughout life, microbes accumulate into the microbiome and incrementally suppress innate immune defenses. Over time, this may eventually cause a patient to develop a unique cluster of symptoms characteristic of an autoimmune disease.
- The familial microbiome is passed down the maternal line.
- Hundreds of protein interactions define a chronic disease, not a defect in any single pathway. Molecular mimicry between microbial and host proteins causes the body to generate antibodies that become autoantibodies by causing collateral damage.
- Immunosuppressive therapies offer short-term palliation, but immunostimulative therapies improve long-term patient prognosis.

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