
Autoimmune disease in the era of the metagenome

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ABSTRACT

Studies of autoimmune disease have focused on the characteristics of the identifiable antibodies. But as our knowledge of the genes associated with the disease states expands, we understand that humans must be viewed as superorganisms in which a plethora of bacterial genomes – a metagenome - work in tandem with our own. The NIH has estimated that 90% of the cells in *Homo sapiens* are microbial and not human in origin. Some of these microbes create metabolites that interfere with the expression of genes associated with autoimmune disease. Thus, we must re-examine how human gene transcription is affected by the plethora of microbial metabolites. We can no longer assume that antibodies generated in autoimmune disease are created solely as autoantibodies to human DNA. Evidence is now emerging that the human microbiota accumulates during a lifetime, and a variety of persistence mechanisms are coming to light. In one model, obstruction of VDR nuclear-receptor-transcription prevents the innate immune system from making key antimicrobials, allowing the microbes to persist. Genes from these microbes must necessarily impact disease progression. Recent efforts to decrease this VDR-pervverting microbiota in patients with autoimmune disease have resulted in reversal of autoimmune processes. As the NIH Human Microbiome Project continues to better characterize the human metagenome, new insights into autoimmune pathogenesis are beginning to emerge.

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1. Introduction

A decade ago microbiologists were generally confident that most of the bacterial species capable of persisting in or on humans had already been identified. However, over the past few years this perception has changed dramatically. Advances in molecular genetic sequencing have revealed the presence of a vast human microbiota, much of which defies detection by culture-based methods.

Homo sapiens was once thought to be the product of one genome. Now, humans are best described as superorganisms in which a multitude of microbial genomes persist in concert with our own [1]. The genomes interact by affecting translation, transcription, and DNA repair in the cytoplasm of infected cells. It is essential that we examine how both human and microbial metabolites (the Human Metabolome) alter the expression of key genes associated with the presentation and progression of autoimmune disease.

2. The human microbiota

According to the NIH, a mere 10% of the cells that comprise the organism known as *Homo sapiens* are human cells. The remaining 90% are bacterial in origin [2]. Thus, *Homo sapiens* is best described as a superorganism in which a large number of different organisms coexist as one [3]. Previously occult bacteria are being found in and on the human body. For example, hydrothermal vent bacteria were found in studies of hip joints during revision arthroplasty [4].

To date, only a fraction of the human microbiota has been genetically characterized and identified, leaving large gaps in our understanding of how it contributes to human health and disease. The NIH Human Microbiome Project aims to use molecular genetic sequencing to catalog the balance of the human microbiome over the coming years. This Project has already succeeded in characterizing over 1,000 novel bacterial genomes [2]. These genetic "fingerprints" allow for a better tracking and understanding of species and any metabolites they produce which might interact with the human genome

Medicine is now comfortable with the bacterial populations that exist in the gut and areas of the body in contact with the external environment, such as the mouth, ears, nose and skin. Yet, components of the human microbiota also likely persist in many other body tissues, including those which become inflamed in autoimmune disease [5]. Such bacteria can persist inside the very cells of the immune system that are supposed to kill them [6], or in biofilm communities in which they are protected from the immune response by a self-created polymeric matrix [7].

These bacteria rapidly and frequently share their DNA with their fellow species – even distantly related species – through horizontal gene transfer. Homologous recombination further muddles genomic coherence. As a result, the diversity and the variability present in the human microbiota is much greater than anticipated [8]. Some argue that the number of microbes created through genetic recombination is so high that the concept of distinct bacterial species may become obsolete [9]. For example, researchers associated with the European Tract Meta Initiative seek to understand how bacteria in the gut may contribute to obesity and inflammatory bowel disease. The goal of the project is simply to examine associations between bacterial genes and human phenotypes. "We don't care if the name of the bacteria is

Enterobacter or *Salmonella*. We want to know if there is an enzyme producing carbohydrates, an enzyme producing gas or an enzyme degrading proteins," explains Francisco Guarner of the project [10]. Such efforts are shifting the focus of microbiology away from the search for single pathogens in a disease state [1]. Instead, an increasing number of researchers are exploring how components of the microbiota may cause disease by interacting together.

3. A metagenome

Because so many bacteria persist in *Homo sapiens*, the microbiota is currently estimated to harbor millions of genes compared with the mere 31,897 [3] that comprise the human genome. In fact, the human genome is barely larger than that of the worm *C. elegans* (23,399 genes) or that of the small flowering plant thale cress (29,388) [3].

Due to their small size, hundreds, or even thousands, of bacterial cells can fit inside a human cell [6]. The combined genetic contributions of these microbes inevitably provide myriad gene products not encoded by our own relatively small genomes. This means that the human genome is only one of the many genomes that affect *Homo sapiens* function. In reality, the organism we call *Homo sapiens* is controlled by a metagenome, a tremendous number of different genomes working in parallel.

Bacterial gene products can be very similar to our own. For example, the metabolism of glucose-6-phosphate by both the human body and *E. coli* is nearly identical, so that remarkably similar metabolites are produced by both species [11]. With this in mind, the interaction between an *E. coli* genome and the human genome, as they exchange nutrition and toxins, increases the complexity of transcription and translation for both species.

4. Bacteria alter the expression of genes that affect the progression of autoimmune disease

When analyzing a genetic pathway, we must study how bacterial and human genes interact, in order to fully understand any process related to the *Homo sapiens* superorganism. Some of these pathways contribute to the pathogenesis of autoimmune disease.

Figure 1 illustrates some of these gene-disease relationships [12]. A number of autoimmune and inflammatory diseases are shown together with the genes that have been associated with each illness. Note that the gene ACE is related to myocardial infarction, renal tubular dysgenesis, Alzheimer's, the progression of SARS, diabetes mellitus, and sarcoidosis. ACE has been shown to be down-regulated by a number of peptides created by *Lactobacillus* and *Bifidobacteria* [13], species of bacteria considered to be innocuous or "friendly." No one would argue that these species aren't present in the human body, yet there has been little study of how they affect chronic inflammatory disease.

For example, PTPN22 is related to rheumatoid arthritis, lupus, and diabetes mellitus. Yet PTPN22 has been shown to be up regulated as part of the innate immune response to mycobacteria [14]. Our population is facing a surge in latent tuberculosis and an increased prevalence of *Mycobacterium avium*. So it is extremely important that we look at how the presence of increased PTPN22 from latent infection, or any of the mycobacteria in the microbiota, might

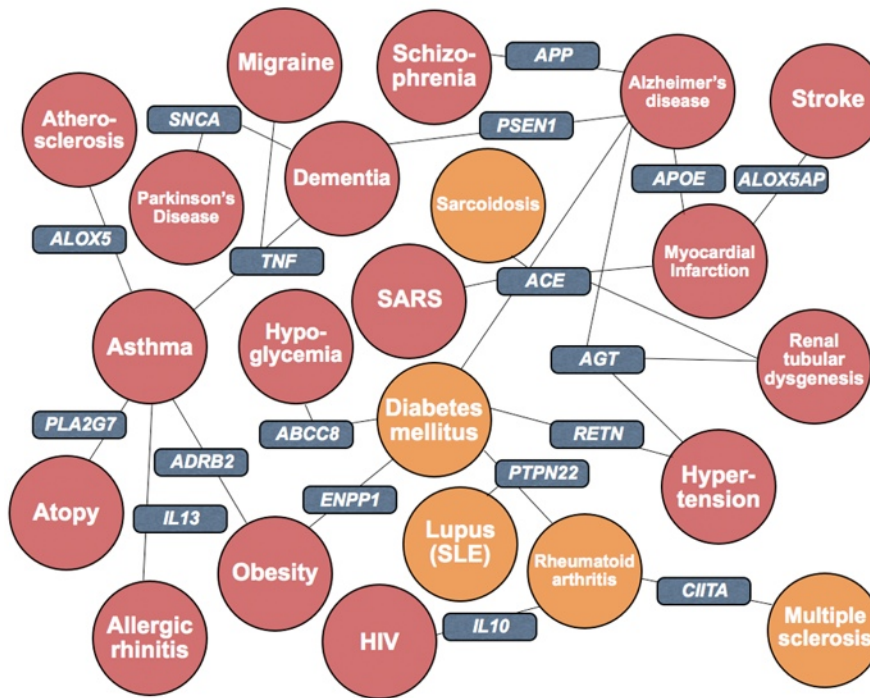


Fig 1. Relationships between diseases and genes, an excerpt from the human disease network [12]. Conditions thought to be autoimmune are shaded orange. Other inflammatory conditions are shaded red.

contribute to the autoimmune process.

5. Capnine and the persistence of the metagenome

Created by the gliding bacteria that are present in biofilm, the sulfonolipid Capnine provides a specific example of how a bacterial metabolite could manipulate human gene expression in order to dramatically alter the progression of autoimmune and other chronic diseases. Capnine has the capacity to disrupt transcription by the VDR, one of the body's most prolific nuclear receptors [15].

The VDR expresses at least 913 genes, many connected to autoimmune conditions and cancers [16]. The Receptor also regulates expression of several antimicrobial peptides (Amps) that play a vital role in allowing the innate immune system to target chronic pathogens [17]. Furthermore, it transcribes TLR2, which enables the innate immune system to recognize gram-positive bacteria [18, 19].

Thus, if Capnine was dysregulating the VDR, it would greatly hamper the innate immune response. VDR dysfunction would cause the active vitamin D metabolite 1,25-D to rise to excessively high levels where it could inhibit expression by the bulk of the body's other nuclear receptors - including alpha thyroid, the glucocorticoid receptor, and the androgen receptor [20]. This would result in hormonal imbalances and also interfere with expression of the dozens of other Amps expressed by these receptors. *In vivo*, the microbiota appears to gradually shut down the innate immune response over a person's lifetime, resulting in the increased accumulation of chronic bacteria and other pathogens [21].

Eventually, genes from the accumulating microbial metagenome may

determine a clinical disease symptomology such as an autoimmune diagnosis, or simply drive the inflammation associated with the aches and pains of aging [5]. This accumulation is an extremely logical evolutionary survival mechanism. Components of the human microbiome have evolved to dysregulate the VDR receptor that would otherwise activate a potent immune response against its presence. As Royston Goodacre comments in *Journal of Nutrition*, we are born with a genome that, aside from the genes of species that can survive in the womb and endometrium [22], is largely human. But we inevitably die with a genome that is at least 90% bacterial [3]. This shift towards an increasingly diverse microbiota over a lifetime is directly correlated with an increase in diseases and symptoms driven by inflammation.

6. Antibodies may be generated in response to microbial DNA

Autoimmune diseases are often regarded as illnesses in which the immune system creates antibodies against itself [23]. Yet now that *Homo sapiens* is understood to be the product of multiple genomes, it is equally possible that the antibodies observed in autoimmune disease result from alteration of human genes and gene products by the bacterial metagenome.

It seems that autoimmune disease is largely the result of the *adaptive* immune system gone awry. However, when a disabled *innate* immune system is forced to respond to the chronic microbiota the resulting cascade of cytokines and chemokines will also stimulate an adaptive response. At this point, the adaptive immune system may likely create antibodies to fragments of DNA that have been generated by phagocytosis or apoptosis of infected cells [5]. Yet, until a much larger portion of the human microbiota has been

characterized, correlation of such antibodies with specific components of the microbiota remains difficult.

7. The human metabolome is a product of its environment

The spectrum of metabolites found in *Homo sapiens* is known as the Human Metabolome [3]. Although many bacteria produce substrates similar to those of their human hosts, others produce metabolites that differ from the byproducts of human metabolism. The human microbiota differs from person to person depending on the unique species of bacteria accumulated over a lifetime. This means that every person's health is distinctly influenced by the specific byproducts created by their particular microbiota. Changes in the metabolome pool are the downstream results of gene expression [3].

Some of the human and microbial metabolites in the *Homo sapiens* superorganism will be manifest in serum and urine samples. For example, mass spectroscopy has been used to identify the non-human metabolites present in the urine of subjects living in three distinct populations - the United States, China, and Japan [24]. The study found that subjects in each population produced very different non-human metabolites [24]. Thus, genetic makeup, nutrition, healthcare, external toxins - factors associated with the acquisition of a particular microbiota - caused the three populations to become substantially different. That environmental factors drive the metabolome is supported by the observation that when five of the Japanese subjects moved to America, their metabolomes adapted to resemble those of the American population [24]. When evaluating the overall operation of *Homo sapiens*, it is thus clear that the composition of the microbiota is far more important than regional variations in the human genome itself.

8. The microbiota can interfere with transcription and translation

Persistent bacteria including *Francisella tularensis* [25], *Mycobacterium tuberculosis* [26], *Rickettsia massiliae* [27], *Brucella spp.* [28], *Listeria monocytogenes* [29], *Salmonella typhimurium* [30] and others, use a variety of mechanisms to evade the immune response and survive inside macrophages and other phagocytic cells. Furthermore, various species of bacteria have been detected inside the cells of patients with juvenile rheumatoid arthritis [31], sarcoidosis [6], and other inflammatory diseases [32]. This suggests that disease-causing microbiota largely persist in the cytoplasm of nucleated cells, where it has access to both the DNA transcription and protein translation machinery of *Homo sapiens*. For example, upon infecting a macrophage, *Brucella spp* down-regulates genes involved in cell growth and metabolism, but up-regulates those associated with the inflammatory response and the complement system [33]. When *Shingella* persists within a macrophage it modulates numerous host signaling pathways, including those that inactivate mitogen-activated protein kinases [34]. According to one analysis, expression of 463 human genes are changed during an infection with *Mycobacterium tuberculosis* [35].

Microorganisms are also capable of integrating their DNA with our own [36]. This results in alteration of the human DNA by the microbiota over time, potentially leading to genetic mutations associated with autoimmune diagnoses. Genetic haplotypes observed

in autoimmune disease frequently have very low statistical significance, as would be expected based on knowledge that the metabolome varies from population to population and individual to individual.

In addition, host DNA repair mechanisms are susceptible to modification by the products of the metabolome. In fact, bacteria may hijack DNA repair mechanisms to generate genetic diversity without losing genomic stability [37, 38]. If the rate of DNA damage or mutation by bacterial metabolites exceeds the capacity of cellular repair, the accumulation of errors can overwhelm the cell and result in early senescence, apoptosis or cancer [39].

9. Discussion

It is becoming apparent that the body of *Homo sapiens* consists not only of the human genome, but also genomes of commensal bacteria, bacteriophages, and viruses. Consequently, the human genome can no longer be studied in isolation. Genes known to be associated with autoimmune conditions are susceptible to modification by the myriad pathogenic metabolites. Thus their activity in disease processes must be studied in the tissues in which they are expressed.

Commensal microbes that were thought to be solely beneficial to man are now known to create metabolites that interfere with the expression of genes associated with autoimmune disease. For example, peptides from of *Lactobacillus* and *Bifidobacteria* affect expression of the ACE gene. Those species that disable VDR gene expression secure their survival by suppressing key antimicrobial peptides. Their persistence may well cause the inflammation and antibody production thought to result from autoimmune processes. Species of pathogens that collect in an individual's microbiota will affect disease presentation and progression. In particular as the innate immune response is compromised by the chronic infection, the body additionally loses its ability to stop the proliferation of opportunistic acute infectious agents.

Lifelong symbiosis between the human genome and persistent components of the bacterial metagenome does not simply result in modification of the metagenome. It results in the accumulation of microbial metabolites in the cytoplasm of infected cells that are capable of interfering with DNA repair and transcription activity. Thus genetic abnormalities such as those observed in autoimmune disease may well be the result of metagenomic activity.

The use of a VDR agonist and subinhibitory antibiotics has demonstrated the ability to restore VDR function and induce recovery in diverse autoimmune diagnoses [21, 40]. This supports a biological description in which a persistent pathogenic component of the microbiota accumulates inside macrophages and other nucleated cells. The use of corticosteroids slows the immune system's ability to target the cause of any chronic inflammation resulting from this persistent infection. This can at best result only in short-term palliation.

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Take home messages

- *Homo sapiens* is a superorganism controlled by both the human genome and a microbial metagenome.
- Bacterial metabolites can up-regulate and down-regulate the expression of genes associated with autoimmune disease.
- The human metabolome varies greatly from person to person depending on microbiota composition. Thus, its ability to alter gene expression varies greatly depending on the individual.
- The microbiota can survive by slowing VDR Nuclear Receptor transcription, and subsequently the expression of ~913 genes key antimicrobials for the innate immune response.
- The microbiota can persist in the cytoplasm of nucleated cells where it has direct access to both the DNA transcription and to the protein translation machinery of *Homo sapiens*.
- We must necessarily study how the metagenome and the human genome interact in order to fully understand any process related to autoimmune disease.