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Abstract

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ABSTRACT
The human microbiome has been shown to play a role in the regulation of human health, behavior, and disease. Data suggests that microorganisms that co-evolved within humans have an enhanced ability to prevent the development of a large spectrum of immune-related disorders but may also lead to the onset of conditions when homeostasis is disrupted. In many conditions, a link between dysbiosis (microbial imbalance or microbiome upset) has been identified and associated with immune conditions such as rheumatoid arthritis (RA). This review provides insight into how an individual's unique microbiome, combined with a genetic predisposition and environmental factors may lead to the onset and progression of RA. While research efforts have been largely focused on *Porphyromonas gingivalis* in the generation of citrullinated products as a trigger in the onset and progression of RA, recent research efforts have also indicated that *Proteus mirabilis* may play a key role in the development of anti-citrullinated antibodies through shared epitope sequences IRRET and ESRRAL. Thus, this review also highlights how targeting dysbiosis with alternative approaches may help to reduce microbial resistance as well as potentially improve outcomes. Further investigation is needed to see if potential future treatments for RA could benefit from personalized medicine based on an individual's unique microbiome.

Keywords: rheumatoid arthritis, microbiom, proteus mirabilis

INTRODUCTION

Background

Individuals with rheumatoid arthritis (RA) present with varying symptomatology and disease severity, which can be attributable to several factors, including a genetic predisposition, environmental factors, and/or unique microbiom[1]. In recent years, researchers have demonstrated that the human microbiome likely plays a role in the regulation of human health, behavior, and disease. Data suggests that microorganisms that co-evolved within humans have an enhanced ability to prevent the development of a large spectrum of immune-related disorders, but they may also lead to the onset of conditions when homeostasis is disrupted. In many conditions, a link between dysbiosis (microbial imbalance or microbiome upset) has been identified and associated with immune conditions such as RA. Citrulline is an amino acid enzymatically created by the conversion of peptidylarginine to peptidylcitrulline, in an irreversible calcium-mediated process by the enzyme peptidylarginine deiminase (PAD).[2] The synovial fluid of patients with RA contains unique hypercitrullinated proteins with intra and extracellular substrates. Autoantibodies that enhance the catalytic ability of PAD4 specifically have been identified in patients with severe RA, lowering calcium required for catalytic reactions. Within an oxidizing extracellular environment, typically PADs would become inactivated. However, in patients with RA the extracellular citrullination occurs via hyperactivation of PADs and the respective autoantibodies, thus contributing to disease pathogenesi.[2] Zhang et al demonstrated that *P. gingivalis* (gram-negative, anaerobic, pathogenic bacterium) of the oral cavity generated citrullinated proteins, which has been shown to be associated with diseases such as RA.[3] Konig et al also demonstrated a link between citrullination and RA. In particular, the presence of leukotoxin A secreted by *Aggregatibacter actinomycetemcomitans* was associated with autoantigen citrullination and was present in RA joints.[4] In this review, we aim to outline the role of the microbiome in RA onset and progression. We also highlight the need for more targeted treatment, especially as evidence continues to grow linking the oral microbiota (presence of bacterium, toxins, etc.) and citrullination development.

Role of the Microbiome Throughout Human Development

Humans have co-evolved with trillions of microbes that coexist within us, creating body-specific, adaptive ecosystems that change based upon the host physiology.[4] Over 1,000 gut bacterial species have been characterized using 16S rRNA gene sequencing and anaerobic culture techniques. Healthy gut microbiomes identified largely consist of the Bacteroidetes and Firmicutes phyla, although the ratios vary greatly between individuals.[5] The Human Genome Project (HGP) study aimed to further define the normal human microbiome by sampling 242 healthy United States volunteers and collecting up to three samples from each individual at various sites.

Over 5,000 samples were analyzed using DNA sequencing techniques to identify the variations within 16sRNA. This research effort identified over 10,000 microbial species in the human ecosystem. The results showed that the overall distribution of microbial metabolic activity appeared to be more important than the species of microbes themselves, and that the microbiome of each individual was unique.[6] HGP researchers reported that the human genome carries approximately 22,000 protein-coding genes, whereas the human microbiome has around eight million unique protein-coding genes. This research is important because specific genes contained within the bacterial sequence allow us to digest food and absorb nutrients for which we do not naturally have the enzymes. Microbes also produce vitamins and anti-inflammatory mediators that our body does not naturally produce, playing a pivotal role in modulating innate and acquired immune systems. This means that individuals can vary significantly in their predisposition to develop and combat different diseases based on their unique microbiome.[6]

Altered Microbiome in RA Patients

Dysbiosis, which occurs due to disruption within the microbiome, has been shown to be associated with onset and progression of cancer, allergies, diabetes, multiple sclerosis (MS), and RA.[5] For RA in particular, multiple factors likely play a role in the onset and progression of the condition. Researchers have identified that a combination of environmental factors such as smoking and infections, genetic predisposition including presence of leukocyte antigen (HLA) alleles, and an individual's microbiome likely contribute to disease activity by altering the presence of autoantibodies.[7] Production of antibodies to citrullinated peptides or proteins in RA patients occurs through peptidylarginine deiminase enzymes (PADS). PADs are activated through cell membrane damage, necrosis, or neutrophil extracellular traps. Processes that lead to overactivation of PAD enzymes are enhanced by smoking and bacteria such as *P. gingivalis* and *A. actinomycetemcomitans*, factors associated with RA.[8.]

A delicate balance is necessary between protective and potentially harmful pro-inflammatory bacteria to prevent a state of dysbiosis, which can lead to detrimental host effects. In the 1970s, researchers demonstrated an imbalance between the microbiota and arthritis pathology in an animal model. The researchers compared the development of arthritis in germ-free rats and conventionally raised rats and found that the germ-free rats developed severe arthritis 100 percent of the time. However, the conventionally raised rats only developed severe arthritis 20 percent of the time.[7] This suggests that exposure to a germ laden environment allows development of disease-protective traits. Further research has shown an increased number of *Prevotella copri* and reduced levels of bacteroides in RA patients, suggesting *P. copri* may be pathogenic, whereas bacteroides may have a

protective effect in the microbiome. Further studies on this topic indicated increased levels of IgA and IgG antibodies produced in response to the presence of *P. copri* in RA patients.[7]

Further research studies have shown that *P. gingivalis* plays a crucial role in the oral microbiome and may also play a role in development and progression of RA. The combination of *P. gingivalis* and an environmental and/or genetic predisposition to the condition increase the risk of developing RA. The reason for this is that *P. gingivalis* has been shown to contribute to the development of citrullinated antigens.[9] Chronic exposure to these citrullinated antigens in susceptible individuals can lead to the generation of citrullinated protein antibodies, which can, over time, lead to the development and onset of RA. Multiple studies have demonstrated an increase in the production of citrullinated antigens by *P. gingivalis* in RA patients compared to healthy controls, suggesting a pathogenic role.[9] For instance, a study performed by Rosenstein et. al found that patients with periodontal infections commonly became exposed to antigens made by PAD. This ultimately led to rheumatoid factor-containing immune complexes and caused inflammation.[10]

Proteus Mirabilis in the Development of RA: *P. mirabilis* has been shown to play a critical role in the intestinal microbiome and may serve as a novel potential target in the management of RA. *P. mirabilis* (a gram-negative bacterium) is commonly associated with urinary tract infections (UTIs), and typically occurs in patients with a long-term catheterization. *P. mirabilis* can cause symptomatic infections of the urinary tract including pyelonephritis, cystitis, and the development of urolithiasis.[11] The majority of UTIs caused by *P. mirabilis* occur from the ascension of bacteria located in the gastrointestinal tract, but UTIs can also be transmitted person-to-person, especially in healthcare settings.[11]

P. mirabilis has also been linked to RA, where individuals develop an autoimmune response to the products of *P. mirabilis*, including hemolysin and urease enzymes.[9] It has been found that rheumatoid factor positive patients with RA had elevated levels of IgA and IgM antibodies to *P. mirabilis*. Elevated antibody levels to various antigens from *P. mirabilis* were discovered in over 1,350 RA patients in 15 different countries in studies performed from 1985 to 2003 (compared to healthy controls).[12.]

P. mirabilis and RA are believed to have co-evolved throughout time, as demonstrated by the ability of *P. mirabilis* to evoke an autoimmune response in RA patients.[13] Specifically, researchers found that the development of antibodies against *P. mirabilis* antigens was significantly higher in RA patients when compared to patients with other disease or healthy controls in 15 different locations worldwide.[14] Further support for the co-evolution occurred following research with synthetic oligonucleotides, that revealed what is now referred to as the RA "susceptibility sequence." This sequence is located in the DRβ1 chain, with coding amino acids at position 70-74. One of the first sequences found in *P. mirabilis*, ESRRAL, was discovered to have similarities to the RA susceptibility sequence at amino acid position 67-74 and demonstrated molecular mimicry.[13] In a study performed by Tiwana et al, it was also found that antiserum against the RA susceptibility sequence EQKRAA was bound to a similar peptide ESRRAL found in *P. mirabilis*. The co-evolution between RA and *P. mirabilis* provides a potential understanding of both the pathogenesis of RA and future targeted therapy. Amino acid sequences such as ESRRAL and IRRET are known to contain arginine doublets acted upon by PAD, leading to harmful catalytic reactions contributing to the pathogenesis of RA.[15] If these harmful amino acid sequences could be detected early in the disease progression, treatment with targeted genomic therapy and biologicals may potentially avoid irreversible joint damage.[15]

P. mirabilis is also more commonly found in patients with RA. Senior et al. evaluated the urine samples of 76 patients with RA and compared them with 48 healthy controls. The investigators found that *P. mirabilis* occurred twice as frequently in RA patients. In addition, *P. mirabilis* was found in 52 percent of the infected urines of the RA patients (as compared with Escherichia coli (E.coli)) and was detected as a pure growth isolate. This research suggests that patients with RA have raised levels of *P. mirabilis* antibodies in the urine.[16] These results suggest that RA patients are likely to have *P. mirabilis* bacteriuria in the system, even before symptoms arise. The presence of this bacterium may act as a trigger in disease onset and progression. These results highlight that inflammation arising from the individual's microbiome can begin years prior to disease onset and may serve as a marker of disease activity and can be used for a more targeted treatment approach and preventive medicine.[16]

A theoretical model developed by Ebringer suggests that UTIs can trigger autoimmunity via epitopes or antigens.[17]The model is a consolidation of research demonstrating the link between *P. mirabilis* and RA based on ten findings. Ebringer's theory includes research supporting the shared epitope sequences IRRET and ESRRAL, demonstrating molecular mimicry, as noted above. Furthermore, it provides evidence that antibodies to *P. mirabilis* were present in RA patients (from 14 different countries) that are RA disease-specific.[17] The *P. mirabilis* antibodies are from both cross reacting and non-cross-reacting sequences, helping to prove active RA infections had been exposed to infection. It was also found that HLA-DR4 lymphocytes injected into rabbits evoked antibodies specific to *Proteus vulgaris* and *P. mirabilis*.[17] Overall, UTIs caused by *P. mirabilis* appear to lead to molecular mimicry and cross-reactivity in RA patients.[18]

Treatment of Proteus Mirabilis: Current research efforts have focused on developing antibiotics and general UTI prevention for RA management. Proper treatment in patients with RA especially early in the course of the disease with the use of antibiotics can benefit the patient by restoring the proper balance in the microbiome and avoiding the subsequent citrullination of arginine.[18] Treatment for an uncomplicated UTI caused by *P. mirabilis* involves treatment with trimethoprim/sulfamethoxazole. If a patient presents with more severe infection, intravenous (IV) antibiotics with ceftriaxone, a fluoroquinolone, or a combination is commonly used initially until symptoms abate (i.e., fever resolves), and followed with oral antibiotics for 10 to 21 days with proper follow-up. However, the incidence of *P. mirabilis* antimicrobial resistance has been increasing in recent years, especially to extended-spectrum beta-lactamases (ESBLS).[18] In a study performed by Ahn et al in South Korea, the researchers evaluated 62 patients diagnosed with Proteus mirabilis, and found that those with ESBLs had a significantly higher 28 day mortality rate (17.74 percent) as compared to those without the presence of the bacterium. While this study highlights the need for early detection and non-invasive plant-based treatment options that can avoid the development of antimicrobial resistance, the use of plant-based medicine and therapy for RA management has been less of a research focus.[18]

Still, a study performed by Cock et al demonstrated a potential, novel, non-invasive therapeutic treatment for *P. mirabilis* and *P. vulgaris*.[19] The researchers used 34 extracts from 12 South African plant species and tested their ability to control for these two bacteria. Low or non-toxic extracts with Proteus inhibitory activity were used in vitro, testing for the presence of resveratrol using Reverse phase high performance liquid chromatography and UV-Vis spectroscopy.[19] Twenty-nine of the extracts were able to inhibit *P. mirabilis* and 23 of them inhibited the growth of *P. vulgaris*. The researchers identified specific leaves, including *Lippia javanica* and *Syzygium coradatum* bark, which tested positive for the presence of reveratrol (an antioxidant produced as phytoalexin by plants including pine trees, grapes, and peanuts), a potential therapeutic target. Plant extracts from *L. javanica leaf*, *S. cordatum* bark and leaf, *Terminalia pruinoides* leaf and *Terminalia sericea* leaf were shown to be significantly stronger than the control antibiotics (ampicillin and chloramphenicol) with a MIC as low as 49 ug/ml.[19] These research efforts suggest that the use of plant-based medicine as seen in the South African plant species would allow for a potentially more accessible and effective alternative to currently available antibiotic approaches.[19] Further research is needed to evaluate the therapeutic benefits and side effects of such treatment.

Table. Proteus mirabilis

Proteus mirabilis	**Key Literary Findings**
Proteus mirabilis Background	*P. mirabilis* is a Gram-negative bacterium that is a frequent cause of UTIs [11].
Genetic Linkage	*P. mirabilis* has been shown to be linked to RA through antibodies against hemolysin and urease enzymes, that are believed to recognize self-antigens in RA patients [9]. It has also been shown to demonstrate similarities to cell membrane proteins of mycoplasma in patients with the sequence ESRRAL at amino acid position 67-74 [13].
Ebringer's Theoretical Model	RA specific antibodies to Proteus have been demonstrated to be present in RA patients from 14 countries. HLA-DR4 lymphocytes injected into rabbits evoked *P. mirabilis* and *P. vulgaris* specific antibodies [17].
Potential Future Treatment	Researchers have demonstrated specific leaves like *L. javanica* and *S. cordatum* bark test positive for resveratrol, an anti-inflammatory antioxidant produced as phytoalexin by plants including pine trees, grapes, and peanuts [19].

CONCLUSION

While research efforts have been largely focused on *P. gingivalis* in the generation of citrullinated products as a trigger in the onset and progression of RA, recent research efforts have also indicated that *P. mirabilis* may play a key role in the development of anti-citrullinated antibodies through shared epitope sequences IRRET and ESRRAL. These findings indicate that a trigger, such as a UTI, which increases *P. mirabilis* levels may disrupt the natural composition of the human microbiome, contributing to the onset and progression of RA. Current treatment of *P. mirabilis* involves the use of trimethoprim/sulfamethoxazole or an oral fluoroquinolone in the treatment of uncomplicated UTIs. However, antimicrobial resistance has led to an increased interest in researching alternative approaches. One such approach is the use of plant based therapy, including the use of resveratrol, an antioxidant from South African plants has been shown to control both *P. mirabilis* and *P. vulgaris*. Further research is needed to evaluate the efficacy and safety of these plant based approaches for RA patients experiencing a dysbiosis including elevated levels of P. mirabilis.

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