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Review Article

The human microbiome in rheumatic autoimmune diseases: A comprehensive review

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ABSTRACT

The human microbiome consists of the total diversity of microbiota and their genes. High-throughput sequencing has allowed for inexpensive and rapid evaluation of taxonomic representation and functional capability of the microbiomes of human body sites. Autoimmune and inflammatory rheumatic diseases are characterized by dysbiosis of the microbiome. Microbiome dysbiosis can be influenced by host genetics and environmental factors. Dysbiosis is also associated with shifts in certain functional pathways. The goal of this article is to provide a current and comprehensive review of the unique characteristics of the microbiome of patients with autoimmune and inflammatory rheumatic diseases, measured using high-throughput sequencing. We also highlight the need for broader studies utilizing a longitudinal approach to better understand how the human microbiome contributes to disease susceptibility, and to characterize the role of the interaction between host genetics and microbial diversity in the pathogenesis of autoimmune diseases, disease manifestations, and progression.

Contents

1. Introduction

The human microbiome is the totality of the microbial species (microbiota) that exist on or in a human being and their genes [1]. The advent of high-throughput sequencing technology allowed for powerful, expedient, and inexpensive assessment of microbial communities including previously unculturable organisms [2]. A number of analysis platforms exist to aid in sequence processing and statistical testing; of note are *mothur* and *QIIME*, which feature active user communities and on-going development [3,4]. Current microbiome studies generally focus on taxonomic classification and representation (sequencing of bacterial 16S rRNA genes), determining the functional capacity of the

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microbiome (metagenomics), or the function of actively transcribed genes (metatranscriptomics) [5]. While taxonomic identification was traditionally useful for detection of pathogenic species in infectious diseases, the value of microbial metagenomics is the determination of the functional capacity of the microbiota present and their abundance [6]. Metagenomics studies provide a way to identify clinically important factors like antibiotic resistance genes and disruptions in the functional networks of body sites by comparison to characterized isolates and *in silico* functional predictions [7]. That is not to say that microbial taxonomy itself lacks value, on the contrary, it is a source of clinical biomarkers in a variety of diseases, like type 2 diabetes, obesity-related disorders, inflammatory bowel disease, and dental caries [8–10].

Considering the intimacy of the host-microbiota relationship, it is not surprising that genetic variation between hosts has been linked to differences in the microbiome, specifically in regions previously identified as being associated with microbiome-related disease like inflammatory bowel disease [11]. For example, drastic differences in the diversity of gut microbiota showed a strong association with the host genotype of the fucosyltransferase 2 gene, *FUT2*, which were consistent with shared patterns of microbial representation known as "enterotypes" [12]. The sex of the host has been associated with specific variations in the microbiota of the human gut, and a link between the microbiome and distinct sex-bias found in some autoimmune disease cannot be ruled out [13]. In addition to genetic factors, the environment also shapes the human microbiome, chief among these being diet, which can change the profile and gene expression of the gut microbiota in a consistent manner on a time scale as short as days [14]. There is also an extensive body of research showing that bacteria, both pathogenic and commensal, can interact with and influence epigenetic systems, including DNA methylation and histone modifications, in human cells through direct and indirect interactions [15].

The human microbiome has the property of resilience, a characteristic allowing stability when challenged by extrinsic assaults like antibiotic therapy, though repeated perturbations over time can cause disruptions that can persist far beyond the duration of the exposure [16,17]. One mechanism for this resilience is the functional redundancy of the constituent microbial taxa of a body site. While taxonomic representation can vary greatly among individuals driven by factors intrinsic and extrinsic to the host, there is less variability when considering the functional capability of the microbiome [18]. In the gut alone the number of bacterial genes exceed human genes by more than two orders of magnitude [19–21].

Much of the literature on the human microbiome focuses on the gut, where the microbiota play a vital role in shaping the gut's structure and function, as seen in gnotobiotic murine models where there is abnormal development and function of immune-related structures like gut-associated lymphoid tissues [22]. Metagenomic sequencing has pointed towards the existence of co-occurring species classified as enterotypes that do not stratify by region or host-specific factors like body mass or gender [23]. The most relevant function of the gut microbiome to autoimmunity is maintenance of the immune system. This is accomplished through the production of metabolic byproducts (short/medium-chain fatty acids, secondary bile salts, and trimethylamines) used by host cells [24]. Short-chain fatty acids (SCFA) derived from dietary fiber by bacterial fermentation interact with colon regulatory T cells (T_{regs}) through G protein-coupled receptor stimulation and histone deacetylase inhibition, promoting T_{reg} differentiation [24,25].

Much of the research currently conducted in the microbiome is limited to bacteria. The contribution of fungi and viruses, both human and bacteriophage, is still being determined, but is no less important to understanding what the role the human microbiome plays in disease [26–28]. Herein, we review the role of the microbiome in human rheumatic autoimmune diseases. This article is limited to microbiome studies conducted in human patients with rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, lupus, Sjögren's syndrome, and vasculitis published in English and accessible through MEDLINE/PubMed.

2. The microbiome in rheumatic autoimmune diseases

2.1. Rheumatoid arthritis

So far, the largest focus of research on the role of the microbiome in autoimmunity has been rheumatoid arthritis (RA). RA is a disease of the synovial joints that is characterized by inflammation and hyperplasia, and the production of the autoantibody rheumatoid factor and anti-citrullinated protein antibodies [29]. The result is the destruction of the joint cartilage and bone, and co-morbidities involving the cardiovascular and pulmonary systems [29]. RA affects approximately 0.5–1.0% of adults living in developed areas and the mortality rates of RA patients are more than twice that of the general population [30]. The genetic risk factors for RA include alleles in the shared epitope (SE) of the *HLA-DRB1* gene and variants in ~100 non-HLA genes including *PTPN22*, *STAT4*, *CTLA4*, and many others [31].

Linking the microbiota of RA patients with disease pathogenesis has focused primarily on the oral environment as past observations found that RA patients had a higher prevalence of periodontitis, an inflammatory damage to the connective tissue and bone of the tooth caused by immune response to microbial plaque, compared to healthy controls. A recent meta-analysis found a significant slightly elevated risk of periodontitis in RA patients (risk ratio = 1.13; $P = 0.006$) [32,33]. It is still unknown if periodontitis is a causative factor in RA, but one suspected mechanism involves the ability of a pathogenic bacteria, *Porphyromonas gingivalis*, to citrullinate arginine residues of proteins by way of peptidyl arginine deiminases, to which the host immune system is exposed [32,34]. *P. gingivalis* is a member of a pathogenic group called the "red complex bacteria" that also includes *Treponema denticola* and *Tannerella forsythia* that have been extensively shown to be causative agents in periodontitis [35]. RA patients and patients with anti-citrullinated protein antibodies (ACPA) have been observed with periodontitis significantly more frequently than healthy people, and ACPA levels were significantly higher in people with subgingival *P. gingivalis* and have a positive correlation with levels of circulating anti-*P. gingivalis* antibodies, which have been found to be elevated in RA patients with ACPA [34,36]. However, a recent study using high-throughput sequencing of the subgingival microbiota of treatment-naïve new onset RA (NORA) patients found no difference in microbial diversity when compared to patients with chronic RA or healthy controls. Differences in taxonomic abundance and the abundance of *Porphyromonas* species correlated with the severity of periodontal disease but not RA status [37]. The authors identified a novel association between ACPA and rheumatoid factor levels, periodontal disease, and a species of the genus *Anaeroglobus*, of which little is known [37]. Species of *Prevotella* and *Leptotrichia* were characteristic of NORA patients, regardless of periodontal disease severity [37]. *P. gingivalis* was found in almost equal abundance in the subgingival plaque of both new onset and chronic RA patients and in healthy controls [37]. While the role of *P. gingivalis* in the pathogenesis of RA is still in question, there has been demonstrated clinical efficacy when treating periodontitis as an adjuvant for RA drug therapy, reducing the severity of RA symptoms and even outperforming anti-TNFα treatment alone in one study [38–41].

As will become a recurrent theme throughout the study of the microbiome of rheumatic diseases, the gut has become a major focus of research. Earlier work in transgenic animal models found that RA could be induced in germ-free animals by exposure to specific bacteria, particularly *Lactobacillus* species and segmented filamentous bacteria (SFB), which stimulate Th17 and depress T_{reg} activity [42]. Species of *Lactobacillus* bind to the mucosal barrier of the gut protecting the host epithelium, and are increased in abundance and diversity in early RA patients [43]. Interestingly, *Lactobacillus casei* 01 has been shown to have immunomodulatory effects in RA patients when administered as a probiotic in double-blind clinical trials [44,45]. Patients in both studies showed significant reductions in serum levels of IL-12 and TNFα, while the serum levels of IL-6 and IL-10 were conflicting, with IL-6