Bacteria in rheumatoid arthritis biomarker research

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Abstract: Rheumatoid arthritis (RA) is a chronic inflammatory disease primarily of joints caused by genetic and environmental factors. Microbiome, highlighted in a lot of research publications, as an environmental factor in inflammatory arthritis and human rheumatic diseases. With the rapid advance in next-generation sequencing technologies (NGS), analyses of gut and oral bacteria between different types and subtypes of arthritis and healthy individuals have been possible. Mi-crobiome stands for a critical environmental factor that can influence autoimmune disease indication. In this review, human gut microbiota and the role of bacteria in RA pathogenesis have been covered. In addition, the traditional and new sequencing techniques to analyze microbiome have been discussed. The approach of manipulating bacteria as potential remedies of RA has been discussed and laid significant importance on.

Key words: Rheumatoid Arthritis (RA); microbiome; gut bacteria; autoimmune disease; biomarker research; next-generation sequencing (NGS).

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease that affects joints primarily(1) . Without efficient treat-ment, RA can lead to pain, chronic joint deformity, disabi-lity, and increased mortality in severe conditions. Though great progress has been made in the biomarker research for RA, the etiology of RA is not fully known. Multiple factors contributes to the pathogenesis of RA, while the in-teraction between genetic and environmental factors have been reported to play an important role(1–3) . Among the possible pathogenic environmental factors, microbiome and the gastrointestinal (GI) bacteria in particular, have been reported to contribute to the pathogenicity of RA(4, 5). Despite that disease-modifying antirheumatic drugs (DMARDs) have been demonstrated to reduce inflamma-tion in RA in clinical studies, the efficacy of RA treatment has been impeded by the lack of accurate biomarkers and an incomplete understanding of factors that trigger the di-sease. Studies on microbiota reveals the potential use of probiotics to mitigate RA symptoms (3) . Fortunately, the sequencing technologies for microbiome has been evol-ving greatly in the past decades. Traditional PCR, high-throughput 16S ribosomal RNA pyrosequencing, Illumina Shotgun Metagenomics, etc have been utilized to analy-ze microbiome composition and enrichment, enabling us to have a better understanding of the role of microbiota in RA pathogenesis especially with the advance of next generation sequencing technologies (NGS) (6– 9) . Ap-proaches for controlling dysbiosis, or the imbalance of bacteria, therefore, can be used as remedies to alleviate RA symptoms(10– 13) .

Human gut microbiome

Human bodies contains 10 times microbial cells com-pared to that of human cells roughly(1) . Tolerance of the gut bacteria by the immune system is required for the health of gut since failure of this tolerance can lead to autoimmune diseases. A healthy microbiome is critical to the overall intestinal health of the host, namely bacteria" homeostasis" (1,2,11, 12). They help to prevent the in-testine from the colonization of "bad" bacteria. The gut microbiota also interacts with mucosal immune cells, promoting the immune maturation(2,5) . Exposure of gut microbiota to mucosa can elicit T cell and B cell response, generating antibodies against those microbial antigens, which constitute a major component of adaptive immu-nity(2, 5, 14). When this microbiota" homeostasis" state is disrupted (" dysbiosis"), the innate and adaptive immune response of the host becomes dysregulated, changing lo-cal T-cell responses and regulating systemic inflamma-tion. Subsequently, those pathological conditions result in multiple diseases, including RA, autoimmune hepatitis, type I diabetes, and aging(2,11– 13, 15,16).

Changes of gut microbiome in RA

Recent studies have demonstrated that alterations of gut microbiota are associated with RA disease progres-sion(1– 5,7). A previous study has reported that there is a strong association of Prevotella with new-onset rheu-matoid arthritis (NORA). This study found that the rela-tive abundance of P. copri in NORA inversely correlated with presence of shared-epitope risk alleles while P. copri is enriched in NORA patients. This is a solid study that has correlated a specific bacteria to the onset of RA(1). In another study carried out by Zhang et al *Nat Med,* Gut mi-crobial dysbiosis(bacteria imbalance) is detectable in RA and is associated with clinical index. They found the gut microbiota of RA patients are distinct from that of healthy individuals. More specifically for instance, the RA gut was enriched in Gram-positive bacteria and depleted of Gram-negative bacteria, including some Proteobacteria

and Gram- negative Firmicutes of the Veillonellaceae fa-mily(3,4). Vaahtovuo et al reported that the fecal micro-biota of RA patients contain significantly less bifidobac-teria and bacteria of the Bacteroides-Porphyromonas-Pre-votella group, B. fragilis subgroup, and the E. rectale – C. coccoides group than the fecal microbiota of patients with non-inflammatory fibromyalgia(2,5). Vaahtovuo pointed out that those bacterial species listed above are known to belong to the most common genera and groups in the human fecal microbiota. Despite that fecal microbiota is not totally representative of the gut microbiota, it offers a way to check the overview of gut bacteria(2). In addition to studies from human samples, there is also evidence to build the link between the microbiome and RA pathoge-nesis in different germ-free animal models. In an old stu-dy when rats reared under germ-free conditions developed severe joint inflammation in an adjuvant-induced arthritis model, while conventionally raised controls showed only mild disease. This study suggests that gut microbiome has immune-suppressive effects in this condition. In a strep-tococcal cell wall-induced rat arthritis model, animals reared conventionally are resistant to joint inflammation, whereas germ-free rats become susceptible to arthritic disease, mainly due to loss of T-cell tolerance(7). Those studies indicate that gut microbiome can be deleterious or beneficial to joint health depending on the interaction between microflora and host. Those studies corroborates the possibility that gut microbes are stimulating immune cells to develop a self-damaging immune response to other parts of the body. Some intestinal bacteria may train the immune system to produce Th17 cells, which produce factors that can cause inflammation and damage of bone in arthritis (2, 8,16, 17). Altogether, the gut microbiota has a role in the pathogenesis of RA and is useful for RA bio-marker research(1-5, 7, 8, 16, 17).

Human oral microbiome and RA

The teeth, gingival sulcus, tongue, cheeks, hard and soft palates, and tonsils all constitute human oral cavity, which are colonized by bacteria (18). There are only a few studies building the connection between oral micro-biota and RA development due to that the oral microbiome is relatively understudied in comparison to the gut micro-biota(3,7, 8). Although dental plaque and salivary samples are more readily obtained than fecal samples, metageno-mic analysis of the oral microbiome and its association with disease has been less known (3). Zhang et al reported that oral microbial dysbiosis is present in RA and is as-sociated with clinical indices such as C-reactive proteins (CRP), anti- Cyclic Citrullinated Peptide (CCP) and Rheumatoid Factor (RF). When they did the enrichment analysis of oral metagenomic linkage groups (MLGs) in dental and salivary samples of RA subjects and controls, distinction between RA and healthy individuals of oral microbiota was shown. They argued that RA is having a state of chronic inflammation, which might be caused and worsened by the expansion of pathogenic bacteria or the lacking in beneficial immune immune-modulating com-mensal bacteria (3).

In the 1900s, the oral sepsis hypothesis became very widespread. The use of teeth extraction became a very po-pular treatment of disease and was used very intensively in the treatment of RA for several decades. Scher et al has

carried out a comparison of richness and diversity of oral microbiota in patients with new-onset RA, patients with chronic RA, and healthy controls. Interestingly, Prevotel-la species and Leptotrichia species were the only charac-teristic taxa observed to be present in the patients with new-onset RA, and were completely absent in the oral microbiota of healthy controls (8). This study proved that specific populations of oral microbiota are associated with the initiation of RA.

Analysis of microbiome in RA

Since the majority of bacteria living in our body ca-vities have never been cultured, scientists have been using culture-independent DNA sequencing for taxono-mic identification and bacterial enzymatic function cha-racterization(7).16S sequencing technologies become very feasible and relatively easy to analyze microbiome nowadays. DNA can be extracted from fecal samples and V1–V2 16S rDNA region can be amplified and sequenced for downstream analysis(1). 16S rRNA conserved re-gion makes it possible to design universal primers and get the complete nucleotide sequence of the 16S rRNA. The hypervariable region renders it feasible to design species-specific primers and enable unbiased bacterial identification through NGS (next-generation sequencing) platforms. There are old approaches utilizing PCR of 16S rRNA region for microbiome sequencing(1). On a small scale, there are studies that have used 16srRNA hybridi-zation method relying on the specific probes needed(3). In addition, Illumina has some 16S rRNA NGS platforms for characterization of bacteria identify. Therefore, 16sRNA is able to characterize the complexity of microflora resi-ding in human body but unable to give deep insights into the molecular machinery encoded by microbial genes. Subsequently, whole-genome shotgun sequencing can be used to identify the metabolic and enzymatic pathways of human microbiome(7). Notably, Zhang et al reported in *Nature Med* using the metagenomic shotgun sequencing of 212 fecal samples (77 treatment- naive individuals with RA and 80 unrelated healthy controls, 17 treatment-naive individuals with RA paired with 17 healthy relatives; and 21 samples from DMARD-treated individuals with RA))
(3). Thus, they were able to show that the redox environ-ment, transport and metabolism of iron, sulfur, zinc and arginine were changed in the microbiota RA patients. This study provided functional analysis of RA-associated mi-crobiome, and answered the question of " what the micro-biota does" in comparison to the question of " what the microbiota are", which is addressed by 16S rRNA sequen-cing(3,7).

Potential remedies targeting bacteria for RA

As discussed above, the advance of sequencing tech-nologies of microbiome enables us a better and deeper understanding of the taxa of the microbiomes and their functional pathways. Subsequently, human interventions as therapeutic approaches have evolved. Medications with antimicrobial properties, such as minocycline and sulpha-salazine, have been used as disease-modifying antirheu-matic drugs (DMARDs) in RA clinics(2). The study publi-shed in *Nature Med* also showed that DMARDs partially restored a healthy microbiome in RA patients compared to

controls(3). Certain antibiotics (e.g., Clarithromycin) can alleviate the symptoms of active RA and show protective effect in DMARD RA non-responders and who are not tolerant of DMARDs. In contrast, certain probiotics could prevent or attenuate RA symptoms(2,3).

New strategy for manipulating the gut microbiota to achieve the balance state of gut and the host might be useful for the therapeutic effect(2, 18). Furthermore, new drugs specifically targeting the gut microbiota dysbiosis (imbalance) in RA still require a more through investiga-tion(2, 11-13, 15).

Acknowledgements

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