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Microbiota analysis in rheumatoid arthritis: News and perspectives

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Abstract

In the last decade, our knowledge regarding the importance of the microbiome (i.e., the genes of the microbiota) in the pathophysiology of Rheumatoid Arthritis (RA) was transformed with the development of next generation sequencing techniques that now allow an overview of the totality of a host microbiome (defined as the species of microorganisms, such as bacteria, phages, viruses and fungi). Indeed, gut dysbiosis, that characterizes RA, evolves during the history of the disease with modifications observed at the preclinical stage and even more at the onset of the clinical disease in untreated and active patients. In those patients receiving treatment a partial correction is reported particularly when remission is achieved. Although incomplete, similar observations are noted when exploring mouth and lung microbiota. Dysbiosis reflects a loss of anti-inflammatory species and an overrepresentation of pro-inflammatory species (e.g. *Prevotella copri, Collinsella aerofaciens*) or species that promote citrullination and, in turn, anti-citrullinated peptide antibodies (ACPA) autoantibody production (e.g. *Cryptobacterium curtum, Haemophillus species*). Moreover, gut, mouth and lung dysbiosis are suspected of being involved in the origin of bacterial nucleic acids detected in the synovium of RA patients. Therefore, better knowledge of the microbiota during the course of the disease can open new perspectives regarding the prevention, diagnosis, and treatment of RA patients as reviewed.

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterized by the presence of long-standing inflamed synovial joints resulting in symmetric polyarthritis, synovial membrane hypertrophy with progressive joint damage as well as bone and cartilage destruction. RA etiology is unknown and involves genetic, epigenetic, and environmental factors, particularly infections and the host microbiome have been implicated in RA [1-3]. The term microbiome refers to the totality of the microbial genes in a host, whether those microbes are commensal or pathogenic, and the development of next generation sequencing techniques in the last decade has contributed to our deeper understanding of the effects of microbiomes. Testing the totality of the microbiota (i.e. the various species of bacteria, phages, viruses and/or fungi) contributes to a better analysis of the relationship between microbiota and the host without the necessity to perform bacterial cultivation.

How does RA impact the gut microbiota?

In healthy individuals and RA patients, the gut microbiota is stably maintained over time although important differences exist between individuals [4]. The first parameters to be explored when conducting a microbiota analysis are related to the density (10^{13}–10^{14} microbes/mL in gut) and the diversity (>1,000 species and >10,000 strains in gut) of the microbiota, which are exceptionally high in gut microbiota and constitute 80% of the total microbial biomass [5]. These parameters can be tested by determining the Shannon's index, for example, which takes into account the number of species present (alpha diversity), referred to as operational taxonomic units (OTU), and their relative frequency (beta diversity). Although initial studies have reported significant differences when testing global gut microbiota density and diversity in RA [6,7], more recent studies were only able to report differences at a lower taxonomic level, suggesting a modest effect of RA on the richness and diversity of the gut microbiota as initially suggested [4,8-10]. At the genera and even better at the species level, the most striking observations reported in RA were related to an altered balance between pro-inflammatory (e.g. Prevotella, Citrobacter rodentium, Collinsella aerofaciens, and Segmented filamentous bacteria) and anti-inflammatory bacteria (e.g. Bifidobacteria, Bacteroides, Porphyromonas, and *Faecalibacterium*), leading to the creation of a pro-inflammatory immune status. Such a concept was further confirmed experimentally as fecal transplantation in mice can promote RA when fecal material is taken from an RA patient (pro-inflammatory bacteria enrichment) while RA-prone mice have a reduced disease when fecal transplantation originated from feces with anti-inflammatory enrichment [9,11].

From microbiota experiments, several pathogenic taxa have emerged such as *Prevotella copri* and *Collinsella aerofaciens*. The prevalence of *Prevotella copri* at high abundance (>5%) is increased at the preclinical stage in RA patients (53%), at onset in non-treated RA patients (75%) when compared to treated RA patients (11.5%) and healthy controls

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(21.4%) [9,12]. Pianta et al. [13] have further demonstrated, *in vitro*, that peptides from *Prevotella copri* were effective in binding the HLA-DR molecules when they contain the "shared epitope" [13]. In patients with RA, it was also observed that the IgA anti-*Prevotella* immune response was correlated with innate (MIP-1), Th1 (IFNγ and IL-12), Th17 (IL-23, IL-17), and anti-citrullinated peptide antibodies (ACPA) sero-positivity [14]. However, *Prevotella copri* is not restricted to RA since it is also associated with other inflammatory diseases including metabolic syndromes such as insulin resistance type II diabetes as well as with plant-rich diets [15,16]. Finally, the *Prevotella copri* capacity to exacerbate colitis was revealed in an antibiotic treated mouse model after colonization with *Prevotella copri* by oral gavage.

The contribution of *Prevotella copri* was not replicated in a study by Chen et al. [7] which demonstrated that *Collinsella aerofaciens* was more abundant in new onset and untreated RA patients than in chronic RA cases and healthy controls [7]. The authors have further tested the pathogenic role of the bacteria by treating mice with *Collinsella aerofaciens,* in the collagen-induced arthritis-susceptible HLA-DQ8 model, leading to an increase in the arthritis incidence but not of the severity. The proposed mechanism relies on the ability of *Collinsella aerofaciens* to increase the gut permeability by reducing the expression of the tight junction protein ZO-1 which, in turn, promotes Th17 cytokine expression.

How does RA impact mouth and lung microbiota?

In contrast to the gut microbiota that is critical for immune homeostasis, both the mouth and lung have been implicated as potential sites where loss of tolerance against self-peptides can occur in individuals presenting the right genetic background (e.g. the HLA-DRB1 "shared epitope") leading to the local production of circulating autoantibodies such as ACPA and anti-IgG antibodies also known as rheumatoid factor (RF) [17] . This is based on the observation of an enrichment in the RA patients' mouths of bacteria with sialydase properties able to induce periodontal diseases (*Porphyromonas*, *Treponema* and *Tannerella*) and, in particular, to *Porphyromonas gingivallis* which has the additional capacity to induce production of citrullinated antigens, a precursor step for ACPA production [18,19]. Further, *Porphyromonas gingivalis* has the capacity to exacerbate a pro-inflammatory response through activation of the Toll like receptor (TLR)2/4 pathway [20]. When using a 16S rRNA gene high-throughput sequencing approach, instead of a classical culture approach, a higher abundance of periodotopathic bacteria was retrieved in the saliva and subgingival dental plaque of patients with RA [21-23]. In their study, Sher *et al* failed to associate *P gingivalis* detection in the oral microbiota of RA patients with the presence and titers of ACPA or RF. In contrast *Prevotella* and *Leptotrichia* species only existed in RA, and *Anaeroglobus geminates* detection was associated with the serum titers of ACPA and RF as well as with the presence of periodontal disease. Similarly, Lopez-Oliva *et al* failed to associate *P gingivalis* with RA in a periodontis cohort but highlight another species, *Cryptobacterium curtum,* with the capacity to produce citrulline from arginine through the arginine deiminase pathway [24]. From the Zhang report, an inverse correlation between the abundance of *Haemophillus* species in dental plaque, saliva and gut was reported with anti-ACPA and RF levels [8].

The role of the lung as an RA trigger has recently emerged based on the report of associations between tobacco smoking, HLA-DRB1 "shared epitope", and an elevated production of ACPA in the sputum. Lung microbiome analysis conducted by Scher et al. [25] has revealed an important reduction of the diversity (40% less OTUs) in RA patients when compared to healthy controls [25]. However, such differences were not seen between RA and patients with sarcoidosis, a typical inflammatory lung disorder, which supports that lung microbiota diversity reduction results from a common inflammatory signature rather than from a disease specific signature. Such reductions involve periontopathic taxa (e.g. *Porphyromonas*, *Treponema* and *Prevotella*) as well as anti-inflammatory taxa (e.g. *Actinomyces*, *Spirochaetaceae*, *Burkholdelia*, *Prevotella*). With regards to specific genus for RA when compared to healthy controls and patients with sarcoidosis, the antifungal and commensal microorganism, *Pseudonocardia,* was increased in RA patients and correlated with higher disease activity and erosion. Another genus previously associated with RA in the gut and detected in the lung microbiota, *Prevotella,* was positively correlated with ACPA titers in the RA group.

Synovial microbiota

The presence of microbes in the synovial fluids and tissues is controversial as some but not all authors have revealed in RA patients intra-articular pathogens including bacteria and viruses [26,27]. To clarify this issue, Zhao *et al* conducted a microbiota analysis of synovial fluids and tissues by comparing RA patients with osteoarthritis (OA) patients, a mechanistic joint disease used as a control [28]. The first observation from this study was related to the detection of bacterial nucleic acid in all synovial fluids and tissues from both RA and OA patients. The second observation supported the concept that more species were reported in the synovial tissues (15 OTUs) than in the synovial tissue (2 OTUs including *Porphyromonas* and *Bacteroides*). Third, *Treponema amylovarum, haemophilus parainfluenzae, Prevotella copri, Fusobacterium* and *Veillonella dispar* were more concentrated in synovial fluids from RA patients which is in agreement with a higher report in the gut of *Prevotella copri*; in the oral cavity of *Porphyromonas gingivalis* as well as *Fusobacterium nucleatum*; and in the respiratory tract of patients with arthritis of *haemophilus* [7,12,29-32].

Relation with disease activity and DMARDs

Disease activity in RA can be estimated by the use of the disease activity score 28-joint count (DAS28) that is a composite score taking into account the number of swollen joints (out of 28), the number of tender joints (out of 28), the patient's global health assessment (from 0=best to 100=worst), and the inflammation based on the blood measure of the erythrocyte sedimentation rate (ESR) or the C-reactive protein (CRP) [33]. A DAS28 of greater than 5.1 reveals active disease, between 5.1 and 3.2 low disease activity, and less than 2.6 a remission. RA activity is correlated with gut dysbiosis and the dysbiotic condition is partially resolved after remission upon treatment with conventional or biologic Disease-modifying anti-rheumatic drugs (DMARDs) [7,10]. Such an observation was confirmed in other diseases with an inflammatory component such as inflammatory bowel disease (IBD) and diabetes. Chen *et al* failed to retrieve dysbiosis when the first-degree relatives of RA patients under treatment were tested indicating that genetic and environmental factors had a lower effect than disease activity on the gut dysbiosis. However, genetic factors could not be neglected as mice expressing the RA-susceptible HLA-DRB1*0401 gene exhibited dysbiotic gut microbiomes dominated by *Clostridium-like bacterium*, whereas mice carrying an RA-resistant gene are enriched for members of the *Porphyromonadaceae* family and *Bifidobacteria* [34]. When assessing the influence of treatment on the oral microbiome of chronic RA patients, the higher impact was observed with steroids when used alone or in association with conventional or biological DMARDs [35].

In RA, the *Euryarchaeota* phyla was correlated with DAS-28 in Picchianti-Diamanti's report [10]. A negative association between HLA-

DRB1 "shared epitope" and the abundance of the pro-arthritogenic bacteria *Prevotella copri* was observed, providing another argument supporting the interplay between microbiota and genetic background [36]. Other parameters influencing gut dysbiosis are related to the body mass index, the disease duration, the inflammation (CRP levels), and RF levels [7].

Relation with antibiotics

From RA-mouse models we know that mice remain healthy when reared in germ-free conditions but this can be reversed through activation of the TLR signaling pathway upon mono-colonization with segmented filamentous bacteria or *Lactobacillus bifidus* in the K/BxN and IL-1RA-/- mouse models, respectively [37-39]. The implication of the microbiota in RA pathogenesis is further supported by the fact that antibiotic prescriptions have been associated with a higher risk of RA development in a large study conducted in the United Kingdom and the fact that the use of macrolide antibiotics, active against anaerobic bacteria, were effective in improving the RA outcome in a double blind trial [40,41]. Similarly, periodontal treatment consisting of root scaling and planning and oral hygiene instruction reduces RA disease activity, serum levels of ACPA and IgG anti-*Porphyromonas gingivalis* [42].

As a consequence, changing the microbiota homeostasis may have therapeutic implications and this is suspected to be the means of effect for methotrexate (MTX), the first and most effective drug for RA. After entering the cell, MTX suppresses dihydrofolate (DHF) reductase, thereby interfering with purine and pyrimidine synthesis which blocks lymphocyte proliferation. However, the gut microbiota produce DHF that may compete with host DHF and, as a consequence, the ratio between DHF low bacteria (e.g. *Prevotella*) and DHF high bacteria (e.g. *Bacteroides*) may be important for MTX response as proposed by Scher et al. [9].

Conclusion

There is compelling evidence to consider that the microbiota plays a key role in RA but, although key evidence has been provided, many questions remain. In particular, in future studies it will be important to determine the influence of the genetic background as well as the influence of the environment (food, water, soil, pollution, and other stressors) and systemic infections. A better understanding of the key actors and mechanisms is also a particular challenge to address in order to propose new strategies for prevention, diagnosis and treatment.

Disclosures

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