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EDITORIAL

Can *Prevotella copri* Be a Causative Pathobiont in Rheumatoid Arthritis?

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Microbes of the same order as the number of human cells inhabit the skin and mucosal surfaces inside and outside the body (1). It is now widely recognized that the host and the microbes coexist in close equilibrium and maintain a symbiotic relationship (1,2). The intestinal tract contains the greatest diversity and density of microbial species, affecting many aspects of the host, including metabolism, circadian rhythm, neurobehavioral development, and immune defenses to pathogens (2). It is also well known that the interaction between host and microbes is necessary for the proper development of the host immune system (2,3). For example, germ-free animals have defects in the development of gut-associated lymphoid tissue and Peyer's patches, formation of a tight junction between intestinal epithelial cells, and secretion of antimicrobial peptides and mucus from epithelial cells (2). Many microbiota, so-called symbionts, have regulatory properties beneficial to the host and prevent colonization by the pathogens, while some microbiota, called pathobionts, induce a proinflammatory state, triggering disease under certain circumstances (2).

Intestinal dysbiosis, a state of microbial imbalance, has been implicated in the pathogenesis of a number of diseases, particularly autoimmune disorders in which the host immune system erroneously attacks its own tissues (2,4). As expected, intestinal dysbiosis in inflammatory bowel disease is characterized by its reduced diversity and, in contrast, increased number of pathobionts (2). Intriguingly, the disturbance of intestinal microbiota can influence the onset and/or progression of autoimmune diseases including rheumatoid arthritis (RA), even though it is localized to tissues far removed from the gut (4,5).

RA is a prototype chronic inflammatory disorder characterized by uncontrolled inflammation of synovial tissue, leading to joint destruction and a wide array of multisystem comorbidities (4). Its pathogenesis is thought to be attributed to complex interplay between multiple genes and diverse environmental factors, including infectious microorganisms (5–7). Interestingly, antibiotic treatment or germ-free conditions interrupted the development of arthritis in several experimental RA models with diverse genetic predispositions, such as zymosan-treated SKG mice, interleukin-1 (IL-1) receptor antagonist–knockout mice, T cell receptor–transgenic K/BxN mice, and HLA–B27–transgenic rats (5). Conversely, arthritis symptoms developed in animals that were housed under germ-free conditions and were colonized with specific bacteria, such as *Lactobacillus bifidus* and segmented filamentous bacteria, and recolonized with feces from other sources (5). Thus, it has now been speculated that the microbiota may become one of the missing links in the pathogenesis of RA.

Over the past few decades, diverse pathogens, including some bacteria (e.g., *Escherichia coli*, *Streptococcus*, and *Mycoplasma*), viruses (e.g., *Parvovirus* and *Retrovirus*), and mycobacterium have been suggested as possible causative agents in RA (6). Moreover, recent studies have shown a close correlation between peptidylarginine deiminase produced by gingival pathogens and the development and

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progression of RA. In particular, *Porphyromonas gingivalis* was shown to citrullinate the host proteins, leading to the formation of immune complexes with anticitrullinated protein antibodies that can mediate local synovial inflammation (4,8). However, another clinical study demonstrated that the presence and abundance of *P gingivalis* in the subgingival site were irrelevant to development of RA (9). Because in-depth and high-throughput sequencing of the human microbiome at affordable costs has been possible with the next-generation sequencing technologies and high-density microarrays, the gut microbiome in RA patients compared with that in healthy controls has been studied (7,10). A metagenome-wide association study using such technology demonstrated that *Haemophilus* species were depleted in fecal, dental, and salivary samples from RA patients, whereas *Lactobacillus salivarius* was overrepresented in the same samples (10).

In this issue of *Arthritis & Rheumatology*, Maeda et al (11) describe their use of 16S ribosomal RNA–based deep sequencing technology to analyze fecal microbiota and observed that *Prevotella*, specifically *P copri*, was in abundance within gut microbiota in Japanese patients with early RA who had not received drug treatment, which is consistent with the results of a study in North American patients with early-onset RA (12). Importantly, Maeda et al provided the first direct evidence that *Prevotella*-dominant feces from patients with RA and *P copri* could aggravate arthritis signs, inducing severe synovitis in SKG mice housed under germ-free conditions that were recolonized with feces from RA patients or *P copri*. Moreover, they identified the pathogenic cellular mechanisms connecting the abundance of *P copri* with arthritis progression. *Prevotella*-dominant fecal content increased the Th17 cell population in SKG mice as well as Th17 cell–biased responses to the arthritis-related autoantigen RPL23A. *P copri* per se had a high capacity to induce Th17 cell–related cytokines, such as IL-6 and IL-23.

Intriguingly, some studies suggested that *Prevotella* might be regarded as a beneficial bacterium and not a pathobiont (13,14). For example, the enrichment of *P copri* was observed in healthy individuals. A decrease in bacteria of the *Bacteroides–Porphyromonas–Prevotella* group was also observed in patients with early RA (15). Moreover, Scher et al reported that the relative abundance of *P copri* was inversely correlated with the presence of the shared epitope risk allele in RA patients (12). In fact, *Prevotella* is a large genus with high species diversity between human hosts; even a single strain of *P copri* shows variation across hosts (16). Therefore, the effects of *P copri* on arthritis progression can be different depending on such strain variations and the context. Further studies are needed to determine whether *P copri* plays a preventive or provocative role in RA pathogenesis.

It may be necessary to consider other microorganisms together with *P copri* to better understand the precise role of *P copri* in host health and disease. In the current study, Maeda et al identified a reduction in the abundance of *Bacteroidaceae* in fecal samples from the RA patients in cluster 4. *Bacteroides* and *Prevotella* species have their own specific preference in the human body, because they are antagonistic (13,16). *Prevotella* has been associated with fiber-rich diets, whereas *Bacteroides* has been linked to increased consumption of protein and fat (13,16). Interestingly, *Bacteroides fragilis* was involved in the production of IL-10, an antiinflammatory cytokine, and the development of Treg cells in the intestine (3). In this regard, a decrease in *Bacteroides* and an increase in *Prevotella* might be contributing to the progression of experimentally induced arthritis observed in this study.

The current study specifies *P copri* as a clinical target pathobiont and supports the plausibility of various clinical approaches to reverse dysbiosis. A number of clinical studies have shown the effectiveness of antibiotic drugs in the treatment of RA (4). Sulfasalazine, a disease-modifying antirheumatic drug (DMARD), possesses not only antibacterial activity against nonsporing anerobes, *Clostridia* and *Enterobacteria*, but also has antiinflammatory properties. These may include suppression of inflammatory cytokines and induction of apoptosis in inflammatory cells (4). Doxycycline and minocycline, which are safe and moderately effective DMARDs for the treatment of early RA, inhibit matrix metalloproteinase and nitric oxide synthase, suppress adaptive immune cells, and increase IL-10 production (4). Because certain intestinal microbiota have a role in RA pathogenesis, particularly *P copri*, it is all the more plausible that improvement in the RA disease course by antibiotic drugs comes, at least partly, from their antibacterial properties. Thus, it will be valuable to examine whether these antibiotics are effective against *Prevotella*, in particular *P copri*.

Because probiotics have the potential to maintain a positive balance that is favorable to a host, several groups of investigators have explored their effect in RA, such as treatment with antibiotics to reduce harmful bacteria (4). *Lactobacillus casei* and *Lactobacillus* GG regulated the expression of proinflammatory and antiinflammatory cytokines and cyclooxygenase 2, resulting in attenuation of symptoms in animal models of RA (17,18). Even though clinical effects of probiotics have been proven in only a handful of conditions, it will be worthwhile to note the effects of probiotic consumption on *Prevotella* in the gut. Bacteriophages also play an active role in shaping the ecology of the bacterial community, although they are a neglected component of the gut microbiota (19). Phage therapy has been used for ~100 years as a treatment of bacterial infections in humans as well as other species with bacteriophages that specifically infect and kill certain bacterial species but only minimally affect nontarget bacteria

or body tissues (19). Such properties could be used to specifically modulate intestinal dysbiosis involving *P copri*.

One important avenue of study will be to determine whether *P copri* plays a role as an etiologic agent in the onset of RA or whether it simply contributes to the perpetuation of RA. Maeda and colleagues clearly showed that *P copri* could facilitate the progression of RA in SKG mice treated with zymosan, based on its proinflammatory properties. However, because the feces analyzed in their study originated from RA patients in whom disease had already developed despite the early stage, it is difficult to infer the contribution of *P copri* to the initiation of RA. All cases of arthritis that cannot be classified in one of the accepted categories are defined as undifferentiated arthritis, and a significant proportion of patients with undifferentiated arthritis progress to RA, while others undergo spontaneous remission (8). To determine the pathobionts responsible for RA onset, long-term monitoring of the alteration of microbiome from undifferentiated arthritis to RA will be needed.

Another critical avenue of research will be elucidation of the mechanisms by which *P copri* predisposes its host to RA in tissue distal to the gut, particularly the joints. Maeda et al showed that *P copri* induces an increase in IL-17–mediated responses and differentiation of Th17 cells in the large intestine and draining lymph nodes but not in the small intestine and spleen. Do the increased numbers of Th17 cells migrate to joint tissues and contribute to the development of arthritis? Unfortunately, this study failed to provide evidence that Th17 cells generated in the gut are transported to the joints, and direct evidence about migration of Th17 cells from the gut to the joints is lacking at this stage. Alternatively, dysbiosis potentially mediates leakage of the immune barrier, leading to penetration of bacteria and/or their components into the entire body (2,4). In support of this notion, bacterial components have been detected in the synovium of RA patients, leading to induction of inflammatory responses and pannus formation in the joints (4). Therefore, it is worthwhile to examine whether *P copri* has the potential to break down the intestinal barrier, which triggers bacterial components in arthritic joints, generating a chain of proinflammatory events leading to arthritis in the joint space.

Taken together, the findings of the study by Maeda et al indicate that *P copri* might be a significant etiologic agent in facilitating the progression of RA, or that the inflammation elicited by a variety of microorganisms, including *P copri* (dysbiosis), might contribute to the perpetuation of RA. In this regard, it seems promising to develop treatments to selectively deplete *P copri* or recover total dysbiosis in RA patients, although the methods for selectively targeting of certain bacteria or a bacterial group are currently limited. In addition, the current study sheds new light on the need for revisiting how probiotics and antibiotics ameliorate the activity and severity of RA. More studies are needed to further investigate interactions between *P copri* and its host and other bacteria in a clinical scenario.

AUTHOR CONTRIBUTIONS

Both authors drafted the article, revised it critically for important intellectual content, and approved the final version to be published.

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