Review

# Microbiota and arthritis: cause or consequence?

G. Cafaro, G. Cruciani, L. Bruno, R. Dal Pozzolo, A. Colangelo, F. Tromby, M. Nicchi, B. Pianese, C. Perricone, R. Gerli, E. Bartoloni

*Rheumatology Unit, Department of Medicine and Surgery, University of Perugia, Italy.*

*Giacomo Cafaro, MD, PhD**
*Giulia Cruciani, MD**
*Lorenza Bruno, MD*
*Roberto Dal Pozzolo, MD*
*Anna Colangelo, MD*
*Francesco Tromby, MD*
*Martina Nicchi, MD*
*Biancamaria Pianese, MD*
*Carlo Perricone, MD, PhD*
*Roberto Gerli, MD*
*Elena Bartoloni, MD*

**Contributed equally.*

*Please address correspondence to:*
*Roberto Gerli*
*U.O. di Reumatologia,*
*Dipartmento di Medicina,*
*Università di Perugia,*
*Piazzale G. Menghini 1,*
*06129 Perugia, Italy.*
*E-mail: roberto.gerli@unipg.it*

*Received on February 22, 2024; accepted in revised form on March 15, 2024.*

*Clin Exp Rheumatol 2024; 42: 1097-1103.*



**Key words:** microbiota, rheumatoid arthritis, psoriatic arthritis

*Competing interests: none declared.*

**ABSTRACT**

*The relationship between intestinal microbiota and arthritis has garnered significant attention, with emerging evidence suggesting a potential association between dysbiosis and various forms of inflammatory arthropathies. While observational studies have provided valuable insights into microbiota alterations in patients with arthritis, establishing causality remains challenging.*

*Observational data, influenced by multiple confounders such as environmental factors, medication effects, and dietary habits, are insufficient to conclusively determine whether microbiota changes are somehow causally linked to arthritis. The heterogeneity of results across independent studies further complicates interpretation. To further support this hypothesis, interventional randomised trials are deemed necessary, yet their implementation in this area presents significant technical limitations.*

*Experimental animal models offer insights into potential pathogenic mechanisms linking dysbiosis to arthritis, including compromised intestinal barrier function, the role of microbiota-derived metabolites and molecular mimicry. However, conflicting findings underscore the complexity of host-microbiota interactions and the challenges in establishing causality.*

*Efforts to modulate the microbiota for arthritis treatment or prevention have shown promise, yet efficacy and applicability remains uncertain. Antibacterial drugs, dietary interventions, probiotics, and faecal microbiota transplantation have been explored, but their clinical utility awaits further validation. In conclusion, while the association between intestinal microbiota and arthritis is increasingly recognised, establishing causality remains elusive.*

## Introduction

Through the millennia, humans developed a close relationship with a huge number of bacteria, mostly hosted in the digestive tract. Gut microbiota, in fact, comprises 1000–5000 diverse species of microorganisms, 99% of whom belong to the phyla *Firmicutes, Bacteroidetes, Proteobacteria*, and *Actinobacteria* (1). The relationship is not passive and biological processes with mutual advantages take place (2). Unfortunately, an association between changes of the microbiota and a wide spectrum of diseases has been unequivocally demonstrated.

Modern DNA sequencing technologies enhabled us to study human microbiota and its alterations in a variety of conditions, including various forms of arthritis, and the correlation between gut dysbiosis and inflammatory arthropathies is now clear. Correlations, however, do not necessarily entail causation, whose ascertainment presents numerous hurdles that are not easily overcome. The most obvious question arising is whether microbiota alterations observed in a disease occur as a consequence of systemic inflammation and autoimmunity, or if they have a causative role in the initial loss of tolerance observed in autoimmune and inflammatory diseases, or, finally, whether no causal relation is present (3-5).

Doubtlessly, from a temporal point of view, changes of the microbiota can occur before disease onset, as clearly shown by multiple studies in pre-clinical rheumatoid arthritis (RA) (6-10). In fact, patients with autoantibodies or genetic risk factors, showed gut microbiota alterations well before RA onset (11). However, these findings are not enough to establish causality, nor its direction.

In this paper, we will try to review and summarise the main data available on

dysbiosis in inflammatory arthritides, with a focus on likely causative mechanisms. We will also try to underline the complexity of this research field and to show why obtaining a final answer to the question is a remote goal.

Current evidence is mostly of observational nature and the results of a significant number of independent studies are very heterogeneous with numerous and distinct bacterial species found to be involved. For this reason and due to the overall poor results obtained with treatment strategies trying to modify gut bacterial composition it is however clear that intestinal dysbiosis cannot be an independent cause of disease development (7, 8).

## Microbiota alterations and inflammatory arthropathies

### *Rheumatoid arthritis*

Doubtlessly, RA is the form of arthritis with the greatest amount of research on this topic. RA is particularly useful as a model to study causality, due to its extended period of seropositivity preceding disease onset, which is usually defined as the pre-clinical phase (3). Evidence from epidemiological and translational studies indicates that mucosal environmental exposures and dysbiosis play causal roles during this period in the development of RA (12-15). Notably, mucosal sites, including the oral and intestinal mucosa, might be the location where autoimmunity begins, due to the ability of microbes and their metabolites to influence and modulate the function of the host immune system (1).

The vast majority of data suggesting a potential relationship between rheumatic diseases and microbiota alterations comes from cross-sectional and case-control studies comparing patients with healthy controls or first-degree relatives (16, 17). Unfortunately, the results of these studies display a certain degree of inconsistency. Albeit most studies found an increase in *Prevotellaceae* (6, 11, 12, 18) and a decrease in *Bacteroides* and *Bifidobacteriaceae* (7, 8, 12), other data show a decreased prevalence of *Bifidobacteria* with a simultaneous increase of *Bacteroides* in RA patients(8). Additionally, other studies found changes in the concentration of other bacterial populations, such as *Collinsella, Eggerthella,* and *Faecalibacterium* (9). Differences in the methodological approach to the analysis of microbiota are the most likely cause of discordance among studies, together with multiple variables with unequivocal impact on microbiota composition.

Nevertheless, we must keep in mind that observational studies do not allow to establish causal relationships, which inevitably require experimental evidence. However, in this area, this is of very difficult applicability in humans, therefore most studies focused on animal models of arthritis. Nonetheless, even experimental models provided conflicting results. As an example, a study by Liu *et al*. found substantial changes in gut microbial community of a collagen induced arthritis (CIA) model, especially an increased representation in *Bacteroidaceae* and *Lachnospiraceae* and a reduction in *Lactobacillaceae* (4, 19). However, in the same model of disease, Rogier *et al*. (20) showed a reduction in *Bacteroidaceae* family in the preclinical phase of CIA, and an increased amount of *Firmicutes* and *Proteobacteria*.

Evidence in favour of dysbiosis as a cause of arthritis arises from other interesting models (10). Inoculating faecal samples from early RA patients into germ-free arthritis prone SKG mice, an increased prevalence of *Prevotellaceae*, especially *Prevotella copri (P. copri)* and reduction of *Bacteroidaceae* was found after 20 weeks of colonisation, compared to mice inoculated with faecal samples from healthy subjects. Additionally, the former mice showed an increased number of intestinal T helper 17 (Th17) cells and a more severe form of arthritis.

Another useful arthritis model is found in K/BxN mice, expressing the transgenic T cell receptor (TCR) KRN and the MHC class II allele Ag7. They uniformly develop severe inflammatory arthritis due to high levels of autoantibodies directed against the glycolytic enzyme glucose-6-phosphate isomerase (GPI) (21). In germ-free K/B×N mice, the severity of arthritis was greatly reduced along with sharp reductions in serum concentrations of autoantibodies and the number of splenic Th17 cells and germinal centre formation. Additionally, when segmented filamentous bacteria (SFB) were subsequently introduced a Th17 cells accumulation in the lamina propria and the development of arthritis was observed (22-25). An increased Th17/Treg ratio is also observed in RA, and the balance of this ratio is known to be strongly regulated by gut microbiota and their metabolites (24, 26).

### *Psoriatic disease*

In patients with psoriasis and psoriatic arthritis (PsA) we may find an increase of *Actinobacteria* and *Firmicutes* and also of the *Firmicutes-to-Bacteroides* (F/B) ratio, expressing impaired gut epithelial barrier, in a similar manner to that observed in patients with inflammatory bowel diseases, obesity, type 2 diabetes and cardiovascular comorbidities (27, 28). More in detail, an underrepresentation of *Faecalibacterium prausnitzii, Bifidobacterium spp., Lactobacillus spp., Parabacteroide*and *Coprobacillus*, and an increase of *Salmonella sp*., *Campylobacter sp., Helicobacter sp., Escherichia coli, Alcaligenes sp*. and *Mycobacterium* has been observed (27). Cho *et al*. (29) showed an elevated F/B ratio in patients producing increased amounts of trimethylamine-N-oxide (TMAO). In fact, a high F/B ratio is associated with an increased abundance of bacteria capable of metabolising carnitine to TMA, which in turn alters cholesterol turnover and induces macrophage activity, consequently promoting atherosclerosis.

Another significant consequence of an increased F/B ratio is an altered production of short-chain fatty acids (SCFA) and medium-chain fatty acids (MCFA) with an increased bacterial synthesis of acetate and decrease synthesis of butyrate. The beneficial role of SCFAs on the balance of intestinal microbiota has been widely demonstrated and will be discussed further, whereas data about MCFAs are not as consistent. Multiple studies, in fact, showed that MCFAs may contribute to the differentiation of murine and human $CD4^+$ naive T cells into Th1 and Th17 phenotypes, thus exerting a pro-inflammatory action. However, this seems to be in contrast with

findings showing a reduced amount of MCFAs in faecal samples of patients with arthritis (28, 30).

*Juvenile idiopathic arthritis*

Various studies have been carried out in patients with Juvenile Idiopathic Arthritis (JIA), mostly showing an increase of *Bacteroidetes*/*Bacteroides*, especially *Bacteroides fragilis*. Results suggesting a reduction of *Firmicutes*, instead, do not show consistency (31-33).

*Ankylosing spondylitis*

Certain bacteria, including *Bacteroides coprophilus, Parabacteroides distasonis, Eubacterium siraeum,* were found to be enriched in AS patients (34). Additionally, *Klebsiella* abundance appears to be linked to disease activity and *Actinobacteria* may be able to promote inflammation by activating NF-κB signalling (35).

Most of the currently available evidence, however, highlights the concept that instestinal gut dysbiosis may increase AS risk by interacting with environmental exposure and various genetic factors,in particular human leukocyte antigen (HLA)-B27; in murine models, the interaction between this allele and gut microbiota lead to the activation of the interleukin (IL)-23/IL-17 axis (36-39). IL-23, which plays a central role in the development of AS,is mostly secreted by dendritic cells and macrophages and promotes the differentiation and maintenance of Th17 cells which, in turn, are responsible of a significant pro-inflammatory cascade in spondyloarthritis (SpA) (38, 40). Additionally, an intereaction between the microbiota and IL-17/IL-22-producing innate lymphoid cells (ILC) type 3, has been demonstrated (39, 41).

*Gout and hyperuricaemia*

Evidence in this field is limited and mostly observational and speculative. Overall, it seems that patients with gout and hyperuricaemia show a reduction in microbiota diversity (42). In fact, a higher F/B ratio and a lower *Prevotella-to-Bacteroides* ratio has been described in patients with hyperuricaemia (43), while gout patients display a relative abundance of *Prevotella, Fusobacterium* and *Bacteroides* and a lower prevalence of *Enterobacteriaceae* and butyrate-producing species (44, 45).

**Pathogenic insights**

As alreday stated, the vast majority of studies performed on the topic are observational. This is likely the main reason why a clear causal relationship between microbiota changes and inflammatory arthritis has not yet been demonstrated in either direction. Most of the evidence in support of the hypothesis that dysbiosis can contribute to the development of inflammatory arthritis comes from observational studies and from animal models. Multiple pathogenic mechanisms have been suggested to contribute to the induction of autoimmunity and to the transition from the preclinical phase of the disease to the initiation and progression of arthritis due to microbiota dysbiosis (1, 4, 19, 20, 46). These encompass compromised intestinal barrier function, metabolites originating from the microbiota, molecular mimicry, immune responses induced by the microbiota, autophagy of intestinal epithelial cells (IECs), and alterations in microRNA (miRNA) expression (46).

*Intestinal barrier dysfunction*

Histological analysis of intestinal tissue from individuals with established RA revealed distinctive features in approximately 15% of patients, such as partial or complete loss of the superficial epithelium, elevated numbers of plasma cells and granulocytes and the presence of vasculitic lesions (4, 47). Similarly, another study enrolling a small group of patients with early RA revealed subclinical gut inflammation in nearly all participants. In particular, an increased prevalence of infiltrating mononuclear cells, T cells, B cells, and CD68+ macrophages was detected, along with lymphoid follicles (48).

A disruption of the intestinal barrier has thus unequivocally been demonstrated as one of the potential mechanisms linking dysbiosis and inflammation.

The primary mechanism linking gut epithelial barrier disruption and microbiota alterations seems to be zonulin production. Zonulin is an enterotoxin secreted by IECs after stimulation by dysbiotic bacteria or diet (*e.g.* gluten may contribute too), which determines the disassembly of the proteins zonula-occludens 1 (ZO1) and occludin from the tight junction complex, thus increasing intercellular permeability. In fact, the transfer of human gut-derived *Prevotella histicola* to mice with CIA resulted in decreased arthritis severity, reduced intestinal permeability and increased expression of ZO1 in the jejunum, ileum and colon (4).

Barrier integrity is also affected by SCFAs, particularly butyrate, which is capable of enhancing its stability by promoting the production of the tight junction protein mucin 2 (49). Consequently, barrier function is impaired in case of reduced butyrate production by the intestinal microbiota. The role of SCFAs has been very briefly mentioned above and will be further discussed in detail in the following paragraph.

*Gut microbiota-derived metabolites*

Metabolites derived from gut microbiota play a role in regulating the integrity of the intestinal barrier through various mechanisms (1, 4, 39, 49-52). As SCFAs are primarly produced by some intestinal bacteria, alterations in gut microbiome composition, particularly the abundance of certain genera like *Collinsella, Fusicatenibacter* and *Megamonas* may contribute to changes in their levels (53). Bacteria such as *Escherichia coli* and *Streptococcus bovis* appear to be linked to higher degradation of ascorbic acid leading to increased serum levels of pro-inflammatory cytokines such as TNF-α and IL-6 in RA patients (46). Additionally, evidence shows a higher abundance of specific bacteria like *P. copri, Verrucomicrobia* and *Akkermansia* in the pre-clinical and clinical phases of RA, altering the metabolite levels and probably contributing to disease development. As an example, *P. copri* correlates to serum concentrations of arachidonic acid-derived inflammatory mediators including prostaglandins and leukotrienes (46, 54).

Decreased levels of SCFAs are detectable in both RA patients and animal models, with SCFAs supplementation showing reduced disease severity in

various RA mouse models, including CIA and K/BxN serum-transfer arthritis (46). SCFAs can induce metabolic alterations in T cells by activating the mammalian target of rapamycin (mTOR) complex and by modulating glucose metabolism (1). In particular, propionic acid, valeric acid and butyric acid are those with the largest amount of supporting evidence in favour of an anti-inflammatory effect. More specifically, propionic acid induces Th2 cell suppression and promotes Treg differentiation (55), valeric acid induces IL-10 production in regulatory B cells through enhanced mTOR activity and suppresses the generation of intestinal Th17 cells (56) and, finally, butyric acid, a functional SCFA produced by the anaerobic gut microbiota, is involved in T cells polarisation towards Treg cells in the spleen, proinflammatory cytokine down-regulation, decrease of systemic Th17 cells and inhibition of autoantibody production (1, 46).

SCFAs also play a key role in psoriasis and PsA, where the intestinal dysbiosis appears to be characterised by a reduction in the occurrence of butyrate-producing bacteria (27, 57). As an example, there is evidence in psoriatic patients of underrepresentation of *F. prausnitzii* and *Akkermansia muciniphila*, which have an important antioxidant activity by producing butyrates and inhibiting NF-κB, also providing energy to the IECs. Moreover, SCFAs are responsible of blocking IL-6 production, thus reducing intestinal inflammation (27, 51).

### *Molecular mimicry and autophagy of IECs*

Molecular mimicry is another classic mechanism involved in the development of autoimmunity in a broader sense and numerous clues suggest it is likely a major aspect of a potential pathogenic role of the intestinal microbiota (46). In fact, many identical peptides between human tissues and gut microbes are able to bind HLA-II alleles (23). Bacterial species showing the highest impact in genetically susceptible individuals belong to the *Firmicutes* and *Proteobacteria* (23).

It is known that peptides from *Bacteroides, Eggerthella, Citrobater* and *Clostridium* may share molecular mimicry with collagen XI and HLA-DRB1*0401. Collagen XI is present in articular cartilage and it can be used to induce arthritis in DBA/1 mice. HLA-DRB1*0401 is involved in the process of arthritogenic self-peptides presentation and, as an example, the presence of shared sequences with *Collinsella* genome suggested that this gut bacteria may contribute to the induction of RA (23, 46).

Another link between the mucosal and joint immunity may be provided by the sequence homology expressed by peptides tipically found in the synovium, like N-acetylglucosamine-6-sulfatase (GNS) with epitopes from sulfatase proteins of *Prevotella* and *Parabacteroides* sp., and another, filamin A (FLNA), with epitopes from proteins of *Prevotella* and *Butyricimonas* sp. (58). According to these data, GNS and FLNA were identified as T- and B-cell-targeted autoantigens in more than 50% of RA patients, representing a mechanism by which *Prevotella* contributes to RA progression (46).

Another potential mechanism that contributes to maintaining the balance of intestinal microbiota is autophagy. In fact, deficiency of colonic epithelial cell-specific autophagy related gene (Atg) results in an imbalance in host microbiome, leading to an increase of B. *fragilis, Clostridium leptum, Eubacterium cylindroides* and *Prevotella* in mice models, along with a decrease in *Lachnospiraceae* and *Ruminococcaceae*, which are known to exert an anti-inflammatory activity (59, 60). Additionally, supplementation of the autophagy inducer spermidine in mouse models promoted the expansion of *Firmicutes* (61).

## Modulating the microbiota to treat arthritis

In light of the evidence in favour of a potential contribution of the microbiota to the development of inflammatory arthritis, it is reasonable to hypothesise that its modulation may have a role in the treatment or prevention of the disease. However, it should be kept in mind that once the disease is established, restoration of the microbiota is unlikely going to be effective. Thus, any potential intervention on the microbiota should take place very early in the disease development, ideally even before it is currently possible to detect autoimmunity (4, 5, 25, 38, 46, 50, 62-66).

One of the possible ways to modulate the intestinal microbiota is through the administration of antibacterial drugs. Studies on CIA models have demonstrated that the elimination of intestinal microbiota may reduce arthritis severity, perhaps via an inhibitory activity on the Th17 axis (65). For instance, tetracyclines like minocycline may reduce microbial taxa tipically overexpressed in RA, such as *Actinobacteria* and *Firmicutes* (67).

It has also been widely demonstrated that the composition of gut microbiota is heavily dependent on diet. Consequently, it is also reasonable to suppose that changing dietary habits may modulate the microbiota and, eventually, affect the onset of autoimmunity (4, 25, 63, 64, 68).

The evidence available on this specific aspect is overall scarcely consistent. The most scientifically reasonable approach, at the moment, seems to be the mediterranean diet (MD) (25, 62), typically rich in vegetables, cereals, legumes, olive oil and a limited amount of dairy products. The MD demonstrated anti-inflammatory and antioxidant properties thanks to the high concentration of n-3 polyunsaturated fatty acids (PUFAs), monounsaturated fatty acids (MUFAs), fibres and polyphenols. Dietary supplementation of n-3 PUFAs has proven to be effective in reducing pain and improving other clinical outcomes such as tender and swollen joints count (68, 69). A better adherence to the MD has been linked to higher concentrations of *Bacteroidetes* and *Firmicutes*, as well as faecal butyrate and propionate (70). Fibre, a foundamental component of MD, has been shown to contribute to an increase of SCFAs concentration by restoring microbial composition (71). SCFAs, in turn, may modulate the behaviour of T cells and ILC3 by directly binding to the free-fatty acid receptor 2 (FFAR2) (52, 72).

The importance of SCFAs in mantain-

ing the balance between gut microbiota and the immune system may be further underlined by examining the data available on the role of butyrate supplementation. It has in fact been demonstrated that butyrate may suppress arthritis by increasing the level of the serotonin-derived metabolite 5-hydroxyndole-3-acetic acid (5-HIAA), which, in turn, activates the aryl-hydrocarbon receptor (AhR), thus supporting Breg function and inhibiting B cell differentiation in germinal centres (50). Finally, berberine, a dietary supplement, seems to be able to promote the growth of SCFAs-producing bacteria and downregulate *Prevotella spp*. (25, 65). However, little research has been done to assess the potential use of SCFAs as modulators of the microbiota (25, 73).

Along with antimicrobial and dietary interventions, the composition of the intestinal microbiota can also be modulated through the use of probiotics. Although data are conflicting and a therapeutic effect of probiotics on arthritis has not been demonstrated (74, 75), modulation of the immune response through probiotics has been demonstrated both in humans and in animal models. These effects seem to be species-specific, with *Lactobacillus casei (L. casei)* that may be able to reduce IL-12 and TNF-α levels and increase IL-10 in RA patients (76), while also reducing the severity of CIA in mice (77, 78). *Lactobacillus acidophilus* and *Bifidobacterium bifidum* have also been linked to an improvement in markers of inflammation (79).

Another significant limitation in the investigation of a link between microbiota and arthritis is due to the confounding effect of pharmacotherapy. It is, in fact, very likely that immunomodulating compounds used for the treatment of arthritis may modulate the microbiota, also independently of their effect on the disease. As an example, oral methotrexate therapy is linked to a relative reduction of *Enterobacteriales* and *Bacteroides fragilis* (80), in favour of *Lactobacillus salivarus* and some *Firmicutes* in the oral cavity (81). Similarly, sulfasalazine seems to protect the integrity of the intestinal epithelium, thus reducing microbial translocation through the intestinal mucosal barrier (4, 65). Moreover, hydroxychloroquine appears to restore bacterial diversity, particularly enhancing *Faecalibacterium spp* (4, 9, 82).

Limited data are also available on TNF inhibitors. As an example, etanercept seems to reduce alpha diversity of intestinal microbiome in CIA mice and an enrichment of *Nostocophycideae* and *Cyanobacteria* and reduction of *Clostridiaceae* and *Deltaproteobacteria* has been demonstrated in faecal samples of RA patients treated with etanercept (82). Finally, one of the most explored area of research in terms of reducing arthritis disease activity through the modulation of the microbiota is the use of faecal microbiota transplanation (FMT). This technique has demonstrated clear efficacy for the treatment of *Clostridium difficile* infection (83), and some initial evidence suggests it may be an interesting approach for the treatment of RA (84).

## Discussion

The intricate interplay between the intestinal microbiota and arthritis has been the focus of extensive research, yet the question of whether microbiota alterations are a cause or consequence of arthritis remains elusive. Observational studies have provided valuable insights into the association between dysbiosis and various forms of arthritis, but establishing causality is challenging due to inherent limitations and biases. Despite the temporal precedence of microbiota changes observed in preclinical stages of RA, causality cannot be definitively inferred from these findings alone.

To ascertain causality, interventional randomised trials are essential, yet their implementation in this research area carries significant technical difficulties. Moreover, observational data are susceptible to numerous sources of potential bias, including environmental influences on microbiota composition (such as diet and physical activity), medication effects (*e.g.* immunosuppressors, antibiotics), and the use of dietary supplements. These confounders make it challenging to discern whether observed microbiota changes are somehow causally linked to arthritis or spurious correlations.

The heterogeneity of results across independent studies further complicates the interpretation of observational data. Contrasting findings and inconsistencies in bacterial species associated with arthritis underline the need for caution in drawing definitive conclusions from observational studies alone.

Pathogenic mechanisms linking dysbiosis to arthritis development have been proposed, including compromised intestinal barrier function, microbiota-derived metabolites and molecular mimicry. However, most of these data are derived from observational studies and experimental models, warranting cautious interpretation.

Efforts to modulate the microbiota for arthritis treatment or prevention have also shown initial promising results, yet challenges persist. Antibacterial drugs, dietary interventions, probiotics, and faecal microbiota transplantation have been explored, but their efficacy remains uncertain. Moreover, the confounding effects of pharmacotherapy further complicate the interpretation of microbiota-modulating interventions.

In conclusion, while the association between intestinal microbiota and arthritis is increasingly recognised, establishing causality remains a challenge. Interventional trials and further research are needed to elucidate the complex mechanisms underlying this relationship and, eventually, to identify effective strategies for modulating the microbiota. Until then, a cautious interpretation of observational data and a multidisciplinary approach integrating clinical, experimental, and translational research are essential in advancing our understanding.

## References

1. XU H, ZHAO H, FAN D *et al*.: Interactions between gut microbiota and immunomodulatory cells in rheumatoid arthritis. *Mediators Inflamm* 2020; 2020: 1430605. https://doi.org/10.1155/2020/1430605
2. ROUND JL, MAZMANIAN SK: The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol* 2009; 9: 313-23. https://doi.org/10.1038/nri2515
3. HOLERS VM, DEMORUELLE MK, KUHN KA *et al*.: Rheumatoid arthritis and the mucosal ori-

gins hypothesis: protection turns to destruction. *Nat Rev Rheumatol* 2018; 14: 542-57. https://doi.org/10.1038/s41584-018-0070-0
4. ZAISS MM, JOYCE WU H-J, MAURO D, SCHETT G, CICCIA F: The gut–joint axis in rheumatoid arthritis. *Nat Rev Rheumatol* 2021; 17: 224-37. ttps://doi.org/10.1038/s41584-021-00585-3
5. BRAVO-BLAS A, WESSEL H, MILLING S: Microbiota and arthritis: correlations or cause? *Curr Opin Rheumatol* 2016; 28: 161-67. https://doi.org/10.1097/bor.0000000000000261
6. KISHIKAWA T, MAEDA Y, NII T *et al*.: Metagenome-wide association study of gut microbiome revealed novel aetiology of rheumatoid arthritis in the Japanese population. *Ann Rheum Dis* 2020; 79: 103-11. https://doi.org/10.1136/annrheumdis-2019-215743
7. VAAHTOVUO J, MUNUKKA E, KORKEAMÄKI M, LUUKKAINEN R, TOIVANEN P: Fecal microbiota in early rheumatoid arthritis. *J Rheumatol* 2008; 35: 1500-505.
8. ZHANG X, ZHANG D, JIA H *et al*.: The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. *Nat Med* 2015; 21: 895-905. https://doi.org/10.1038/nm.3914
9. CHEN J, WRIGHT K, DAVIS JM *et al*.: An expansion of rare lineage intestinal microbes characterizes rheumatoid arthritis. *Genome Med* 2016; 8: 43. https://doi.org/10.1186/s13073-016-0299-7
10. MAEDA Y, KURAKAWA T, UMEMOTO E *et al*.: Dysbiosis contributes to arthritis development via activation of autoreactive T cells in the intestine. *Arthritis Rheumatol* 2016; 68: 2646-61. https://doi.org/10.1002/art.39783
11. ALPIZAR-RODRIGUEZ D, LESKER TR, GRONOW A *et al*.: *Prevotella copri* in individuals at risk for rheumatoid arthritis. *Ann Rheum Dis* 2019; 78: 590-93. https://doi.org/10.1136/annrheumdis-2018-214514
12. SCHER JU, SCZESNAK A, LONGMAN RS *et al*.: Expansion of intestinal Prevotella copri correlates with enhanced susceptibility to arthritis. *eLife* 2013; 2: e01202. https://doi.org/10.7554/eLife.01202
13. SCHER JU, JOSHUA V, ARTACHO A *et al*.: The lung microbiota in early rheumatoid arthritis and autoimmunity. *Microbiome* 2016; 4: 60. https://doi.org/10.1186/s40168-016-0206-x
14. SCHER JU, UBEDA C, EQUINDA M *et al*.: Periodontal disease and the oral microbiota in new-onset rheumatoid arthritis. *Arthritis Rheum* 2012; 64: 3083-94. https://doi.org/10.1002/art.34539
15. CAFARO G, ALUNNO A, VALENTINI V *et al*.: The onset site of rheumatoid arthritis: the joints or the lung? *Reumatismo* 2016; 68: 167-75. https://doi.org/10.4081/reumatismo.2016.892
16. VAN DIJKHUIZEN EHP, DEL CHIERICO F, MALATTIA C *et al*.: Microbiome analytics of the gut microbiota in patients with juvenile idiopathic arthritis: a longitudinal observational cohort study. *Arthritis Rheumatol* 2019; 71: 1000-10. https://doi.org/10.1002/art.40827
17. VAN DE WIELE T, VAN PRAET JT, MARZORATI M, DRENNAN MB, ELEWAUT D: How the microbiota shapes rheumatic diseases. *Nat Rev Rheumatol* 2016; 12: 398-411. https://doi.org/10.1038/nrrheum.2016.85
18. WELLS PM, ADEBAYO AS, BOWYER RCE *et al*.: Associations between gut microbiota and genetic risk for rheumatoid arthritis in the absence of disease: a cross-sectional study. *Lancet Rheumatol* 2020; 2: e418-27. https://doi.org/10.1016/S2665-9913(20)30064-3
19. LIU X, ZENG B, ZHANG J *et al*.: Role of the gut microbiome in modulating arthritis progression in mice. *Sci Rep* 2016; 6: 30594. https://doi.org/10.1038/srep30594
20. ROGIER R, EVANS-MARIN H, MANASSON J *et al*.: Alteration of the intestinal microbiome characterizes preclinical inflammatory arthritis in mice and its modulation attenuates established arthritis. *Sci Rep* 2017; 7: 15613. https://doi.org/10.1038/s41598-017-15802-x
21. MONACH PA, MATHIS D, BENOIST C: The K/BxN arthritis model. *Curr Protoc Immunol* 2008 . https://doi.org/10.1002/0471142735.im1522s81
22. WU H-J, IVANOV II, DARCE J *et al*.: Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. *Immunity* 2010; 32: 815-27. https://doi.org/10.1016/j.immuni.2010.06.001
23. ZHAO T, WEI Y, ZHU Y *et al*.: Gut microbiota and rheumatoid arthritis: Ffom pathogenesis to novel therapeutic opportunities. *Front Immunol* 2022; 13: 1007165. https://doi.org/10.3389/fimmu.2022.1007165
24. CHENG H, GUAN X, CHEN D, MA W: The Th17/Treg cell balance: a gut microbiota-modulated story. *Microorganisms* 2019; 7: 583. https://doi.org/10.3390/microorganisms7120583
25. ATTUR M, SCHER JU, ABRAMSON SB, ATTUR M: Role of intestinal dysbiosis and nutrition in rheumatoid arthritis. *Cells* 2022; 11: 2436. https://doi.org/10.3390/cells11152436
26. AZIZI G, JADIDI-NIARAGH F, MIRSHAFIEY A: Th17 cells in immunopathogenesis and treatment of rheumatoid arthritis. *Int J Rheum Dis* 2013; 16: 243-53. https://doi.org/10.1111/1756-185X.12132
27. OLEJNICZAK-STARUCH I, CIĄŻYŃSKA M, SOBOLEWSKA-SZTYCHNY D, NARBUTT J, SKIBIŃSKA M, LESIAK A: Alterations of the skin and gut microbiome in psoriasis and psoriatic arthritis. *IJMS* 2021; 22: 3998. https://doi.org/10.3390/ijms22083998
28. MYERS B, BROWNSTONE N, REDDY V *et al*.: The gut microbiome in psoriasis and psoriatic arthritis. *Best Pract Res Clin Rheumatol* 2019; 33: 101494. https://doi.org/10.1016/j.berh.2020.101494
29. CHO CE, TAESUWAN S, MALYSHEVA OV *et al*.: Trimethylamine- *N* -oxide (TMAO) response to animal source foods varies among healthy young men and is influenced by their gut microbiota composition: A randomized controlled trial. *Mol Nutr Food Res* 2017; 61: 1600324. https://doi.org/10.1002/mnfr.201600324
30. KOMAROFF AL: The microbiome and risk for obesity and diabetes. *JAMA* 2017; 317: 355. https://doi.org/10.1001/jama.2016.20099
31. TEJESVI MV, ARVONEN M, KANGAS SM *et al*.: Faecal microbiome in new-onset juvenile idiopathic arthritis. *Eur J Clin Microbiol Infect Dis* 2016; 35: 363-70. https://doi.org/10.1007/s10096-015-2548-x
32. XIN L, HE F, LI S, ZHOU Z-X, MA X-L: Intestinal microbiota and juvenile idiopathic arthritis: current understanding and future prospective. *World J Pediatr* 2021; 17: 40–51. https://doi.org/10.1007/s12519-020-00371-3
33. DI PAOLA M, CAVALIERI D, ALBANESE D *et al*.: Alteration of fecal microbiota profiles in juvenile idiopathic arthritis. Associations with HLA-B27 allele and disease status. *Front Microbiol* 2016; 7: 1703. https://doi.org/10.3389/fmicb.2016.01703
34. ZHOU C, ZHAO H, XIAO X *et al*.: Metagenomic profiling of the pro-inflammatory gut microbiota in ankylosing spondylitis. *J Autoimmun* 2020; 107: 102360. https://doi.org/10.1016/j.jaut.2019.102360
35. WEN C, ZHENG Z, SHAO T *et al*.: Quantitative metagenomics reveals unique gut microbiome biomarkers in ankylosing spondylitis. *Genome Biol* 2017; 18: 142. https://doi.org/10.1186/s13059-017-1271-6
36. HAMMER RE, MAIKA SD, RICHARDSON JA, TANG J-P, TAUROG JD: Spontaneous inflammatory disease in transgenic rats expressing HLA-B27 and human β2m: an animal model of HLA-B27-associated human disorders. *Cell* 1990; 63: 1099-1112. https://doi.org/10.1016/0092-8674(90)90512-d
37. GLATIGNY S, FERT I, BLATON MA *et al*.: Proinflammatory Th17 cells are expanded and induced by dendritic cells in spondylarthritis-prone HLA-B27-transgenic rats. *Arthritis Rheum* 2012; 64: 110-20. https://doi.org/10.1002/art.33321
38. SONG Z-Y, YUAN D, ZHANG S-X: Role of the microbiome and its metabolites in ankylosing spondylitis. *Front Immunol* 2022; 13: 1010572. https://doi.org/10.3389/fimmu.2022.1010572
39. JIAO Y, WU L, HUNTINGTON ND, ZHANG X: Crosstalk between gut microbiota and innate immunity and its implication in autoimmune diseases. *Front Immunol* 2020; 11: 282. https://doi.org/10.3389/fimmu.2020.00282
40. TAUROG JD, CHHABRA A, COLBERT RA: Ankylosing spondylitis and axial spondyloarthritis. *N Engl J Med* 2016; 374: 2563-74. https://doi.org/10.1056/nejmra1406182
41. VECELLIO M, COHEN CJ, ROBERTS AR, WORDSWORTH PB, KENNA TJ: RUNX3 and T-Bet in immunopathogenesis of ankylosing spondylitis – novel targets for therapy? *Front Immunol* 2019; 9: 3132. https://doi.org/10.3389/fimmu.2018.03132
42. YANG H-T, XIU W-J, LIU J-K *et al*.: Gut microbiota characterization in patients with asymptomatic hyperuricemia: probiotics increased. *Bioengineered* 2021; 12: 7263-75. https://doi.org/10.1080/21655979.2021.1976897
43. KIM HW, YOON E-J, JEONG SH, PARK M-C: Distinct gut microbiota in patients with asymptomatic hyperuricemia: a potential protector against gout development. *Yonsei Med J* 2022; 63: 241. https://doi.org/10.3349/ymj.2022.63.3.241
44. CHU Y, SUN S, HUANG Y *et al*.: Metagenomic analysis revealed the potential role of gut microbiome in gout. *npj Biofilms Microbiomes* 2021; 7: 66. https://doi.org/10.1038/s41522-021-00235-2
45. WANG Z, LI Y, LIAO W *et al*.: Gut microbiota remodeling: A promising therapeutic strategy to confront hyperuricemia and gout. *Front Cell Infect Microbiol* 2022; 12: 935723. https://doi.org/10.3389/fcimb.2022.935723

46. LIN L, ZHANG K, XIONG Q *et al*.: Gut microbiota in pre-clinical rheumatoid arthritis: From pathogenesis to preventing progression. *J Autoimmun* 2023; 141: 103001. https://doi.org/10.1016/j.jaut.2023.103001
47. PORZIO V, BIASI G, CORRADO A *et al*.: Intestinal histological and ultrastructural inflammatory changes in spondyloarthropathy and rheumatoid arthritis. *Scand J Rheumatol* 1997; 26: 92-98. https://doi.org/10.3109/03009749709115825
48. TAJIK N, FRECH M, SCHULZ O *et al*.: Targeting zonulin and intestinal epithelial barrier function to prevent onset of arthritis. *Nat Commun* 2020; 11: 1995. https://doi.org/10.1038/s41467-020-15831-7
49. STURGEON C, FASANO A: Zonulin, a regulator of epithelial and endothelial barrier functions, and its involvement in chronic inflammatory diseases. *Tissue Barriers* 2016; 4: e1251384. https://doi.org/10.1080/21688370.2016.1251384
50. ROSSER EC, PIPER CJM, MATEI DE *et al*.: Microbiota-derived metabolites suppress arthritis by amplifying aryl-hydrocarbon receptor activation in regulatory B cells. *Cell Metab* 2020; 31: 837-51.e10. https://doi.org/10.1016/j.cmet.2020.03.003
51. MEIJER K, DE VOS P, PRIEBE MG: Butyrate and other short-chain fatty acids as modulators of immunity: what relevance for health?: *Curr Opin Clin Nutr Metab Care* 2010; 13: 715-21. https://doi.org/10.1097/mco.0b013e32833eebe5
52. SMITH PM, HOWITT MR, PANIKOV N *et al*.: The microbial metabolites, short-chain fatty acids, regulate colonic $T_{reg}$ cell homeostasis. *Science* 2013; 341: 569-73. https://doi.org/10.1126/science.1241165
53. YU D, DU J, PU X *et al*.: The gut microbiome and metabolites are altered and interrelated in patients with rheumatoid arthritis. *Front Cell Infect Microbiol* 2022; 11: 763507. https://doi.org/10.3389/fcimb.2021.763507
54. PIANTA A, ARVIKAR S, STRLE K *et al*.: Evidence of the immune relevance of *Prevotella copri*, a gut microbe, in patients with rheumatoid arthritis. *Arthritis Rheumatol* 2017; 69: 964-75. https://doi.org/10.1002/art.40003
55. DUSCHA A, GISEVIUS B, HIRSCHBERG S *et al*.: Propionic acid shapes the multiple sclerosis disease course by an immunomodulatory mechanism. *Cell* 2020; 180: 1067-80.e16. https://doi.org/10.1016/j.cell.2020.02.035
56. LUU M, PAUTZ S, KOHL V *et al*.: The short-chain fatty acid pentanoate suppresses autoimmunity by modulating the metabolic-epigenetic crosstalk in lymphocytes. *Nat Commun* 2019; 10: 760. https://doi.org/10.1038/s41467-019-08711-2
57. YAN D, ISSA N, AFIFI L, JEON C, CHANG H-W, LIAO W: The role of the skin and gut microbiome in psoriatic disease. *Curr Derm Rep* 2017; 6: 94-103. https://doi.org/10.1007/s13671-017-0178-5
58. PIANTA A, ARVIKAR SL, STRLE K *et al*.: Two rheumatoid arthritis-specific autoantigens correlate microbial immunity with autoimmune responses in joints. *J Clin Invest* 2017; 127: 2946-56. https://doi.org/10.1172/jci93450
59. YANG L, LIU C, ZHAO W *et al*.: Impaired autophagy in intestinal epithelial cells alters gut microbiota and host immune responses. *Appl Environ Microbiol* 2018; 84: e00880-18. https://doi.org/10.1128/aem.00880-18
60. TSUBOI K, NISHITANI M, TAKAKURA A, IMAI Y, KOMATSU M, KAWASHIMA H: Autophagy protects against colitis by the maintenance of normal gut microflora and secretion of mucus. *J Biol Chem* 2015; 290: 20511-26. https://doi.org/10.1074/jbc.m114.632257
61. MA L, NI Y, WANG Z *et al*.: Spermidine improves gut barrier integrity and gut microbiota function in diet-induced obese mice. *Gut Microbes* 2020; 12: 1832857. https://doi.org/10.1080/19490976.2020.1832857
62. BRANDL C, BUCCI L, SCHETT G, ZAISS MM: Crossing the barriers: revisiting the gut feeling in rheumatoid arthritis. *Eur J Immunol* 2021; 51: 798-810. https://doi.org/10.1002/eji.202048876
63. DOURADO E, FERRO M, SOUSA GUERREIRO C, FONSECA JE: Diet as a modulator of intestinal microbiota in rheumatoid arthritis. *Nutrients* 2020; 12: 3504. https://doi.org/10.3390/nu12113504
64. BARATI M, GHAHREMANI A, NAMDAR AHMADABAD H: Intermittent fasting: a promising dietary intervention for autoimmune diseases. *Autoimmun Rev* 2023; 22: 103408. https://doi.org/10.1016/j.autrev.2023.103408
65. HORTA-BAAS G, SANDOVAL-CABRERA A, ROMERO-FIGUEROA M DEL S: Modification of gut microbiota in inflammatory arthritis: highlights and future challenges. *Curr Rheumatol Rep* 2021; 23: 67. https://doi.org/10.1007/s11926-021-01031-9
66. MEI L, YANG Z, ZHANG X *et al*.: Sustained drug treatment alters the gut microbiota in rheumatoid arthritis. *Front Immunol* 2021; 12: 704089. https://doi.org/10.3389/fimmu.2021.704089
67. ZAURA E, BRANDT BW, TEIXEIRA DE MATTOS MJ *et al*.: Same exposure but two radically different responses to antibiotics: resilience of the salivary microbiome versus long-term microbial shifts in feces. *mBio* 2015; 6: e01693-15. https://doi.org/10.1128/mbio.01693-15
68. DAWCZYNSKI C, DITTRICH M, NEUMANN T *et al*.: Docosahexaenoic acid in the treatment of rheumatoid arthritis: A double-blind, placebo-controlled, randomized cross-over study with microalgae vs. sunflower oil. *Clinical Nutrition* 2018; 37: 494-504. https://doi.org/10.1016/j.clnu.2017.02.021
69. MILES EA, CALDER PC: Influence of marine *n*-3 polyunsaturated fatty acids on immune function and a systematic review of their effects on clinical outcomes in rheumatoid arthritis. *Br J Nutr* 2012; 107: S171-84. https://doi.org/10.1017/S0007114512001560
70. GUTIÉRREZ-DÍAZ I, FERNÁNDEZ-NAVARRO T, SÁNCHEZ B, MARGOLLES A, GONZÁLEZ S: Mediterranean diet and faecal microbiota: a transversal study. *Food Funct* 2016; 7: 2347-56. https://doi.org/10.1039/c6fo00105j
71. HÄGER J, BANG H, HAGEN M *et al*.: The role of dietary fiber in rheumatoid arthritis patients: a feasibility study. *Nutrients* 2019; 11: 2392. https://doi.org/10.3390/nu11102392
72. CHUN E, LAVOIE S, FONSECA-PEREIRA D *et al*.: Metabolite-sensing receptor Ffar2 regulates colonic group 3 innate lymphoid cells and gut immunity. *Immunity* 2019; 51: 871-84.e6. https://doi.org/10.1016/j.immuni.2019.09.014
73. PEÑA-RODRÍGUEZ M, VEGA-MAGAÑA N, GARCÍA-BENAVIDES L *et al*.: Butyrate administration strengthens the intestinal epithelium and improves intestinal dysbiosis in a cholestasis fibrosis model. *J Appl Microbiol* 2022; 132: 571-83. https://doi.org/10.1111/jam.15135
74. OLIVIERO F, SPINELLA P: Benefits of probiotics in rheumatic diseases. *Front Nutr* 2020; 7: 157. https://doi.org/10.3389/fnut.2020.00157
75. AQAEINEZHAD RUDBANE SM, RAHMDEL S, ABDOLLAHZADEH SM, ZARE M, BAZRAFSHAN A, MAZLOOMI SM: The efficacy of probiotic supplementation in rheumatoid arthritis: a meta-analysis of randomized, controlled trials. *Inflammopharmacol* 2018; 26: 67-76. https://doi.org/10.1007/s10787-017-0436-y
76. PAUL AK, PAUL A, JAHAN R *et al*.: Probiotics and amelioration of rheumatoid arthritis: significant roles of lactobacillus casei and lactobacillus acidophilus. *Microorganisms* 2021; 9: 1070. https://doi.org/10.3390/microorganisms9051070
77. SO J-S, KWON H-K, LEE C-G *et al*.: Lactobacillus casei suppresses experimental arthritis by down-regulating T helper 1 effector functions. *Mol Immunol* 2008; 45: 2690-99. https://doi.org/10.1016/j.molimm.2007.12.010
78. BUNGAU SG, BEHL T, SINGH A *et al*.: Targeting probiotics in rheumatoid arthritis. *Nutrients* 2021; 13: 3376. https://doi.org/10.3390/nu13103376.
79. ZAMANI B, GOLKAR HR, FARSHBAF S *et al*.: Clinical and metabolic response to probiotic supplementation in patients with rheumatoid arthritis: a randomized, double-blind, placebo-controlled trial. *Int J Rheum Dis* 2016; 19: 869-79. https://doi.org/10.1111/1756-185X.12888
80. MAIER L, PRUTEANU M, KUHN M *et al*.: Extensive impact of non-antibiotic drugs on human gut bacteria. *Nature* 2018; 555: 623-28. https://doi.org/10.1038/nature25979
81. ZARAGOZA-GARCÍA O, CASTRO-ALARCÓN N, PÉREZ-RUBIO G, GUZMÁN-GUZMÁN IP: DMARDs-gut microbiota feedback: implications in the response to therapy. *Biomolecules* 2020; 10: 1479. https://doi.org/10.3390/biom10111479
82. PICCHIANTI-DIAMANTI A, PANEBIANCO C, SALEMI S *et al*.: Analysis of gut microbiota in rheumatoid arthritis patients: disease-related dysbiosis and modifications induced by etanercept. *IJMS* 2018; 19: 2938. https://doi.org/10.3390/ijms19102938
83. MOAYYEDI P, SURETTE MG, KIM PT *et al*.: Fecal microbiota transplantation induces remission in patients with active ulcerative colitis in a randomized controlled trial. *Gastroenterology* 2015; 149: 102-9.e6. https://doi.org/10.1053/j.gastro.2015.04.001
84. ZENG J, PENG L, ZHENG W *et al*.: Fecal microbiota transplantation for rheumatoid arthritis: a case report. *Clin Case Rep*2021; 9: 906-9. https://doi.org/10.1002/ccr3.3677