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Gut microbiota in pre-clinical rheumatoid arthritis: From pathogenesis to preventing progression

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by progressive polyarthritis that leads to cartilage and bone damage. Pre-clinical RA is a prolonged state before clinical arthritis and RA develop, in which autoantibodies (antibodies against citrullinated proteins, rheumatoid factors) can be present due to the breakdown of immunologic self-tolerance. As early treatment initiation before the onset of polyarthritis may achieve sustained remission, optimize clinical outcomes, and even prevent RA progression, the pre-clinical RA stage is showing the prospect to be the window of opportunity for RA treatment.

Growing evidence has shown the role of the gut microbiota in inducing systemic inflammation and polyarthritis via multiple mechanisms, which may involve molecular mimicry, impaired intestinal barrier function, gut microbiota-derived metabolites mediated immune regulation, modulation of the gut microbiota's effect on immune cells, intestinal epithelial cells autophagy, and the interaction between the microbiome and human leukocyte antigen alleles as well as microRNAs.

Since gut microbiota alterations in pre-clinical RA have been reported, potential therapies for modifying the gut microbiota in pre-clinical RA, including natural products, antibiotic therapy, fecal microbiota transplantation, probiotics, microRNAs therapy, vitamin D supplementation, autophagy inducer-based treatment, prebiotics, and diet, holds great promise for the successful treatment and even prevention of RA via altering ongoing inflammation.

In this review, we summarized current studies that include pathogenesis of gut microbiota in RA progression and promising therapeutic strategies to provide novel ideas for the management of pre-clinical RA and possibly preventing arthritis progression.

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by polyarthritis that leads to cartilage and bone damage that can severely impair an individual's physical function. The development of RA is a long process that ranges from the susceptibility stage due to genetic factors to pre-clinical RA with broken immunological tolerance, and eventually to the occurrence of clinical arthritis [1].

Achieving long-term remission or low disease activity, preventing joint damage and disability, and decreasing the incidence of complications, are the aims of RA treatment. Currently available drugs include glucocorticoids, non-steroidal anti-inflammatory drugs, disease-modifying anti-rheumatic drugs (DMARDs) and small molecule inhibitors. Although treat to target strategies, DMARDs and early treatment have improved clinical the outcomes of RA patients, more than 60% of individuals do not achieve true remission once clinical arthritis occurs [2–5]. As an early treatment initiation before the onset of clinical arthritis may prevent severe radiographic damage and help achieve sustained remission, the pre-clinical RA stage is showing its prospect to be the window of opportunity for RA treatment [6,7].

Pre-clinical RA is a prolonged state before clinical arthritis and RA development, in which autoantibodies (including antibodies against citrullinated proteins and rheumatoid factors) can be present due to the

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Abbreviations

AhR	aryl hydrocarbon receptor
Atg	autophagy related gene
β2GPI	β2 glycoprotein I
CIA	collagen-induced arthritis
Cox-2	cyclooxygenase 2
CTSs	clematis triterpenoid saponins
CII	type II collagen
DMARDs	disease-modifying anti-rheumatic drugs
FLNA	filamin A
FMT	fecal microbiota transplantation
GCA	glycocholic acid
GCDCA	glycochenodeoxycholic acid
GM-CSF	granulocyte-macrophage colony-stimulating factor
GNS	N-acetylglucosamine-6-sulfatase
HIF-1α	hypoxia-inducible factor-1α
HLA	human leukocyte antigen
3-HAA	3-hydroxyanthranilic acid
5-HIAA	5-hydroxyindole-3-acetic acid
IL	interleukin
JNK	c-Jun N-terminal kinase
LPS	lipopolysaccharide
MMP	matrix metalloproteinase
MAPK	mitogen-activated protein kinase
miRNAs	microRNAs
mTOR	mammalian target of rapamycin
NF-κB	nuclear factor κB
RA	rheumatoid arthritis
RPL23A	60S ribosomal protein L23a
SAA	serum amyloid A
SCFA, s	short-chain fatty acids
Tfh	follicular helper T
TGP	total glucosides of paeony
TLR	Toll-like receptor
TNF-α	tumor necrosis factor-α
Tregs	regulatory T cells

breakdown of immunological tolerance [8,9].

A growing body of studies has shown that gut microbiota plays a pivotal role in the progression from pre-clinical RA to clinical RA in both patients and mouse models [10–13]. Previous studies reported that intestinal bacteria dysbiosis may potentially induce the breakdown of immunologic self-tolerance and aggravate RA progression through molecular mimicry [14–16]. In particular, some microbial peptides expressed by intestinal bacteria are similar to proven RA auto-epitopes, leading to the production of autoantibodies through cross-reaction and subsequent damage to the joint cartilage [17]. In a multi-omics analysis of the microbiome and metabolomics, gut-microbiota-derived metabolites were found to act as immunomodulatory compounds that modulate immune cell differentiation and function [18,19]. Moreover, gut microbiota-mediated alterations in intestinal barrier function in the pre-clinical RA stage reportedly lead to the subsequent transmigration of immune cells from the gut to the joints, [20].

Lipopolysaccharide (LPS), a structural component of intestinal commensal gram-negative bacteria, reportedly triggers the cytokine cascade and drives T-cell mediated pathogenesis of arthritis [21]. However, LPS produced by some bacteria may have protective effects on immune cells [22]. Furthermore, interactions between gut microbiota and microRNAs (miRNAs), human leukocyte antigen (HLA) alleles, and gut autophagy are key contributors to the regulation of the local and general inflammatory processes (Fig. 1).

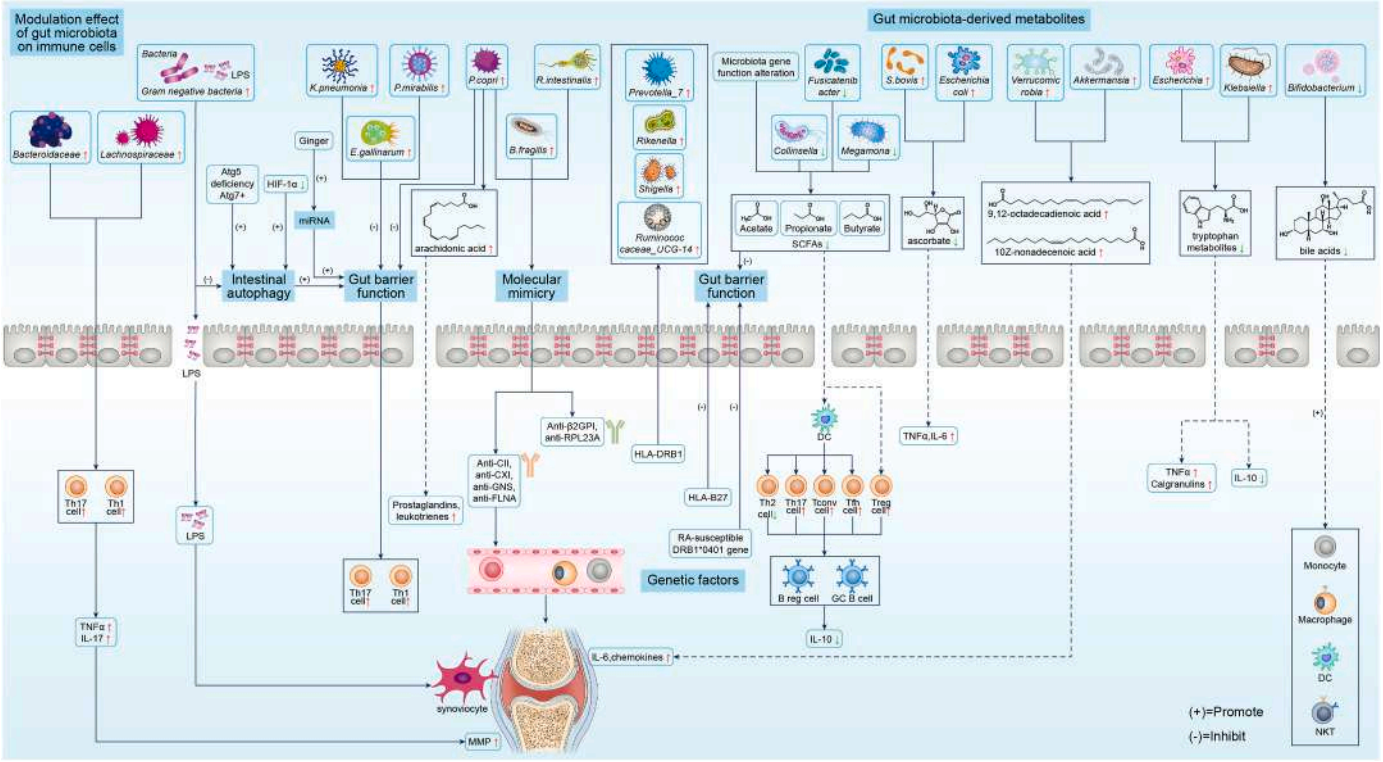

Fig. 1. Potential implication of gut microbiota in the etiology of RA.

Regarding the essential role of the gut microbiota in RA progression, interventions targeting the gut microbiota in the pre-clinical RA stage may provide novel insights into new promising therapeutics for RA (Fig. 2). Extensive research has examined intestinal microbial treatments for RA including probiotics, prebiotics and diet, antibiotic therapy, fecal microbiota transplantation (FMT) and natural products derived from herbal medicine [23–27]. Nevertheless, research to date tended to focus on the effects of intestinal microbial treatments in clinical RA patients and mouse models rather than in the pre-clinical stage, which is calling for further research to confirm their efficacy in pre-clinical RA stage to prevent RA development.

This review explores the interaction between gut microbiota dysbiosis and disruption of immune homeostasis in asymptomatic RA patient from an evidence-based perspectives for new prophylactic strategies aiming to disrupt RA progression in the early stage.

2. Gut microbiota and pre-clinical RA

Studies over the past several years have provided important information about the correlation between gut microbiota and pre-clinical RA in patient and mouse models (Table 1). In an earlier study, colonization with fecal microbiota from collagen-induced arthritis (CIA)-susceptible mice (i.e. mouse develops arthritis after type II collagen [CII] immunization) showed the ability to induce arthritis in germ-free mice [14]. This result was confirmed by subsequent study using pre-clinical germ-free mice harboring microbiota from RA patients [16]. On the other hand, in a pre-clinical RA mouse model, elimination of the gut microbiota by antibiotic treatment suppressed inflammatory factor expression and interleukin (IL) 17-producing T helper 17 cell counts, both of which reportedly play influential roles in RA development [28]. Additionally, via network analysis, Rooney and colleagues reported that microbial taxa in pre-clinical individuals who progressed to RA showed commonality several months before disease onset [13]. Therefore, gut microbiota dysbiosis may play a pivotal role in RA progression.

The increased richness and reduced evenness of alpha-diversity was observed in a pre-clinical mouse model, while beta-diversity was reported to be different between pre-clinical RA individuals and healthy control [13,15]. Interestingly, a high abundance of the bacterial family Prevotellaceae, particularly *Prevotella copri* was observed in pre-clinical RA mice as well as in pre-clinical RA individuals and RA patients [10,16,29]. Furthermore, Prevotellaceae was positively correlated with RF titer in a study that compared the gut microbiota between anti-citrullinated protein positive individuals and healthy controls [10]. A recent study analyzed the gut microbiota of 1650 individuals from the TwinsUK cohort and discovered that a group of gut microbiome bacteria including *Prevotella_7*, *Veillonella*, *Streptococcus*, Ruminococcaceae UBA1819, and *Coprobacter* was correlated with a positive HLA-DRB1 shared epitope, while another group (*Prevotella_7,* Ruminococcaceae_UCG-14, *Rikenella* and *Shigella)* was associated with a high polygenic risk score of RA. The high abundance of Prevotellaceae may play an influential role in systemic inflammation development and onset of arthritis [29]. A high relative abundance of Lachnospiraceae, Ruminococcaceae and *Bacteroidaceae* is another distinctive feature of gut microbiota change in pre-clinical RA which reported by earlier observations in CII immunized mice models of pre-clinical RA, individuals positive for anti-citrullinated protein and new-onset untreated RA patients [10,13,14,16,28]. Several studies also suggested an inverse correlation between specific bacterial families (i.e. Muribaculaceae, Lactobacillaceae) and RA progression. However, further research is required to determine whether those results occurred in the pre-clinical stage [16].

An earlier study by Liu et al. suggested a pro-inflammatory profile of the gut microbiota by uncovering increased Th17 cell proportions and IL17 expression in pre-clinical RA mouse microbiota harbored germ-free mice [14]. A subsequent study reported an autoimmune induction profile of the gut microbiota of pre-clinical RA mice showing the enhanced transformation of intestine-derived lymphocytes to highly

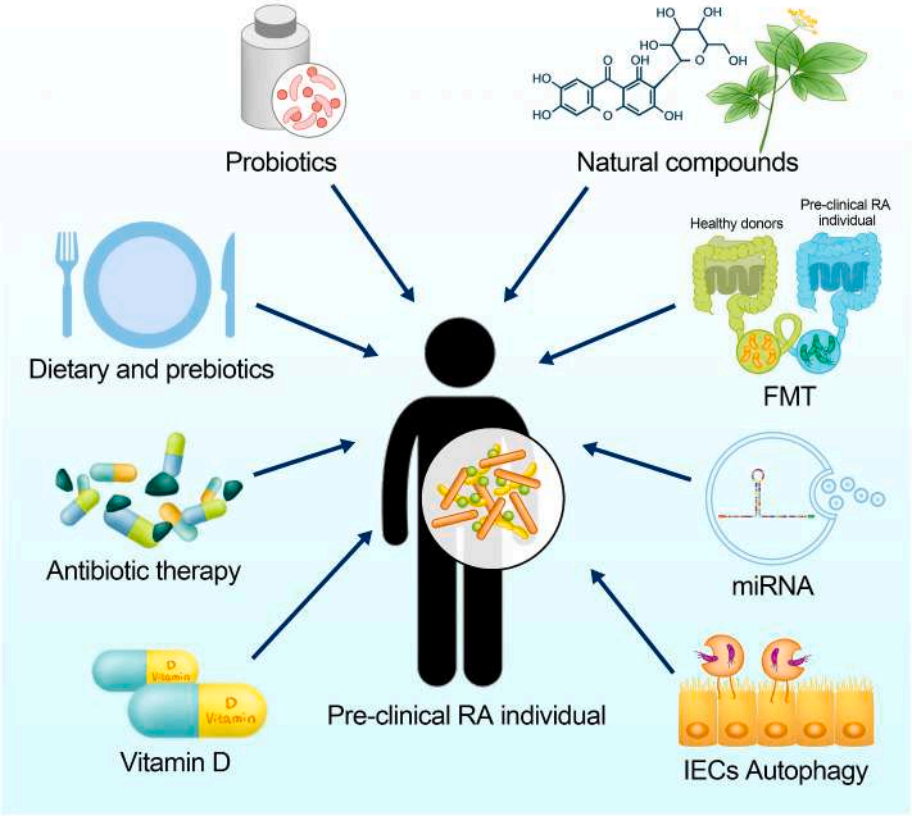

Fig. 2. Potential strategies for gut microbiota based intercention of individuals with pre-clincial RA.

Table 1
Summary of studies on gut microbiota dysbiosis in pre-clinical RA individuals and mouse model.

Study	Method	Subjects	Gut microbiota in pre-clinical RA	Additional key findings	Reference
Liu X, et al. (2016)	16S rRNA (V3–V4)	Pre-clinical CIA-susceptible mice vs pre-clinical CIA-resistant mice	**Family**: *Bacteroidaceae, Lachnospiraceae, Muribaculaceae*↑ *Lactobacillaceae*↓	Microbiota from CIA-susceptible mice showed ability to increase proportion of T regulatory cell and Th17 cells expression in spleen	[14]
Maeda Y, et al. (2016)	16S rRNA (V5–V6)	RA patient microbiota harbored SKG mice vs HC microbiota harbored SKG mice	**Family**: *Prevotellaceae*↑ *Lachnospiraceae Bacteroidaceae*↓ **Species**: *P. copri*↑	Increased number of $CD4^+$ T cells and IL-17−producing $CD4^+$ T cells were found in the large intestine of pre-clinical RA-SKG mice lymphocytes from regional lymph nodes and large intestine of pre-clinical RA-SKG showed high IL-17 responses to RPL23A	[16]
Rogier R, et al. (2017)	16S rRNA (V4)	CII immunized DBA/1J mice	**Phylum**: *Firmicutes, Proteobacteria*↑ *Bacteroidetes*↓ **Family**: *Lachnospiraceae, Ruminococcaceae, Desulfovibrionaceae*↑ *Muribaculaceae, Bacteroidaceae, Paraprevotellaceae, Lactobacillaceae, Erysipelotrichaceae*↓ **Genus**: Oscillospira, Ruminococcus↑ *Bacteroides, Prevotella, Lactobacillus*↓ **Species**: *R. gnavus*↑ *L. reuteri*↓	Antibiotics treatment in pre-clinical RA mice lead to gut microbiota partial elimination, suppressed proinflammatory Th cell in intestinal lamina propria, reduced expression of SAA1 and SAA2 in gut and eventually staving off progression of RA	[28]
Jubair WK, et al. (2018)	16S rRNA (V1–V2)	CII immunized DBA/1J mice	**Family**: *Lactobacillaceae*↑ *Muribaculaceae*↓	Increased richness and reduced evenness of alpha-diversity was observed in pre-clinical mice model Overall microbiota composition showed significant change during progression of RA	[15]
Alpizar-Rodriguez D, et al. (2019)	16S rRNA (V4) sequencing	Pre-clinical RA (83) First-degree relatives (50)	**Family**: *Prevotellaceae*↑ **Genus**: *Prevotella*↑ **Species**: *P. copri*↑	High relative abundance (>1%) of *Prevotellaceae* was correlated with higher prevalence of RF positivity	[10]
Wells PM, et al. (2020)	16S rRNA (V4) sequencing &GWAS	Pre-clinical RA (83) First-degree relatives control (50) First-degree relatives validation (1650)	**Family**: *Prevotellaceae*↑ **Genus**: *Lactobacillus, Butyrivibrio, Ruminococcaceae, Enterococcus, Veilllonella*↑ **Species**: *Clostridiales bacterium DTU089*↑, *P. copri*↑	High abundance of *Prevotella_7*, *Veillonella*, *Streptococcus*, *Ruminococcaceae UBA1819*, and *Coprobacter* were associate with positive HLA-DRB1 shared epitope *Prevotella_7, Ruminococcaceae_UCG-14, Rikenella, Shigella* were positively associated with rheumatoid arthritis PRS	[29]
Rooney CM, et al. (2021)	16S rRNA (V4)	Pre-clinical RA (25) HC (44)	**Family**: *Helicobacteraceae, Erysipelotrichaceae, Ruminococcaceae, Peptostreptococcaceae, Bifidobacteriaceae, Gracilibacteraceae, Planococcaceae, Deferribacteraceae, Victivallaceae, Lachnospiraceae, Peptococcaceae*↑ *Bacteroidaceae, Barnesiellaceae, Methanobacteriacea*↓ **Genus**: *Coprococcus, Oscillospira, Lachnospira, Absiella, Roseburia, Allobaculum, Faecalibacterium, Bifidobacterium, Mucispirillum, Helicobacter, Flexispira, Oxobacter, Gracilibacter, Peptococcus*↑	Beta diversity was different between pre-clinical RA individuals compared with healthy controls Pre-clinical RA individuals who progressed to RA clustering in a phylogenetic network	[13]

CIA, collagen-induced arthritis; CII, type II collagen; GWAS, genome-wide association study; HC, health control; HC-SKG mice, HC microbiota harbored SKG mice; IL, interleukin; *L reuteri*, *Lactobacillus reuteri*; *P. copri*, *Prevotella copri*; PRS, polygenic risk score; RA-SKG mice, RA patient microbiota harbored SKG mice; RPL23A: 60S ribosomal protein L23a; *R. gnavus*, *Ruminococcus gnavus*; SAA, serum amyloid A.

arthritis-related antigen autoreactive T cells [16].

Collectively, gut microbiota dysbiosis in individuals with pre-clinical RA may not be characterized by diversity alterations. Meanwhile, an increased proportion of intestine-derived autoreactive lymphocytes due to impaired intestinal barrier function may cause arthritis and exacerbates its progression. Furthermore, a high abundance of specific bacteria species such as *P copri* may be significantly related to RA progression.

3. Potential mechanisms linking gut microbiota to pre-clinical RA

The existing body of research on microbial flora in RA patients suggests the presence of gut microbiota alteration and a potential link between RA development and intestinal dysbiosis [30–38]. However, there is little published data on whether intestinal dysbiosis in RA patients is the result of complex interaction between genetic susceptibility and environmental factors, or the consequence of intestine-involved systemic inflammation. Recent clinical studies of pre-clinical RA individuals (including those with autoantibodies or genetic risk factors) showed surprising results that gut microbiota alteration occurred before RA onset [10,29].

A metabonomic analysis reported significant changes in microbiome-derived metabolites concentrations in RA patients, and proposed that similar changes may occur in the pre-clinical stage [11,39–41]. In the gut, the sensing of toxic components secreted by gram-negative bacteria with Toll-like receptor (TLR) 4 and downstream pathway activation could induce intestinal inflammation in pre-clinical RA individuals [42]. Indeed, gut microbiota alteration reportedly induce autoreactive T cell activation and may act as a hidden trigger of systemic inflammation [16]. The specific mechanism underlying for the role of gut microbiota dysbiosis in RA will be elaborated below.

3.1. Molecular mimicry

Molecular mimicry has been suggested to play a vital role in the development of multiple autoimmune disease including multiple sclerosis, systemic lupus erythematosus, ankylosing spondylitis, type 1 diabetes, antiphospholipid syndrome and autoimmune hepatitis

[43–47]. By molecular mimicking self-antigen, the gut microbiome might be capable of triggering autoimmune response and systemic inflammation, which eventually result in tissue damage [48]. Therefore, certain intestinal bacteria with self-antigen-like epitope structures in pre-clinical RA individuals can lead to cross-reactive autoantibody production Table 2.

It was previously observed in pre-clinical RA individuals that intestinally rich bacteria including *Citrobacter, Bacteroides, Eggerthella* and *Clostridium*, present epitope which may have molecular mimicry with collagen XI and HLA-DRB1*0401 [14,29,49]. Collagen XI, a classical fibril-forming collagen found in the articular cartilage, regulates cartilage formation, while immunization with collagen XI can be used to induce arthritis in DBA/1 mice [50]. HLA-DRB1*0401 is associated with RA susceptibility through the presentation of arthritogenic self-peptides [51]. Collectively, gut microbiome molecular mimicry with these RA-related antigens may result in immunological tolerance breakdown and early joint cartilage destruction.

In a study of ankylosing spondylitis, peptides from *Bacteroides fragilis* 3_1_12, a bacterial strain of the Bacteroidaceae family, might mimic peptides from CII [45]. Being the primary structures of articular cartilage, CII has been used to establish mouse model for RA [52]. Thus, CII mimicked by peptides from *Bacteroidaceae* can induce the production of autoantibodies against CII and aggravate RA progression [53].

Additionally, a cross-reaction between *Roseburia intestinalis* and anti-β2 glycoprotein I (β2GPI) antibody-reactive memory Th1 cells, resulting in autoantibody production, has been reported in an individual with antiphospholipid syndrome [47]. The presence of β2GPI in the serum of RA patients may also be induced by an *R. intestinalis* cross-reaction, eventually contributing to RA pathogenesis [54]. However, further studies are required to determine the role of β2GPI in RA development as well as the link between an abundance of *R. intestinalis* and the activation of β2GPI production in a pre-clinical RA model.

Enhanced IL-17 responses to 60S ribosomal protein L23a (RPL23A), an autoantigen that reacted to T cells and autoantibodies from RA patient, occurred in lymphocytes from the intestines of pre-clinical RA mice [16,55]. *P. copri* might present an epitope that mimics the structure of RPL23A as that co-culture of T cells from an RA model with *P. copri* induced a high response to RPL23A. Interestingly, another study reported that *Prevotella*-epitopes showed high sequence homology with HLA-DR–presented T cell epitopes of N-acetylglucosamine-6-sulfatase (GNS) and filamin A (FLNA) [56]. As GNS and FLNA are enriched in RA patients' inflamed joints, their autoantibody development can be a hallmark of an autoimmune activation state and joint damage in RA patients [57–59]. Thus, molecular mimicry of GNS and FLNA is a likely mechanism for the contribution of *Prevotella* to RA progression during the clinical phase.

In summary, bacterial molecular mimicry is a key factor in RA progression that induces autoimmune dysfunction. Regarding the enrichment of *Prevotella* in pre-clinical RA individuals, we hypothesized that molecular mimicry of the present *Prevotella* epitope is strongly associate with systemic autoimmunity expansion in pre-clinical RA and potentially induce joint damage.

Table 2
Molecular mimicry between gut microbiota and RA antigen.

Gut microbiota	Self-antigen	Function
Citrobacter, Bacteroides, Eggerthella and *Clostridium*	Collagen XI HLA-DRB1*0401	Regulate cartilage formation. Presentation of arthritogenic self-peptides.
Bacteroides fragilis 3_1_12	Collagen II	Primary structures of articular cartilage.
Roseburia intestinalis	Anti-β2 glycoprotein I	Autoantigen of antiphospholipid syndrome.
Prevotella copri	60S ribosomal protein L23a	Autoantigen react to T cells and autoantibodies from RA patient.
Prevotella spp.	N-acetylglucosamine-6-sulfatase and filamin A	Enrich in RA patients' inflamed joints.

3.2. Gut microbiota-derived metabolites

Metabolites derived from the gut microbiota are mainly synthesized in three ways: (1) metabolites produced by gut bacteria through the degradation and fermentation of dietary components; (2) metabolites synthesized *de novo* by gut bacteria; (3) modification of host-derived metabolites by gut bacteria. The regulator role of gut microbiota-derived metabolites in the host's immune system as well as the link between gut microbiota-derived metabolites and RA was established in recent years [39,60–64] Table 3.

Short-chain fatty acids (SCFA) are a class of immunomodulatory compounds that are strongly associated with RA [63]. A decreased SCFA level has been reported in both RA patients and animal models. Furthermore, reduced disease severity was reported in RA mouse models including CIA, antigen-induced arthritis and K/BxN serum-transfer arthritis with SCFAs supplementation. SCFAs can be divided into six groups by structure, including formic acid, acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid and isovaleric acid. Among them, propionic acid is capable of inducing Th2 cells suppression via receptor GPR41-mediated dendritic cell functional alterations and further shapes the course of autoimmune disease [65,66]. A recent animal study confirmed the immunomodulatory properties of propionic acid by uncovering promoted regulatory T cells (Treg) differentiation and increased IL-10 levels in the propionic acid treated group [67].

Another type of SCFA, valeric acid, showed the ability to induce IL-10 production in regulatory B cells through enhanced mammalian target of rapamycin (mTOR) activity and promoted glucose oxidation. By inhibiting histone deacetylase activity in $CD4^+$ T cells, valeric acid suppresses the generation of intestinal Th17 cells, leading to the amelioration of autoimmune inflammation [68]. The potential mechanism of butyric acid in modulating the inflammation response among pre-clinical RA individuals has been extensively investigated. The mechanisms of butyrate involvement in balancing the immune system include Treg polarization as well as proinflammatory cytokine down-regulation and conventional Th cells suppression [69]. Moreover, butyrate can inhibit autoantibody production, likely via the suppression of follicular helper T (Tfh) cell mediated germinal center B cell differentiation [63]. By regulating histone deacetylase expression and proinflammatory cytokine production, butyrate treatment showed a protective effect on RA development and the suppression of osteoclast differentiation [69].

A microbiota gene function analysis by Yu et al. revealed depleted amino acid biosynthesis pathways in untreated RA patients that resulted in decreased production of branched amino acids [70]. As branched amino acids have long been recognized as precursors of intestinal microbiota mediated SCFA synthesis, depleted amino acid biosynthesis pathways in the gut microbiota could be a potential mechanism leading to SCFA mediated RA regulation. As SCFA are primarily produced by a small number of intestinal bacteria, the alteration of gut microbiome composition is a key factor contributing to reduced SCFA levels in RA patients. *Collinsella*, a genus of the gut microbiome that can regulate isoprenoid metabolism and produce various SCFA including butyric acid, acetic acid, formic acid, and lactic acid, was reported at the higher relative abundance in RA patients [71,72]. Moreover, a decreased abundance of *Fusicatenibacter* and *Megamonas* in RA patients was strongly correlated with reduction in traumatic acid, N-alpha-acetyl-L-lysine and carbohydrate fermentation that eventually antagonized SCFA metabolism [70]. Of note, *Firmicutes* (the family of *Fusicatenibacter*) produced nearly half of the lysine-acetylated derivatives in the gut [73]. Thus, decreases in the intestinal metabolites riboflavin and sphingolipids, which are beneficial for maintaining intestinal homeostasis and the Firmicutes populations, may trigger the depletion of *Fusicatenibacter* by decreasing SCFA production.

Table 3
Gut microbiota-derived metabolites in pre-clinical RA.

Gut microbiota-derived metabolites	Related gut microbiota	Function
SCFA	*Collinsella*	Induce Th2 cells
Propionic acid		suppression via receptor GPR41 mediated dendritic cell; promote regulatory T cells differentiation and IL-10 production
Valeric acid		Induce mammalian target of rapamycin activation, glucose oxidation promotion that improve regulatory B cells IL-10 production; inhibit $CD4^+$ T cell histone deacetylase activity and suppress intestinal Th17 cells generation
Butyric acid		Enhance Tregs polarization, inhibit proinflammatory cytokines and autoantibody production, conventional Th cells suppression, hinder differentiation of germinal center B cell, regulate histone deacetylase expression
Amino acids		Precursors of intestinal
Branched amino acids		microbiota mediated SCFA synthesis
Traumatic acid, N-alpha-acetyl-l-lysine	*Fusicatenibacter*, *Megamonas*	Antagonized SCFA metabolism
Kynurenic acid		Inhibit inflammatory mediators (TNF-α and calgranulins) production.
5-Hydroxyindole-3-acetic acid		Regulate AhR-dependent gene transcription and IL-10 transcription in B cells
3-hydroxyanthranilic acid		Anti-inflammatory
Tryptophan	*Klebsiella, Escherichia*	Mediate pathogenicity in RA
Histidine		Inhibit nuclear translocation of NF-κB and peroxisome proliferator-activated receptor γ receptor γ activation
Riboflavin and sphingolipids	*Firmicutes*	Maintaining intestinal homeostasis and *Firmicutes* populations
Vitamin		
Ascorbate	*Escherichia coli* and *Streptococcus bovis*	Regulate TNF-α, and IL-6 production, decrease oxidative and nitrative stress, inhibit inflammatory cytokines
Fatty acid		
Arachidonic acid	*Prevotella copri*	Enhance production of inflammatory mediators including prostaglandins and leukotrienes
9,12-octadecadienoic acid and 10Z-nonadecenoic acid	*Verrucomicrobia and Akkermansia*	Induce secretion of IL-6 and chemokines in subchondral bone
Bile acids	*Lactobacillus, Bifidobacterium, Clostridium and Bacteroides*	Regulate monocytes, macrophages, dendritic cell and natural killer T cells through activation of farnesoid X receptor and protein-coupled bile acid receptor 1
Glycocholic acid and glycochenodeoxycholic acid	*Bifidobacterium*	Induce IL-6 expression

AhR, aryl hydrocarbon receptor; NF-κB, nuclear factor κB; TNF-α, tumor necrosis factor-α.

Evidence shows enhanced ascorbate degradation of gut microbial function was a hallmark of the RA group and positively associated with serum levels of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and IL-6 [74]. As an antioxidant, ascorbic acid provides a protective effect on oxidative and nitrative stress and works against inflammatory cytokines in RA patients [75]. Moreover, *Escherichia coli* and *Streptococcus bovis* were the driving contributors to ascorbate degradation [76]. The high relative of *S. bovis* abundance in the early RA group and gradually decreased as RA progressed suggested a more crucial role of *S. bovis* in the pre-clinical RA stage. Data from several studies suggested a higher abundance of *P copri* in individuals in the pre-clinical phase of RA [77]. As the abundance of *P. copri* is positively related to serum concentrations of arachidonic acid-related metabolites, increased arachidonic acid biosynthesis by *P. copri* in pre-clinical RA individuals could contribute to RA progression through the production of arachidonic acid-derived inflammatory mediators including prostaglandins and leukotrienes [78]. Another study by Chen et al. showed that 9,12-octadecadienoic acid and 10Z-nonadecenoic acid, two long-chain fatty acids, were associated with increased population of *Verrucomicrobia* and *Akkermansia* in the RA group [40]. By inducing the secretion of IL-6 and chemokines in subchondral bone, 9,12-octadecadienoic acid and 10Z-nonadecenoic acid could affect RA development and progression [79].

Lower levels of tryptophan metabolites including N-methylserotonin, 5-hydroxyindole-3-acetic acid (5-HIAA), kynurenic acid, xanthurenic acid and 3-hydroxyanthranilic acid (3-HAA) have been reported in RA patients versus healthy controls [70]. As an antagonist of ionotropic glutamate receptors, kynurenic acid exerts its anti-inflammatory effect through inhibiting the production of inflammatory mediators (TNFα and calgranulins) in whole blood cultures from RA patient [80]. Nevertheless, kynurenic acid reportedly exists in the synovial fluid of patients with RA. Thus, considerably more work needs to be done to determine the role of kynurenic acid in joint cavity tissue.

The immunoregulatory function of 5-HIAA in arthritis was recently reported by determining the upregulation of aryl hydrocarbon receptor (AhR)-dependent gene transcription and IL-10 transcription in B cells *ex vivo* [63]. Likely due to excessive disposal in the urine, serum levels of 3-HAA decreased as arthritis progressed in a CIA animal model that eventually hampered the anti-inflammatory action of 3-HAA [81]. In a clinical study of 16S rDNA sequencing and nontargeted metabolomic profiling in 26 untreated RA patients, tryptophan synthesis was involved in *Klebsiella* and *Escherichia* mediated pathogenicity in RA [82]. Gut microbiota-mediated bile acids played a crucial role in immune regulation among monocytes, macrophages, dendritic cells and natural killer T cells through activation of the farnesoid X receptor and protein-coupled bile acid receptor 1 [82–84].

Microbial regulation of bile acid metabolism occurs mainly through regulating the expression of bile acid formation related enzymes and bile salt hydrolase secretion by *Lactobacillus, Bifidobacterium, Clostridium* and *Bacteroides.* A recent study reported that amino acid analog treatment reduced glycocholic acid (GCA) and glycochenodeoxycholic acid (GCDCA) levels through plasma metabolomic analysis in an RA model [85]. GCA and GCDCA reportedly induce IL-6 expression, which might further provoke RA-related inflammation. Thus, a possible therapeutic effect occurs through the hydrolysis of GCA and GCDCA by secreted bile acid hydrolase derived from enriched *Bifidobacterium* in the gut [86].

Furthermore, as suggested by Qi et al. studies of the phenylalanine, indoleacetic acid, and glucuronic acid metabolic pathways would promote an understanding of the pathogenesis of RA [87]. This result was confirmed by a further study suggesting a potential role of these pathways in immune system activation [88]. Interestingly, a decreased serum level of histamine, an anti-inflammatory mediator, was noted in RA patients. Supplemental histidine led to decreased nuclear translocation of nuclear factor κB (NF-κB) and peroxisome proliferator-activated receptor γ receptor γ activation [89]. Moreover, an inverse correlation was noted in histidine level with RA disease

activity [88]. As histidine modification and digestion were demonstrated in gut microbes, gut microbiota mediated histidine metabolism may be the underlying mechanism of the pathogenesis of RA.

Increasing research into the anti-inflammatory effect of SCFAs provides a plausible direct elucidation of the molecular mechanisms by which the gut microbiome participates in RA development through regulating SCFAs metabolism [90,91]. Moreover, an alerted gut microbiome could regulate the autoinflammatory state through tryptophan, long-chain fatty acids and arachidonic acid metabolism induced activation of inflammatory mediator; however, the precise mechanism of this phenomenon remains unclear.

3.3. *Intestinal barrier function*

Intestinal barrier function protects the intestinal mucosa by blocking the entry of pathogens, toxins, and food antigens and maintains a symbiotic relationship with commensal bacteria. The front line of this barrier is compressed by the epithelial cell layer, which is linked by tight junction proteins [92]. The transport of small molecules through the intestinal barrier is regulated by the intracellular signaling transduction system and extracellular signaling molecule in the modulation of tight junction protein complexes [93].

An impaired intestinal barrier function can increase intestinal permeability, leading to a leaky gut. The occurrence of a leaky gut and subsequent translocation of the gut bacteria and exterior antigens into the host lead to uncontrollable immune reactions in the local intestinal environment and systemic immune responses [94]. Of note, the disruption of intestinal barrier function and increased intestinal permeability were reported in RA patients, especially those with higher disease activity [95]. This finding was confirmed in CIA mice, as an elevated serum dextran level suggested intestinal barrier impairment alone with RA development [15]. A further study in arthritis-prone C57BL/6 mice reported abnormal intestinal barrier function induced by *P. copri* colonization [77]. In autoimmune hepatitis, enrichment of several gut bacteria species including *Enterococcus gallinarum, Proteus mirabilis*, and *Klebsiella pneumonia* is suggested as highly related to abnormal intestinal permeability [96]. On the other hand, decreased population of certain species of gut bacteria that maintain normal gastrointestinal tract was another mechanism linking gut microbiota alterations and impaired gut barrier function. Gut microbiota-derived metabolites also regulate intestinal barrier integrity through multiple mechanism. SCFAs, especially butyrate, improved barrier function through adenosine monophosphate-activated protein kinase mediated tight junction protein mucin 2 production [97]. Similarly, improved tryptophan metabolites production could induce higher levels of tight junction protein production via AhR activation, which may even reverse barrier dysfunction [98].

In colitis mice, treatment with *Lactobacillus plantarum* derived conjugated fatty acids contribute to upregulated tight junction protein in colonic tissues, suggesting a mitigation effect on colitis [99]. Polyamines, which are mainly synthesized by *E. coli*, *Bacteroides*, and *Fusobacterium*, are suggested important in intestinal maturation. A recent study revealed that polyamine-induced regulation of the gut barrier was related to intracellular $Ca2^{+}$-dependent E-cadherin production [100].

Zonulin production may be the mechanism linking gut microbiota alterations and intestinal epithelial barrier disruption. Zonulin is an enterotoxin secreted by IECs after gut bacteria or dietary stimulation that causes intercellular tight junction disassembly in the intestinal barrier [101]. Moreover, increased zonulin levels were found in "arthritogenic" immunization mice with altered gut microbiota. Of note, increased intestinal barrier permeability accompanied by Th1 and Th17 cell infiltration in the lamina propria was identified in patients and mice before RA onset [20].

Collectively, we hypothesized that gut microbiota induced impaired intestinal function is the potential cause of intestinal immune activation and the subsequent transmigration of immune cells from the gut to the joints. However, further studies are required to confirm our findings.

3.4. *Modulation effect of gut microbiota on immune cells*

Gut commensal gram-negative bacteria can penetrate the gut barrier without disrupting its function or structure. Extra-intestinal gram-negative bacteria reportedly induce microbiota-specific immunoglobulin G (IgG) production by systemic dissemination despite intact intestinal barriers. TLR4 mediated B cell and T cell activations are involved in the secretion of microbiota-specific IgG. TLR is a pattern recognition receptor that can recognize pathogen-associated molecular patterns especially LPS displayed on the outer membrane of gram-negative bacteria. Among the TLR family, TLR4 is responsible for activating the innate and adaptive immune system through NF-κB activation [94], and important regulator in T cell development and antigen-independent differentiation through transcriptional regulation of TNF-α, IL-6, IL-1β and cyclooxygenase 2 (Cox-2) expression [102]. T cell activation and pro-inflammatory cytokine activation, including TNF-α, play a crucial role in the pathophysiology of RA.

The mitogen-activated protein kinase (MAPK) signaling pathways are another key transcriptional pathway of RA mediated by TLR. Along with regulating pro-inflammatory cytokine expression, MAPK family proteins (such as c-Jun N-terminal kinase [JNK] and p38) are directly involved in the proliferation and invasion of synoviocytes, cartilage degradation and joint destruction, which suggests a crucial role in its onset of arthritis [103]. In addition, several treatments targeting the MAPK and NF-κB pathways exerted an antiarthritic effect in an RA model [104–106]. Thus, pro-inflammatory and T cell regulation characteristics of gut gram-negative bacteria may promote a systemic immune response and joint destruction in the pre-clinical RA stage.

A high abundance of *P. copri* is among the most significant changes to the gut microbiota that occur in pre-clinical RA. The presence of *P. copri* 16S rDNA in the synovial fluid of patients with early RA, suggests its potential role in inducing joint inflammation through systemic spread within phagocytic cells. *P. copri* antibody production is specific to RA patients and rarely identified in those with other rheumatic diseases. In addition, the magnitude of *P. copri* antibody responses were directly linked with Th17 immune response related cytokines [78]. Being a gram-negative bacterium, the recognition of LPS secreted by *P. copri* by TLR4 may be responsible for triggering the production of cytokines and T cell-mediated autoantibodies. Th17 cells are a distinct Th lineage characterized by the secretion of IL-17, TNF-α, IL-22, IL-21, and granulocyte-macrophage colony-stimulating factor (GM-CSF) [107]. In RA patients, the population of Th 17 cells and levels of Th17-secreted cytokines were directly related to disease activity, indicating a crucial role of Th17 cells in RA pathogenesis [108,109]. More importantly, Th17 cell derived cytokine IL-17 was highly expressed in the synovial fluid of individuals in the early RA stage [110]. It was previously reported that IL-17 mediated the matrix metalloproteinase (MMP) production of synovial fibroblasts that facilitate tissue destruction in RA patients [111]. This result was confirmed by a subsequent animal study in which enrichment of Bacteroidaceae and Lachnospiraceae in the gut microbiota induced Th1 and Th17 cell polarization, and a positive relationship was found between the secretion of Th17 associated cytokines such as IL-17 and TNF-α and joint destruction [14].

The intestinal lamina propria contains large populations of Th17 cells with the capacity to migrate to the peripheral lymph nodes or synovial tissues after gut bacteria induced activation. A higher abundance of Th17 cells was found in the lamina propria in a pre-clinical CIA model versus naïve mice. Partial elimination of the intestinal microbiota with antibiotic treatment reduced the severity of arthritis by inhibiting Th17 cell differentiation and serum amyloid A (SAA) production [28]. Similarly, decreased levels of Th17 related cytokines including IL-17A and IL-22 resulted from early antibiotic treatment. Antibiotic treatment also decreased the production of anti-CII antibodies in early CIA mice and significant glycosylation changes accompanied by an impaired

complement activation ability of anti-CII antibodies [15].

Accordingly, the gut microbiome induces Th17 activation in the intestinal lamina propria, which subsequently evoke a systemic inflammatory response and aggravates synovial inflammation, and finally facilitates osteoarthritic cartilage degradation and induces autoantibody activation and has been implicated in pre-clinical RA.

3.5. IEC autophagy

To date, few reports have detailed the relationship between autophagy and intestinal bacteria in RA. There has been significant progress toward elucidating the crucial role of autophagy in maintaining intestinal barrier function and gut microbiota homeostasis in other autoimmune diseases. One of the pathways contributing to the protective effect of autophagy on intestinal barrier function was through triggering lysosomal degradation of the tight junction protein claudin 2 [112]. Xenophagy through autophagy-mediated elimination of intracellular bacteria, was another autophagy mediated pathway that maintains normal intestinal permeability [113].

Hypoxia-inducible factor-1α (HIF-1α) is a critical regulator of hypoxic conditions, that plays an important role in maintaining intestinal barrier function. When co-cultured with butyrate, intestinal cell HT 29 autophagy greatly increased through a HIF-1α mediated pathway, which further alleviated colitis damage in inflammatory bowel disease (IBD) mouse model. The absence of intestinal epithelial HIF-1α in C57BL/6J mice led to RA-like gut microbiota composition changes and metabolite profile alterations characterized by the enrichment of *Bacteroides* and a reduced butyric acid concentration [114]. HIF-1α regulated autophagy is closely related to alterations in the gut microbiota and metabolite profiles in RA and affects its progression.

Autophagy, on the other hand, is crucial in the regulation of gut microbiota composition. Colonic epithelial cell-specific autophagy related gene (Atg) 7 deficiency resulted in an increased gut microbiome population with a specific enrichment in *Eubacterium cylindroides*, *B. fragilis*, and *Clostridum leptum* [115]. Th17 cells polarization related *Candidatus athromitus* enrichment as well as decreased abundance of anti-inflammatory bacteria such as Lachnospiraceae and Ruminococcaceae were exhibited in Atg5-deficient mice [116]. Dysregulation of gut microbiota may also contribute to defective autophagy. TLR4-MAPK and NF-κB pathway activation through sensing of LPS from intestinal gram-negative bacteria has been extensively reported in RA patients. Zhou M, et al. provided a deeper insight into the relationship between LPS induced MAPK and NF-κB pathway activation and autophagy, by uncovering LPS activated mTOR via upstream TLR4-MyD88-MAPK signaling and inhibiting autophagy through the NF-κB pathway, which further results in intestinal cells oxidative injury and inflammation [117].

Although the certain mechanism of autophagy in RA pathogenesis is still unclear, the disruption of autophagy mediated regulation in gut microbiota and intestinal barrier function may lead to aggravated RA progression. Interaction between gut microbiota and IECs autophagy may also play a crucial role in inducing impaired intestinal barrier function and affecting the progression of RA.

3.6. *MicroRNAs*

MiRNAs are single-stranded non-coding RNA that are implicated in post-transcriptional gene expression regulation. The most recent study in pre-clinical RA individuals revealed that miRNAs were importantly associated with RA pathogenesis and onset of arthritis [118,119]. Apart from enhancing inflammatory pathway signaling and increasing the secretion of pro-inflammatory cytokines, mechanisms of miRNAs in promoting RA development also include altered gut microbiota and impaired intestinal barrier function. The alterations of gut microbiota in deficient IEC miRNA (Dicer1 $^{\Delta IEC}$) mice were characterized by enriched Bacteroidaceae and Helicobacteraceae and decreased abundance of Prevotellaceae, Porphyromonadaceae, Lachnospiraceae and Ruminococcaceae. This study further revealed the ability of miRNAs to enter *E. coli* and affect bacterial gene transcripts and growth [120]. In addition, Dicer1 $^{\Delta IEC}$ mice exhibited higher IL-17 expression and intestinal permeability related epithelial tight junction molecules productions (Zo-1, occludin-1, claudin-1, claudin-2, and claudin-5), which suggests a strong association between miRNAs deficiency and impaired intestinal function [120].

On the other hand, gut microbiota depletion through antibiotic treatment in mice result in miRNA dysregulation, suggesting a regulatory function of the gut microbiota in miRNA expression and post-transcriptional modification [121]. Two probiotics, *Lactobacillus fermentum* and *Lactobacillus salivarius,* induced increased miRNA-495-155 and miRNA-495-223 expression in a colitis model, contributing to alleviated inflammation [122]. Mice harbored gut microbiota from Prader-Willi syndrome patients exhibited significant differential expression of miRNA in the colon tissues and induced an immune response similar to that seen in Prader-Willi syndrome related inflammation [123].

Taken together, the interaction between gut microbiota and miRNA may affect RA disease progression, but the causal relationship remains unresolved.

3.7. Genetic factors and microbiota

HLA alleles are major susceptibility factors for RA, as more than 70% of RA patients carry positive HLA-DRB1 molecules [124]. Significant alteration of the microbiome were identified in healthy individuals carrying the RA risk allele HLA–DRB1 [125]. More specifically, in an ongoing cohort study of first-degree relatives of patients with RA, *Prevotella_7* was the only species from the Prevotellaceae family that is positively associated with the HLA-DR allele [29]. Of note, *Prevotella_7* had the strongest association among other species including *Ruminococcaceae_UCG-14*, *Rikenella*, and *Shigella*, whichare positively associated with a high genetic risk of RA. On the other hand, mice carrying the RA-susceptibility *DRB1*0401* gene showed a significant increase in gut permeability and Th17 cytokine transcription compared to naïve mice and those carrying the RA-resistant *DRB1*0402* gene [51]. HLA-B27, an ankylosing spondylitis risk genetic factor, reportedly to induces intestinal permeability and inhibits epithelial tight junction protein expression [126]. Thus, carrying HLA-B27 is a critical condition that leads to the activation of bacterial products related to immune responses [127].

These results suggest that the HLA-DRB1 RA risk allele may account for gut microbiota dysbiosis and intestinal barrier function dysregulation in RA, but the underlying mechanism remains to be clarified. More broadly, research is also needed to determine the mechanism of the genetic predisposition of RA mediated changes in gut microbiota abundance.

4. Gut microbiota-based intervention for pre-clinical RA

The most widely used approaches to manage RA currently include DMARDs, glucocorticoids, non-steroidal anti-inflammatory drugs and small molecule inhibitors including TNF-α-inhibitors, co-stimulation modifiers and IL-6-inhibitors [128]. However, those available treatments carry substantial side effects including cytopenia, transaminase elevation and infection. As described in several recent reviews, intestinal bacteria-based intervention was a promising treatment that could be used during the pre-clinical RA stage [34,129,130]. Dietary supplements and prebiotics have been applied as a clinical treatment for RA by rebalancing the gut microbiota composition. Prophylactic treatment with dietary supplements and prebiotics can regulate T cell polarization, improve Treg and Th1/Th17 numbers, reduce pro-inflammatory cytokine levels and decrease the incidence of RA. The oral administration of probiotics can competitively inhibit the colonization of pathogenic *Prevotella* and suppress autoantibody production [131]. Regulating

microbiome mediated metabolism is another characteristic of many intestinal bacteria-based intervention such as probiotic and natural compounds that prevent RA progression [132]. This section will discuss several potential therapies for the interaction of the gut microbiota in the treatment of pre-clinical RA.

4.1. Dietary intervention and prebiotics

Representing a major factor influencing the microbiota's composition, dietary intervention can prevent RA development. Fish oil, a mixture of polyunsaturated fatty acids, is an important contributing factor in the attenuation of inflammation and regulation of autoimmunity. Significant improvements in tender joints and shortened morning stiffness time have been reported in several studies of fish oil supplementation in RA patients [133–135]. Recent studies focusing on the effect of fish oil supplements as immune regulators found that ω-3 polyunsaturated acids derived from fish oil exerted anti-inflammation benefit by reducing the TNF-α, IL-17, IL-1β and IL-6 production in humans and animals [136,137]. An analysis of CIA mice supplemented with tuna oil found that dietary intervention of tuna oil restored gut microbiome dysbiosis by increasing the abundance of Bacteroidales, Clostridiales, Lactobacillales and Desulfovibrionales.

A dose-dependent intervention of tuna oil also relieved arthritis severity and joint bone erosion with decreased pro-inflammatory cytokine levels. Of note, the anti-inflammatory effects of a horizontal fecal transfer from mice treated with tuna oil intervention were similar to those in the tuna oil treatment group, with reduced serum pro-inflammatory cytokine levels and alleviated arthritis-related traits. Since the horizontal fecal transfer resulted in significant microbiota changes between mice that received fecal transplants from the group with or without tuna oil treatment, the protective effects of tuna oil supplementation in recipient CIA mice were mediated via the gut microbiota [24]. Therefore, fish oil supplementation may be a preventive approach for RA, but this strategy requires confirmation in further studies.

Diets rich in vegetables, fruits, and fiber have long been considered a protective factor in RA development. Häger et al. reported restored immune dysbiosis including increased numbers of Tregs and an increased Th1/Th17 ratio in RA patients with short-term high fiber supplementation [138]. The regulation of immune cells by a high fiber diet resulted in significantly decreased levels of anti-citrullinated vimentin p18 peptide and markers of bone erosion. Moreover, a reduced level of the intestinal barrier function markers-calprotectin and zonulin after a high fiber diet indicated improved intestinal homeostasis [138]. The mechanistic investigation of the high fiber supplement pointed to the shift in the *Firmicutes*-to-*Bacteroidetes* ratio [139]. Diets rich in resistant starch, a type of soluble dietary fiber, had a preventive effect on arthritis development and bone damage in CIA mice. Changes to the gut microbiome induced by resistant starch treatment and the associated IL-10 production and splenic Treg expansion were the key factors contributing to the improvements in disease outcomes in CIA mice [140]. Importantly, SCFAs, produced by resistant starch fermentation in the intestine, are crucial executors of the gut microbiota mediated by resistant starch treatment on CIA alleviation [141].

Treatment with one of the most popular prebiotics, inulin, greatly inhibited the production of pro-inflammatory cytokines SAA and TNF-α in CIA mice compared to the control group. The immunoregulatory role of inulin reduced the levels of RA-related autoantigen fibrinogen and further inhibited the development of paw swelling induced by complete Freund's adjuvant [142]. Dietary intervention with inulin induced altered the abundances of *Anaerostipes*, *Bilophila* and *Bifidobacterium* in a double-blind-randomized-cross-over intervention study, indicating a promising novel target for research specifically focusing on the link between inulin-mediated gut microbiota alterations and RA prevention [143].

Collectively, these findings suggest the possibility that dietary and prebiotics-mediated manipulation of the gut microbiota would be a plausible preventive strategy for individuals susceptible to RA.

4.2. Probiotics

The use of probiotics is another way to improve the prognostic value of arthritic processes. The long-term use of probiotics has immune-modulating action and protects bones from destruction, thereby lessening arthritis severity.

A mixture of five probiotics – *Lactobacillus acidophilus* La-14, *Lactobacillus casei* Lc-11, *Lactococcus lactis* Ll-23, *Bifidobacterium lactis* Bl-04, and *Bifidobacterium bifidum* Bb-06 showed beneficial effects in inflammation as well as oxidative and nitrosative stress [144]. However, the disease severity of RA patients treated with these probiotics for 60 days remained stable, which suggests that a different route of administration of probiotic supplements and the use of multi-strain probiotics may affect the efficacy of probiotic treatment [145].

A newly isolated commensal bacterium, *Prevotella histicola*, modulated the systemic immune response in transgenic mice expressing DQB1*0302/DQA1*0301 (DQ8) inhibiting the expression of pro-inflammatory cytokines IL-2, IL-17, TNF-α, GM-CSF, and monocyte chemoattractant protein-1. Prophylactic treatment with *P. histicola* significantly reduced the incidence of arthritis in transgenic mice, with a protective effect on RA by inhibiting the production of RA-related antibodies and the RA antigen-specific T cell response. Importantly, *P. histicola* modulation of arthritis is dependent on the regulation of IL-10 producing Tregs and antigen presentation by dendritic cells in the gut. The influence of *P. histicola* on the gut includes increasing intestinal barrier function, inhibiting cytokine expression, and restoring gut microbiota dysbiosis. Moreover, in an *in vivo* RA study, human derived *P. histicola* alleviated disease severity without harming the intestinal tissue, indicating a therapeutic potential of *P. histicola* [25]. A subsequent study using DQ8 mice revealed the colonization of *P. histicola* largely in the duodenum of the small intestine and competitively reduced the abundance of pathogenic *Prevotella* [146].

On the other hand, duodenum colonized *P. histicola* might be involved in restored population of *Allobaculum* in the host gut with augmenting butyrate production thus increased SCFA levels contribute to maintaining immune homeostasis [131]. These findings suggest that the introduction of *P. histicola* can impact the onset of arthritic inflammation and support that early treatment with *P. histicola* can interrupt RA progression in the pre-clinical stage.

In RA patients, levels of *Bifidobacterium* species, a group of widespread probiotics organisms, are inversely related to the pro-inflammatory cytokine level [147,148]. In a pre-clinical model, therapy with *Bifidobacterium longum* RAPO for 7 weeks inhibited the development of arthritis by reducing cytokines IL-17 levels and increasing Treg populations. Th17 related genes in peripheral blood mononuclear cells from RA patients including *IL-17A*, *IRF4*, *RORC*, *IL-21*, and *IL-23* were downregulated after culturing with *B. longum* RAPO [71]. In a human avatar arthritis model, oral *B. longum* RAPO therapy inhibited the Th17 polarization and severity of arthritis damage, leading to RA protection benefits in mice [71]. The experimental investigation also reported that oral treatment of *B. longum* RAPO reduced RA-related antibody and inflammation factor levels, thus alleviating inflammation status in a CIA model [71].

The preventive administration of another *Bifidobacterium* species, *Bifidobacterium adolescentis*, 2 weeks before CII immunization significantly suppressed arthritis development in more than half of the CIA rats. Early probiotic administration of *B. adolescentis* rebalanced the immune response by reducing TNF-α, IL-6 and IL-17A productions and recovered the Treg population, thus modulating anti-CII IgG1 production. Similar to *B. longum* RAPO therapy, *B. adolescentis* supplementation partially restored arthritis induced gut microbiota dysbiosis and improved intestinal barrier function. Moreover, a gut microbial metabolism analysis revealed role of *B. adolescentis* in modulating

metabolism and promoting SCFAs production. The preventive effect of *B. adolescentis* arthritis may occur through the regulation SCFA production as SCFA levels are positively correlated with Tregs and negatively correlated with pro-inflammatory cytokines levels [149].

The results of a study evaluating the modulatory effect of *Bifidobacterium* spp. in adjuvant arthritis mice confirmed the prophylactic effect of *Bifidobacterium breve, B. longum, B. bifidum* in RA by controlling oxidative stress and improving the *Bifidobacteria* population [132]. Moreover, reduced levels of inflammatory mediators and bone hydrolytic enzymes also detected, such as Cox-2, hyaluronidase, elastase and MMP, suggesting that *Bifidobacterium* spp. improves joints and bones mainly through modulating bone hydrolytic enzyme production and inflammation activation in synovial tissue [150]. As *B. breve* showed better antiarthritic effects than the other *Bifidobacteria* species, further studies with a greater focus on the putative positive impact of *B. breve* on RA prevention are suggested.

Lactobacilli share several common properties with *Bifidobacteria*, including being lactic acid-producing and non-spore-forming. Since first reported in 1998, the oral administration of *Lactobacillus casei* for RA treatment has attracted much interest [151]. The immunomodulatory activity of *L. casei* increased anti-inflammatory cytokine (IL-10) levels and inhibited pro-inflammatory cytokine production (TNF-α and IL-6) [152]. A histopathology examination reported reduced bone destruction and lymphocyte infiltration with a lower arthritis score in CIA mice who received *L. casei* treatment [153].

An *in vitro* study by So, et al. suggested that the exerted anti-inflammatory effect of *L. casei* was the result of NF-κB inactivation, suppressed $CD4^+$ T cell proliferation and inhibited Th1 effector functions [153]. Their result was confirmed by a subsequent study of an experimental rodent model of osteoarthritis that demonstrated that tissue inhibitors of MMP1 mediated its chondroprotective action [154]. Similarly, 30-day oral treatment with *L. casei* significantly prevented arthritis induction and progression, partially by maintaining the redox balance of oxidative stress. Moreover, *L. casei* supplementation prevented gut dysbiosis by increasing the abundance of several *Lactobacillus* strains which was strongly correlated with reduced pro-inflammatory cytokines levels [12].

Another study compared the therapeutic effect of *L. casei* CCFM1074 and CCFM1075 and reported significantly higher SCFA production resulting from the carbohydrate metabolism function of CCFM1074 and a subsequently shaped Treg expansion mediated by SCFAs. Plasma levels of metabolites such as docosapentaenoic acid and eicosapentaenoic acid were increased after CCFM1074 treatment, indicating the involvement of gut microbiota mediated system metabolites synthesis in promoting inflammation resolution and ameliorating arthritis [41]. Of note, as CCFM1075 could not mitigate arthritis by reducing pro-inflammatory cytokine production, regulating Treg polarization and rebalancing the gut microbiota, further studies are required to confirm the preventive effect on strain level. *Lactobacillus sakei* showed an anti-inflammatory property in an RA model characterized by regulating Th17 cells and regulatory B cell differentiation. Seven weeks of oral treatment with *L. sakei* 1 μg/mL led to a significantly reduced number of osteoclasts, the down-regulation of osteoclast-related genes (tartrate-resistant acid phosphatase and calcitonin receptor), and inhibited expression of cathepsin K, supporting its therapeutic potential for RA [155]. However, unlike the therapeutic effect of *L. sakei* from health individuals, *L. sakei* from RA patient did not improve arthritis in CIA model, indicating that the protective effect of *L. sakei* might depend on the bacterial function.

A study of *Lactobacillus helveticus,* a probiotic mainly found in fermented foods, reported its ability to potently regulate the anti-inflammatory immune response. Five-dose weekly oral treatment with *L. helveticus* before CII immunization suppressed autoantibody production, delayed disease onset, and reduced arthritis symptoms. It was concluded that the oral administration of *L. helveticus* inhibited the recruitment of mononuclear cells to the synovial joints, which could result in reduced CIA development rates in mice [156]. Yamashita et al. evaluated the efficacy of the intraperitoneal inoculation and oral administration of *L. helveticus* in reducing CIA incidence and progression. The intraperitoneal inoculation of *L. helveticus* significantly alleviated hind paw thickness and reduced the incidence of arthritis [157]. A further study revealed inhibited IL-6 and CII-specific antibody production with a decreased number of RA related immune cells were the result of *L. helveticus* mediated JNK signaling pathway suppression and induced A20 expression via TLR2 signaling [158,159].

On the other hand, significant clinical improvement was observed with other *Lactobacillus* species including *L. fermentum*, *Lactobacillus rhamnosus* and *L. salivarius* [156,157,160,161]. A comprehensive study evaluating the abilities of different *Lactobacillus* species to improve arthritis found a protective effect of *Lactobacilli* occurring through different pathways [162]. More specifically, *L. rhamnosus* and *L. fermentum* targeted Th17 mediated immune responses, while *L. reuteri* and *L. casei* inhibited Th1 mediated immune responses. Discrepant *Lactobacillus* species effects were also found in changes to the gut microbiota and SCFAs [163]. Therefore, identifying the ideal combination of probiotic species for multispecies therapy to alleviate arthritis through regulating immune response, metabolism, and gut microbiota should be the basis of future research. Besides, recombinant Hsp65-producing *L. lactis* prevented CII-induced arthritis development through immune tolerance induction by Hsp65, which reduced inflammatory cytokine production and autoantibody synthesis through the TLR2 signaling pathway [164]. Thus, more research is needed to determine the preventive effect of RA with recombinant immunodominant antigen based probiotic treatment. Improved arthritis with decreased pro-inflammation cytokine levels and suppressed paw swelling were reported in an adjuvant-induced arthritis model with *Bacillus coagulans* treatment [165]. Of interest, infection with *Clostridium difficile* promote mesenteric Tregs and Th2 polarization and consequently prevented RA development [166].

Overall, prophylactic probiotics is a promising prevention approach of RA. Prophylactic use of certain probiotics such as *L. casei*, *B. adolescentis* and *P. histicola* may exert immune regulatory function by ameliorating gut microbiota dysbiosis and regulating gut microbiome mediated metabolite production. As probiotic treatment may prevent the onset of arthritis through individual pathways, the use of multispecies probiotic therapy in the pre-clinical RA stage requires further study to identify the ideal combination. The benefits and risks of recombinant probiotics for preventing RA development require assessment, and their application in clinical practice requires further research [26].

4.3. Antibiotic therapy

Antibiotic treatments have profound and long-lasting effects on microbiota composition since several studies reported that the microbiome reverts to the pretreatment status after nearly 1 year after treatment cessation [167–169]. The influence of antibiotic treatment on the gut microbiota not only involved a loss of diversity and drastic shifts in community composition, but it also depleted the levels of *Ruminococcus*, *Facecalibacterium*, *Bacteroides*, which subsequently resulted in decreased gut metabolites (uracil, primary bile acids, and essential amino acids) [170,171].

In the past two decades, the addition of oral antibiotics to standard therapy has significantly improved active RA. For example, the combination of 4 weeks of clarithromycin and standard methotrexate induced remission in the majority of RA patients [26]. In patients with early RA, doxycycline plus methotrexate significantly reduced the American College of Rheumatology 50 score compared to placebo [172]. A single-blind randomized trial reported that combination therapy with intravenous clindamycin and oral tetracycline may improve arthritis [173]. A meta-analysis of tetracycline treatment in RA patients was conducted by Mandel et al. This study reviewed 10 randomized controlled trials (N = 535). A statistically significant reduction in

disease activity was noted with minocycline treatment [174]. A preliminary study exploring the potential mechanisms of antibiotics for RA treatment reported that tobramycin, an aminoglycoside antibiotic, induced significant gut microbiota alterations characterized by a decreased abundance of *Helicobacter*, *Flexispira*, *Clostridium*, and *Dehalobacterium* and reduced synovial inflammation and cartilage destruction. Thus, the beneficial effects of antibiotics in RA may occur through the amelioration of gut microbiota dysbiosis [175]. However, several studies revealed that antibiotic prescriptions were associated with a higher risk of RA, suggesting that disrupted gut microbiota induced by antibiotic use in health population is a contributing factor for in RA onset [176,177].

In conclusion, the use of antibiotic as a pre-clinical RA intervention may be still risky. Further studies must confirm the prophylactic effect of antibiotics in clinical practice.

4.4. Fecal microbiota transplantation

FMT restores micro ecological homeostasis through a gut microbiome transplantation from a healthy donor to another patient's intestine [178]. FMT has been highly regarded since 2013, being listed in expert guidelines of standard practice for treating recurrent and refractory *C. difficile* infection [179]. As FMT for patient with recurrent *C. difficile* infection surprisingly improved their celiac symptoms, its use has been extended to autoimmune diseases [180]. Several studies applying FMT in an animal model of the autoimmune disease reported alleviated gut dysbiosis, improved autoreactive $CD4^+$ T cells and subsequently reduced clinical disease scores [181–185]. Similarly, rebalanced gut microbiota and improved production of beneficial SCFAs were reported in patients with multiple sclerosis and psoriatic arthritis [186,187]. In contrast, clinical studies of FMT did not always report successful outcomes; for instance, one study reported no significant changes in a Sjögren's syndrome patient after the receipt of donor gut flora, while another study reported less improvement in physical function among an FMT group with psoriatic arthritis [188–190].

The protective effects of FMT in RA have been studied in recent years [23,24]. A tuna oil supplement could reportedly rebalance gut microbiota homeostasis and improve intestinal epithelial barrier function in CIA mice, while the gut microbiota modulated by tuna oil was used for FMT to treat control CIA mice [24]. After investigating the beneficial effects of elastin peptides - the degradation product of elastin from tuna - the transplantation of gut flora from tuna elastin peptide-treated mice exerted a therapeutic effect by inhibiting the production of proinflammatory cytokines (IL-1β, TNF-α) and inducing anti-inflammatory cytokine production (IL-2, IL-10) [191]. The gut microbiota composition was restored at the phylum level after FMT, especially Firmicutes which increased from 52.50% to 75.01%. At the genus level, mice receiving FMT from tuna elastin peptide-treated mice had a significantly decreased abundance of *Muribaculum* while the abundance of *Lactobacillus* significantly increased from 31.51% to 59.16% [191]. Negatively correlation between *Prevotella* sp., *Helicobacter typhlonius* and *Lactobacillus hilgardii* with valeric acid, butyric acid and acetic acid were reported after Spearman's correlation analysis of the species abundance and SCFAs.

On the contrary, the relative abundance of *Clostridium aerotolerans* was positively correlated with valeric acid production [191]. In 2021, the first report of FMT use for RA treatment showed a good therapeutic effect of FMT in refractory RA therapy with a reduced arthritis score and RF level [23]. A randomized, double-blind study evaluating FMT efficacy and safety for refractory RA therapy started in 2019, but the result was not published yet (NCT03944096). Another randomized, placebo-controlled exploratory trial aiming to confirm the safety and efficacy of FMT in multiple autoimmune diseases including RA was started in 2022 and is expected to end in 2024 (NCT04924270). However, the donor screening procedure should be strictly reviewed, as several adverse infectious events through FMT have been reported [192,193]. Through the evaluation of the long-term safety of FMT in a *Clostridioides difficile* infected patient, the overall safety of FMT was confirmed, although a patient who received it demonstrated an increased incidence of myocardial infarction [194]. Furthermore, a recent cross-sectional study suggested that patients with common autoimmune diseases including RA, systemic lupus erythematosus, and psoriasis are less likely to know about FMT than those with ulcerative colitis or Crohn's disease ($p > 0.001$) [195]. Moreover, as general knowledge of FMT was the major factor influencing its acceptability ($p > 0.001$), educational programs offered by physicians to bolster patient literacy and avoid misunderstanding of FMT are highly demanded [195].

In conclusion, considerably more work is required to determine the benefits and risks of FMT as an intervention for pre-clinical RA development.

4.5. Natural compounds

The history of the natural compounds-based treatment has been traced back 2000 years. Recent studies assessing traditional herb-derived natural compounds in RA treatment revealed the potential role of natural compounds in gut microbiota structure modulation and RA amelioration [27]. The daily intragastric administration of total glucosides of paeony (TGP), a mixed natural compound derived from the roots of *Paeonia lactiflora* Pall, partly restored microbial function, increased the abundance of beneficial symbiotic bacterium and regulated intestinal mucosa immune response [27]. TGP-induced intestinal microbiota and functional changes were accompanied by STAT3 signaling pathway mediated Th1, Th17 and Tfh cells differentiation inhibition [196,197]. Another study revealed that 14-day TGP administration significantly reduced levels of MMP-1 and MMP-3, which were considered as key enzymes in the pathologic destruction of cartilage in RA, thus suppressing joint destruction in CIA rats [198]. These results revealed the potential mechanism of TGP for alleviating inflammation, inhibiting the immune response, and preventing bone destruction.

Alternatively, another study found that treatment with clematis triterpenoid saponins (CTS) prepared from *Clematis mandshurica* Rupr significantly ameliorated arthritis symptoms by balancing the gram-positive and gram-negative bacteria ratio in the gastrointestinal tract [199]. A further study suggested that the metabolism change induced by CTS treatment was associated with ameliorated synovial tissue swelling and articular pathologic status [200]. More specifically, metabolic pathways modulated by CTS including inflammatory mediators (Cox-2, prostaglandins) production-related arachidonic acid metabolism and proteolytic enzymes and reactive oxygen species production-related glycerophospholipid catabolism, and SCFA production related amino acid metabolism [200].

An isoquinoline derivative alkaloid, berberine, has been used to treat gastrointestinal disorders for centuries in China. The oral administration of berberine for 4 weeks suppressed MAPK activation including extracellular signal-regulated kinase, p38, and the JNK plus NF-κB pathway, maintaining immune tolerance and preventing RA progression [201]. Gut microbiota dysbiosis-related activation of MAPK and NF-κB pathways is considered as potential mechanism leading to RA progression. Thus, modulating MAPK and NF-κB through berberine-induced *Blautia*, *Butyricicoccus*, and *Parabacteroides* expansion and reduced abundance of *Prevotella*, *Paraprevotella*, and *Coprococcus* may partially explain the anti-RA efficacy of berberine [202]. The influence of berberine on the CIA rat intestine also includes inhibiting the expression of inducible nitric oxide synthase, a M1 macrophage marker that contributes to nitrate production, rebalancing M1 and M2 macrophage populations by inhibiting M1 macrophage polarization [203]. The modulatory effect of berberine on macrophages, a group of important innate immune cells with properties of phagocytosis, antigen presentation and cytokine production, may be the underlying mechanisms contributing to berberine-mediated anti-inflammation function. The influence of

berberine treatment on macrophages was characterized by reduced expressions of pro-inflammation cytokines (TNF-α, IL-1β, IL-6) and improved production of anti-inflammatory factors (transforming growth factor-β1, IL-10). Improved gut microbiota-mediated SCFA production might also contribute to inflammatory cytokine regulation after berberine treatment [202]. Moreover, altered SCFA production could also affect the T helper, Tfh and Treg cell differentiation resulting in the reduced incidence of arthritis, suggesting a potential role for berberine as a prophylactic supplement for RA [204,205].

The oral administration of the Chinese traditional herb *Atractylodes koreana* significantly ameliorated gut microbiota dysbiosis and an SCFA metabolic disorder, decreased levels of inflammatory factors, and suppressed synovial infiltration in RA rats [206]. A study in *Paederia scandens,* a traditional herbal medicine used to treat RA, exerted therapeutic effects in RA mice by reducing the abundance of inflammatory-related bacteria in the gut and thus suppressing the expressions of pro-inflammatory cytokines and mediators (TNF-α, IL-1β, IL-6, IL-7, and IL-23) [207]. A metabolic signaling pathway and functional analysis of the gut microbiome of *Aralia echinocaulis* treated CIA mice reported a protective effect of *A. echinocaulis* derived total polysaccharide and glycoside involved alteration of multiple gut microbiota functions [208].

These studies suggest that natural compound mediated inflammatory response inhibition and arthritis improved by regulating the composition and metabolic function of the gut microbiota.

4.6. Autophagy regulation

Autophagy is crucial for maintaining IEC mediated intestinal mucosal homeostasis and modulating gut microbiota homeostasis. Impaired IEC autophagy has been proposed as a crucial event in RA. Therefore, regulating IECs autophagy may help improve intestinal barrier function and gut microbiota composition for the treatment of pre-clinical RA.

An early study by Haq et al. revealed the manipulation of gut microbiota with autophagy regulation as a plausible strategy to improve gut inflammation. Transformation of the microbiota from mice with IEC-specific Atg7 knock out directly increased the abundance of *Dubosiella* and *Turicibacter* at the genera level, followed by increased severity of dextran sodium sulfate-induced colitis. In contrast, improved autophagy, by which gut bacterial composition might be skewed toward homeostasis, prevents intestinal inflammation [209]. Some preliminary studies assessed the protective mechanisms conferred by autophagy inducers using an IBD mouse model. Supplementation of the physiological autophagy inducer spermidine promoted the expansion of *Firmicutes* and induced potential alterations to gut microbiota function mainly involving an amino acid, nucleotide, and lipid metabolism showing beneficial effects of improving the expression of the tight junction related genes (Tjp1, Cldn7, Cldn1, and Tjp3) [210]. Also, the administration of a natural autophagy inducer, galangin, protected mice from colon mucosal inflammation by inducing the expansion of *Lactobacillus* spp. with promoted SCFA production, especially acetic and butyric acids [211].

Mechanistically, improved autophagy may prevent intestinal inflammation in pre-clinical RA individuals through restoring the gut microbiome composition, inducing the synthesis of anti-inflammatory metabolites, and improving intestinal barrier function. Additionally, specific changes to the microbiota composition including expansion of *Bifidobacterium dentium* and Verrucomicrobia*,* Akkermansia may improve autophagy through a TLR4-mTOR dependent pathway that subsequently suppress inflammation by enhancing the intestinal mucus layer and goblet cell function and inhibiting NF-κB, oxidative stress, and cytokine releases [212,213]. Hence, combination administration with probiotics and some individual species that specifically regulate IEC autophagy might be a promising strategy for suppressing RA progression from the early stage. However, a recent study reported that enhanced intestinal autophagy could induce polarization of M1 macrophages by modulating the gut microbiota and inducing Paneth cell metaplasia, indicating that the overactivation of autophagy aggravates IBD in individuals with high psychosocial stress [214].

Given the sophisticated downstream pathway of IEC autophagy and complicated interaction mechanisms between autophagy and the gut microbiome, considerably more work will be required to assess the benefits and risks of IEC autophagy regulation as an intervention for pre-clinical RA. In addition, improving autophagy inducer screenings should be prioritized to provide ideal anti-inflammatory effects and prevent RA progression with minimal risk of inducing IBD.

4.7. MiRNAs

Recent studies noted that miRNAs may be involved in the development of early RA [215–218]. Multiple miRNAs (including miRNA-449, miRNA-27b-3p, miRNA-495, miRNA- 34a-3p, miRNA-340–5p and miRNA-17–5p) reportedly suppress RA synovial fibroblast proliferation by targeting the STAT3/β-catenin pathway, suppressing cell division cycle protein expression, and inhibiting histone deacetylase 1 production [219–225]. However, the study of miRNA intervention in the treatment of pre-clinical RA is still in its infancy, but it can learn from other dysbacteriosis-associated diseases and predict future regimens in the treatment of pre-clinical RA. Through exploring the efficacy of exogenous miRNA treatment in IBD, studies reported that miRNA treatment offers a protective effect for ameliorating intestinal inflammation through modulating the gut microbiota composition and intestinal immune response [226,227].

Distinctive microbial features were observed in an experimental colitis model administered with mesenchymal stem cell derived miRNA treatment. An alpha diversity analysis reported increased indexes of observed operational taxonomic units, Chao1, and Abundance-based Coverage Estimator after miRNA-181a treatment [226]. At the genus level, miRNA treatment restored the abundance of *Enterorhabdus*, *Lactobacillus* and *Akkermansia* and decreased *Bacteroides* counts [227, 228]. As for the relative abundance of species at the family level, miRNA-181a treatment promoted growth of Lactobacillaceae and Bacteroidales S24-7 but inhibited the growth of Clostridiaceae [226]. In addition, the administration of miRNAs increased the percentage of IL-10^{+}Foxp3^{+} Treg and reduced that of Th17 cells in the intestinal mucosa, with inhibited pro-inflammatory cytokine production (including IL-1β, IL-6, IL-2, IL-18, IL-17, IL-6, and TNF-α) [226,227, 229]. More interestingly, an increased abundance of *Enterococcus*, *Turicibacter*, *Helicobacter*, *Desulfovibrionaceae*, *unclassified Desulfovibrionaceae*, and *Mogibacteriaceae* is correlated with greater expression of proinflammatory cytokines and activation of immunoinflammatory pathways [227]. These findings revealed a potential role of miRNAs in regulating intestinal immune homeostasis through modulating gut microbiota composition.

On the other hand, Teng and colleagues hypothesized that miRNA may contribute to an anti-inflammatory environment and improve gut barrier function through direct binding of the *L. rhamnosus* monooxygenase ycnE gene, which may induce the production of IL-22, a critical regulator that maintains intestinal homeostasis through promoting intestinal epithelial regeneration, inducing pathogen clearing chemokine and cytokine expression, and promoting goblet cell proliferation [217–220]. Their hypothesis was confirmed by the down-regulation of ycnE after ginger derived mdo-miR7267–3p treatment and the subsequent inhibition of indole-3 acetamide (I3AM), an inhibitor of the indole-3-carboxaldehyde (I3CA) precursor, and thus result in profound I3CA production. Comparably, IL-22 production remains constant in AhR knock-out mice treated with I3AM, suggesting the critical role of AhR in I3AM induced inhibition of *L. rhamnosus* I3CA mediated IL-22 expression [228]. Thus, miRNAs might be a valuable tool for maintaining intestinal barrier function and immune homeostasis through regulating metabolites of specific intestinal bacteria.

In addition, miRNAs may enhance gut barrier function by improving its structural integrity. The overexpression of miRNA-602 markedly alleviated IBD in a mouse model with an improved diarrhea score and promoted the expression of intestinal tissue integrity associated genes (*ZO-1*, *MUC2* and *MUC3*) [229]. Restored expression of tight junction proteins claudin-1 and ZO-1 were also reported in mice with induced colon injury that received mesenchymal stem cell-derived miRNA-181a [226]. Downregulated transcriptional and protein expressions of *L. rhamnosus* pilus-specific protein SpaC were reported after ginger-derived miRNAs exposure, which significantly reduced *L. rhamnosus* accumulation in the gut mucosa, implicating that an interaction between that miRNAs and specific gut bacteria may improve resistance to pathogen colonization [228].

Collectively, these results indicated that manipulation of the gut microbiota with miRNAs might be a plausible strategy for improving the stability of the microbial symbiont community, relieving intestinal inflammation, and enhancing the intestinal barrier function of pre-clinical RA individuals. Future clinical studies are needed to evaluate the efficacy and safety of miRNA intervention and provide new insight as well as more evidence of the efficacy of miRNA therapy.

4.8. Vitamin D

Vitamin D is a steroid hormone that is largely known as a critical factor responsible for regulating calcium and phosphorus homeostasis. Extensive studies over the past decade revealed other biological effects of vitamin D involving antiproliferative, antibacterial and anti-inflammatory properties [230]. Vitamin D deficiency has long been recognized as a risk factor for many autoimmune diseases. Although the mechanism of vitamin D deficiency in promoting autoimmune diseases progression remains unclear, recent studies exploring vitamin D supplementation and changes in the autoimmune disease microbiome propose a link between vitamin D supplementation and microbiome composition [231].

In a study of vitamin D supplementation for colitis treatment, *Megasphaera*, *Lactobacillus* and *Enterobacteriaceae* enrichment occurred in the vitamin D intervention group with significantly improved disease activity [232,233]. In healthy individuals, serum vitamin D metabolite levels were positively associated with improved alpha diversity and abundant butyrate producing bacteria [234]. Similarly, the gut microbiota in postmenopausal women with low serum vitamin D levels exhibited significantly lower alpha diversity with decreased relative abundances at the family level including *Christensenellaceae*, *Eggerthellaceae*, *Defluviitaleaceae*, and *Izimaplasmatales*. In addition, vitamin D deficiency induced alteration of the microbiota subsequently reduced the abundance of several metabolites including N-acetyl-L-glutamate, glycocholic acid, and trimethylamine N-oxide [235]. On the other hand, vitamin D can strengthen the epithelial barrier by upregulating of tight junction related proteins ZO-1, occludin, claudin-1, and claudin-15 [236–238].

Further studies proposed that reduced autophagy serves as a potential mechanism mediating vitamin D deficiency and impaired intestinal barrier function. Through establishing intestinal vitamin D receptor knockout model, Wu et al. reported that intestinal epithelial vitamin D deficiency could inhibit the expression of *Atg16L1*, an autophagy gene in ubiquitin-like molecule LC3 lipidation to promote autophagosome formation [239]. Meanwhile, studies confirmed that downregulated *Atg16L1* expression led to abnormalities Paneth cells, which are essential for intestinal antimicrobial peptide production and maintaining homeostasis at the intestinal-microbial interface [240–242]. The impaired Paneth cell function significantly changed the bacterial abundance, demonstrating increased *E. coli* and *Bacteroides* abundance and decreased butyrate-producing bacteria abundance [241]. Being another potential mechanism linking vitamin D with autophagy regulation, the promotion of apoptotic cascades of initiators-cleaved caspase-3 and apoptosis *Bax* genes in vitamin D deficiency intestinal epithelial organoids contributed to autophagy reduction by decreasing Beclin-1 expression [243]. The expression of *Atg16L1* in *Salmonella*-infected IECs was upregulated with vitamin D supplementation and the subsequent LC3II formation was accompanied by inhibited IL-1β expression [244]. Therefore, the above results suggest that vitamin D supplementation may inhibit early RA progression by activating autophagy signaling pathways, rebalancing gut microbiota composition, and improving intestinal barrier function.

However, excessive vitamin D intake could aggravate intestinal microbiota dysbiosis with a worsened colitis phenotype [245]. Thus, RA models might help determine the optimal dose of vitamin D and answer the key question of whether its supplementation can ameliorate the gut microbiota dysbiosis and impaired intestinal function in RA.

5. Future perspectives

Gut microbiota dysbiosis occurs in the RA pre-clinical stage and is closely related to the onset of arthritis. Here we identified several pathways including molecular mimicry, microbiome-derived metabolites, impaired intestinal barrier function, microbiome-induced intestinal immune response, IEC autophagy and miRNA expression that link microbiota dysbiosis with RA progression.

Traditional treatment for RA begins only after its onset with long-term use resulting in the accumulation of multiple drug adverse effects. Therefore, targeting intestinal bacteria in the pre-clinal stage may be a breakthrough. Dietary and prebiotic interventions such as a fiber-rich diet, fish oil supplementation, and inulin intake may modulate microbiome dysbiosis and rebalance the immune system in pre-clinal RA individuals, but long-range research in pre-clinical individuals is needed to confirm its ability to prevent arthritis development. Oral antibiotic administration can ameliorate arthritis, but prophylactic antibiotic treatment may lead to intestinal microbiota dysbiosis and induce RA development. Similarly, the ability of FMT to restore intestinal bacterial balance and intestinal barrier function was confirmed in a CIA model, but the use of FMT before arthritis onset may be questionable due to the risks of infectious event and gut microbiota disruption.

We also propose new insights into the regulation of gut microbiota in pre-clinical RA, including probiotics, natural compounds, autophagy regulation, miRNA treatment and vitamin D therapy. Firstly, natural compounds, that are commonly used in RA, can ameliorate intestinal dysbacteriosis with reliable safety. Second, regulation of the gut microbiota may enhance the efficacy of natural compounds in the intervention of RA progression and modulate immune cell polarization. Third, regulating IEC autophagy can restore gut microbiota composition and intestinal barrier function, thus interrupting RA progression in the pre-clinical phase, but further research is needed. The microbiota modulation property of miRNAs and vitamin D, suggests a preventive function in treating pre-clinical RA individuals. Finally, in previous studies, the probiotics-induced modulation of the gut microbiota has shown some ability to relieve RA symptoms. As animal studies report a potent preventive effect of probiotics in RA progression, probiotics-induced regulation of the gut microbiota in pre-clinical RA is a promising direction for future studies. Moreover, the combination of probiotics and dietary intervention has the potential to enhance the preventive effect of RA. Therefore, combination therapy of probiotics, dietary intervention, natural compounds, and vitamin D therapy in regulating the microbiota to prevent RA progression would be a fruitful area for further work.

Here, we summarized novel insights into the mechanisms of microbiota alteration in pre-clinical RA, which may improve our understanding of the regulatory mechanisms that cause immune system dysfunction in the pre-clinal RA stage. We compiled several promising therapeutic strategies that may eventually improve RA remission by preventing disease progression in the pre-clinical phase.

Authorship

LL, KZ, JC and **QX** wrote and conceptualized the manuscript. **JZ, BC, ZH, BY, JC** and **BW** revised the manuscript for important intellectual content. **QN** conceived the concept and design of the paper and contributed to the preparation of the Figure.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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