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Review

‘All disease begins in the gut’—the role of the intestinal microbiome in ankylosing spondylitis

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Abstract

Ankylosing spondylitis is a chronic, debilitating arthritis with a predilection for the axial skeleton. It has a strong genetic predisposition, but the precise pathogenetic mechanisms involved in its development have not yet been fully elucidated. This has implications both for early diagnosis and for effective management. Recently, alterations in the intestinal microbiome have been implicated in disease pathogenesis. In this review, we summarize studies assessing the intestinal microbiome in AS pathogenesis, in addition to synthesizing the literature exploring the postulated mechanisms by which it exerts it pathogenic potential. Finally, we review studies analysing manipulation of the microbiome as a potential therapeutic avenue in AS management.

Key words: ankylosing spondylitis, spondyloarthropathy, intestinal microbiome, intestinal microbiota, intestinal dysbiosis, pathogenesis

Key messages

- AS is a complex, debilitating arthropathy, whose pathogenesis is not yet fully elucidated.
- Advancements in analytical methods have enhanced understanding of the intestinal microbiome and its pathogenic potential in AS.
- The role of the intestinal microbiome in AS is likely to involve a complex interplay of genetic, immune-mediated and microbial metabolic dysfunction.

REVIEW

Introduction

Ankylosing Spondylitis (AS) is an insidiously progressive, chronic, immune-mediated arthritis, characterized by inflammation of the axial skeleton, with early involvement of the SI joints [1]. Its prevalence ranges from 0.2 to 1.6%, depending on the geographical location and population studied [2], with 90% of patients developing symptoms before 40 years of age [3].

AS is the prototype of a class of seronegative spondyloarthritides (SpA), which also include ReA, PsA, arthritis associated with IBD and undifferentiated spondyloarthritis [4]. The pathogenesis of AS has not yet been elucidated fully. HLA-B27, present in $\leq$90% of those with AS, is a major risk factor for the development of the disease [3]. However, despite extensive research, the precise pathogenic mechanism by which HLA-B27 is involved in AS development remains unclear, and genetic predisposition alone fails to explain AS pathogenesis adequately. This paucity of information concerning the causality of AS development has catalysed an expanding field of research analysing alternative pathogenic mechanisms and predisposing factors. One such area under investigation is the intestinal microbiota or, more precisely, intestinal dysbiosis. More than 2000 years ago, Hippocrates supposedly declared that ‘all disease begins in the gut’, and most certainly, in recent years there has been an explosion of scientific literature pioneering the intestinal microbiome as crucial to the pathogenesis of a number of systemic autoimmune and inflammatory disorders [5]. Interest in the pathogenic potential of the intestinal microbiota in AS stems from evidence that ~60% of those with AS have subclinical intestinal inflammation [6], with a further 4–16% developing clinically evident IBD [7], thus establishing

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involvement of the gastrointestinal tract in disease pathogenesis. Furthermore, the implication of a number of bacterial suspects, including *Salmonella*, *Shigella*, *Yersinia* and *Campylobacter*, in the initiation of ReA [8] provides further evidence of a link between the intestinal microbiota and SpA. In addition to this, *Klebsiella pneumoniae*, an intestinal bacterium with both commensal and pathogenic potential, has long been implicated as a precipitating factor for AS [9], further strengthening the association of the intestinal microbiota and AS pathogenesis.

In this review article, we aim to delineate the current understanding of the microbiome in AS disease pathogenesis, in addition to providing a summary of potential biological mechanisms by which it exerts its pathogenic potential. To conclude, with a greater understanding of the pathogenic role of the intestinal microbiome, we will explore methods by which the intestinal microbiome can be exploited for therapeutic benefit in AS management.

Methods

Relevant literature pertaining to the intestinal microbiome and AS was identified by keyword searches of Medline (via PubMed) and Embase (OVID) from inception until 1 February 2021. Keywords included: (ankylosing spondylitis); (axial spondyloarthritis); (spondyloarthropathy); (intestinal microbiome); (intestinal microbiota); (intestinal dysbiosis) and (pathogenesis). In addition, manual searching of reference lists from primary articles was performed. Articles published in English were included, and there were no restrictions on study type.

Review

The intestinal microbiome

The intestinal microbiota describes the highly diverse microbial flora, including commensal, symbiotic and pathogenic microorganisms, inhabiting our intestine, and the intestinal microbiome refers to their collective genome and gene products [10].

The microbiota is predominantly composed of bacteria, with Frimicutes and Bacteroidetes representing the two most abundant phyla in healthy adults [11]. An increasing body of evidence has confirmed that the intestinal microbiome is essential for health, exerting pleiotropic roles in immune system modulation, nutrition and metabolism [12]. Owing to these essential functions, the intestinal microbiota has been coined the 'microbial organ' [13]. Intestinal dysbiosis is a consequence of altered diversity, composition or function of the intestinal microbiota [14]. Such dysregulation of the intestinal microbiota has been implicated in the initiation and perpetuation of a myriad of autoimmune and inflammatory diseases, including the spondyloarthritides [5].

Analysis of the intestinal microbiome

The analysis of the intestinal microbiome has undergone a significant paradigm shift in recent decades and, correspondingly, our understanding of the microbiome and its role in health and disease has increased. In the 1970s, Carl Woese analysed the 16S ribosomal RNA (16S rRNA) genes of prokaryotes and successfully elucidated microbial phylogeny, creating the field of molecular phylogenetics and later catalysing the concept of metagenomics [15]. Before the work of Woese, the study of the microbiome was significantly limited, given the inability of $>$70% of bacteria to be cultured readily [11]. The 16S rRNA genes have highly conserved regions common to most bacteria, interspersed with hypervariable regions unique to individual bacterial taxa [16]. 16S rRNA gene sequencing uses primers that target the highly conserved sequences within the hypervariable regions, enabling PCR amplification and subsequent sequencing, taxonomic assignment and community comparisons of bacterial species [17]. After sequencing, highly similar sequences are categorized into a single group, referred to as operational taxonomic units, which are then either compared with existing reference sequences, enabling taxonomic assignment, or compared based on sequence similarity, referred to as '*de novo* operational taxonomic unit clustering' [18]. The latter, although sometimes used for taxonomic assignment, is more frequently used in studies of community diversity. The 16S rRNA sequencing approach is the most commonly used method for microbiome analysis, owing to its relatively low cost and large body of archived reference data, thus supporting large-scale microbiome analyses. However, it has a number of shortcomings that must be considered. Taxonomic classification is reliant on the quality of the pre-existing reference database. This precludes characterization of previously unknown microbiota, in addition to the increased potential for inaccurate taxonomic classification. There is a further risk of inaccurate taxonomic classification owing to chimera formation or the intrinsic error rate of sequencing [19]. However, undoubtedly, one of the biggest limitations of this method is the inability of operational taxonomic units to allow for accurate identification at the species level [20].

Metagenomic sequencing, an alternative method of microbiome analysis, captures not only fragments of genes, but focuses on the entire genome [21]. One such approach is that of high-throughput whole-genome shotgun sequencing (WGS), which uncovers the complete genetic information of microbes, providing specific taxonomic information down to the species level, in addition to describing functional profiling and enzymatic capabilities [22]. However, WGS is expensive and time consuming, requiring extensive data analysis [23].

Where metagenomics has the potential to describe the potential functional capabilities of the intestinal microbiome, methodology is expanding to analyse the real functional activity of a chosen intestinal microbiome. Metatranscriptomic methods analyse the RNA transcribed by the microbiota [24], and metabolomic

and metaproteomic approaches describe the metabolites and proteins, respectively, present in the microbiome [25, 26]. These evolving areas in microbiome analysis are providing further insight into the relationship of the gut microbiome with the host, and although somewhat in its infancy, the multiomic approach is providing increased understanding of the gut–host symbiotic interaction central to health and to disease states.

Another consideration in microbiome analysis concerns the type of sample analysed. The majority of studies examining the intestinal microbiota analyse faecal samples, given their ease of obtainment *vs* mucosal samples from intestinal biopsies. However, using faecal samples alone fails to account for the spatial heterogeneity of distinct microbial habitats along the intestinal tract [27]. In particular, ileal inflammation is common in AS, and the ileal microbiome analysed using mucosal samples obtained at biopsy differs significantly from the microbial profile of faecal samples [28]. This is an important consideration when planning a study examining the intestinal microbiota in those with AS, especially to ensure reproducibility and authenticity of findings. Future studies examining intestinal microhabitats are essential.

Evidence of altered intestinal microbiota in AS

To date, no distinct AS microbiome signature has been characterized. However, through the advancements in microbiome analysis outlined above, it has been demonstrated that the intestinal microbiota composition of those with AS is altered *vs* healthy controls. There is variation in the nature of this intestinal microbial dysbiosis between studies, dependent on the study population, mode of microbiome analysis and type of sample analysed [29]. Table 1 highlights findings of human studies to date displaying altered intestinal bacterial composition in those with AS.

The intestinal microbiome and as pathogenesis

Although there is strong evidence to implicate changes in the intestinal microbiome in AS disease pathogenesis, the biological mechanisms by which it contributes to development of AS are still under investigation. Multiple postulations, including interplay with genetics, an altered intestinal epithelial and mucosal barrier with associated immune dysregulation, and altered bacterial function with dysregulation of microbial metabolites, have been proposed in an attempt to link the intestinal microbiome with the pathogenesis of AS.

HLA-B27 and the microbiome

The association of the major histocompatibility complex class I (MHC-1) HLA-B27 with AS is well established. Up to 90% of patients with AS possess the HLA-B27 allele [3]. However, only 5% of the healthy population who are positive carriers will develop AS [39]. Despite intensive research, the precise pathogenic role of HLA-B27 in AS remains unknown. However, several hypotheses have been interrogated, including arthritogenic peptide presentation, cell surface HLAB27 dimer recognition by NK receptors, and HLA-B27 misfolding with subsequent activation of pro-inflammatory endoplasmic reticulum stress [40].

Recently, an alternative hypothesis, linking the interaction between the HLA-B27 allele and the intestinal microbiota, coined ‘B-27 shaped flora’ [8], and the increased risk of development of AS was proposed. Evidence for this concept is supported by the demonstration of HLA-B27 transgenic rats raised under germ-free conditions failing to develop an arthritic phenotype [41]. Interestingly, once recolonized with commensal microbiota, >80% of the HLA-B27 transgenic rats developed both arthritis and colitis [42], establishing a causative association between the HLA-B27–microbiota interaction and disease penetrance. Furthermore, the substantial influence that HLA-B27 allele carriage alone has on alteration of the intestinal microbiome of healthy individuals without disease was recently described [43]. One may thus postulate that HLA-B27-dependent intestinal dysbiosis potentially occurs before AS phenotypic development, and not merely as a consequence of disease, thus playing a potential role in AS disease initiation. However, evidence to date is circumstantial, and the precise mechanistic role of HLA-B27 in the development of gut dysbiosis and subsequent development of AS requires further investigation.

Alteration of the intestinal epithelial barrier

The intestinal epithelium plays a key role in tissue homeostasis, functioning as an effective physical and biochemical barrier against both pathogenic and commensal microorganisms [44]. Tight junctions form connections between adjacent intestinal epithelial cells and tightly regulate the paracellular movement of water, ions and solutes across the epithelium [45]. Of note, in normal circumstances these tight junctions preclude the passage of bacteria, pathogens and toxins [45]. If the integrity of the tight junction is compromised, there is a corresponding increase in intestinal permeability, leading to a leaky gut phenomenon [46]. Such dysregulation of tight junctions and subsequent increased intestinal permeability has been demonstrated to be present in both AS patients and their first-degree relatives [47]. The disruption of intestinal epithelial integrity, dysbiosis and intestinal inflammation are likely to be interrelated closely, both temporally and spatially. Studies in HLA-B27 rat models suggest that both intestinal inflammation and impaired intestinal barrier function develop simultaneously [48]. It remains to be elucidated whether the integrity of the intestinal epithelium is compromised by intestinal inflammation or whether dysbiotic changes that precipitate epithelial breakdown (such as the intestinal bacterial upregulation of the tight junction modulator zonulin [49]) culminate in intestinal inflammation, or perhaps an interplay of both, are responsible for increased intestinal permeability.

It is postulated that once there is an increase in intestinal permeability, increased translocation of intestinal microbes to the systemic circulation is facilitated, with the subsequent priming of immunological reactions,

Table 1 Human studies depicting altered intestinal bacterial composition in AS compared with healthy controls

Study reference	Study population	Sample analysed	Analysis method	Increased microbiota abundance AS	Decreased microbiota abundance AS
[30]	Italy AS ($n = 9$) HC ($n = 9$)	Terminal Ileal biopsies	16S rRNA sequencing	Lachnospiraceae, Ruminococcaceae, Rikenellaceae, Porphyromonadaceae, Bacteroidaceae families	*Veillonellaceae*, *Prevotellaceae families*
[31]	China AS ($n = 97$) HC ($n = 114$)	Faecal samples	WGS	*Prevotella* spp., *Prevotella copri* *Bifidobacterium* spp., *Bifidobacterium bifidum*	*Bacteroides* spp.
[32]	Sweden AS ($n = 150$) HC ($n = 17$) UC ($n = 18$)	Faecal samples	16S rRNA sequencing	Proteobacteria, Enterobacteriaceae, Bacilli, *Streptococcus* spp., Actinobacteria	*Bacteroides*, *Lachnospiraceae*
[33]	China AS ($n = 41$) HC ($n = 19$)	Faecal samples	16S rRNA sequencing	*Prevotella*, *Dialister*, *Comamonas*, *Collinsella*, *Streptococcus*, *Alloprevotella*	*Eubacterium ruminantium*, *Ruminococcus gnavus*, *Lachnospira*, *Bacteroides*
[34]	China AS ($n = 103$) HC ($n = 104$)	Faecal samples	16S rRNA sequencing	Bacteroidetes, *Meg*, *Dorea*, *Blautia*	*Lachnospira*, *Ruminococcus*, *Clostridium*
[35]	China AS ($n = 22$) HC ($n = 16$)	Faecal samples	16S rRNA sequencing	Proteobacteria Enterobacteriaceae	Bacteroidetes
[36]	China AS ($n = 127$) HC ($n = 123$)	Faecal samples	WGS	*Clostridiales bacterium*, *Clostridiales bolteae*, *Clostridiales hathewayi*	*Bifidobacterium adolescentis*, *Coprococus comes*, Lachnospiraceae
[37]	China AS ($n = 29$) HC ($n = 37$)	Faecal samples	WGS	*Flavonifractor plautii*, *Oscillibacter*, *Parabacteroides distasonis*, *Bacteroides nordii*	
[38]	China AS ($n = 85$) HC ($n = 62$)	Faecal samples	WGS	*Bacteroides coprophilus*, *Parabacteroides distasonis*, *Eubacterium siraeum*, *Acidaminococcus fermentans*, *Prevotella copri*	*Enterococcus faecium*, *Eubacterium hallii*, *Coprococcus catus*, *Faecalibacterium prausnitzii*, *Coprococcus eutactus*

HC: healthy control; WGS: whole-genome metagenomic shotgun sequencing; UC: ulcerative colitis.

such as leucocyte recruitment and activation and release of soluble mediators [10, 50]. This theory is supported by the demonstrated high levels of lipopolysaccharide, a bacterial endotoxin, in the serum of those with AS [51]. Furthermore, translocated intestinal microorganisms might themselves precipitate an inflammatory cascade at extra-intestinal sites, as evident in endotoxin-induced uveitis [52].

Microbiota-induced immune dysregulation

There is an increasing body of evidence to suggest that alterations in the composition of the intestinal microbiota lead to dysregulation of the mucosal immune balance, with implications in AS pathogenesis. The IL-23/Th cell 17 (Th17) signalling axis has been demonstrated to be central in the pathogenesis of AS [53]. Th17 cells are a subtype of effector T cells proposed to play a crucial role in immunological defence against microbial infections [54]. The differentiation of Th17 cells is stimulated by a number of cytokines, with IL-23, in particular, implicated in driving its pathogenic potential through the expression of the key transcription factor retineic-acid-receptor-related orphan nuclear receptor gamma (ROR-γt) [55].

Th17 subsequently mediates its effects through the release of cytokines, including IL-17, of which IL-17A is the signature cytokine of the lineage [56]. The pathogenic potential of this type 17 response is evident in the DBA/1 murine model, which spontaneously develops AS-like enthesitis in normal circumstances, but upon neutralization of IL-17A it fails to do so [57].

Its role is further supported in human studies by the demonstration of increased levels of both IL-17 and IL-

23 in the serum of those with AS [58], in addition to increased numbers of circulating Th17 cells in those with AS [59]. Furthermore, the serum IL-17 concentrations of patients with AS have been shown to be correlated closely with their BASDAI score, further implicating IL-17 in disease activity, but also catalysing interest in its future potential as a disease biomarker [60]. Excitingly, IL-17A has also been exploited successfully as a therapeutic target, with the monoclonal antibody targeting IL-17A, secukinumab, demonstrating efficacy in the management of AS [61, 62].

One caveat to IL-17 antagonism is its associated increased risk of IBD exacerbation [63]. This highlights the differences in the immunopathogenic pathways driving these conditions, despite their high degree of co-familiality. Furthermore, surprisingly, two clinical trials [64, 65] evaluating IL-23 inhibition as a therapeutic target in AS failed to achieve their primary endpoints. This has stimulated further research into the precise pathogenic role of IL-23 in AS, particularly its varying degrees of significance at different sites of inflammation, including the axial skeleton and entheses [66].

Furthermore, what exactly precipitates IL-23/Th17 axis activation remains under investigation. Several immunological studies have postulated a role of the intestinal microbiota as a pathogenic link between IL-23/Th17 and AS development. One hypothesis suggests a link with intestinal villi Paneth cells, which specialize in the secretion of antimicrobial peptides, including defensins, lysozymes and cathelicidins.

These antimicrobial peptides are produced following exposure to pathogenic microorganisms (pathobionts) and have an essential role in modulating microbial composition and enteric pathogen invasion [67]. The antimicrobial peptide defensin has proved to be crucial to the defence against pathobionts and modulation of microbiota composition [68]. Defensins exert their effect via chemoattraction of macrophages, T lymphocytes and mast cells [68], in addition to the production of pro-inflammatory cytokines and chemokines [69]. Murine models deficient in alpha defensin, compared with those overexpressing the human Paneth cell alpha defensin 5 (DEFA-5), demonstrated significantly different microbiota composition [70]. Furthermore, those with overexpression of DEFA-5 displayed a reduction in colonization with segmented filamentous bacteria and, subsequently, reduced Th17 skewing [70]. Segmented filamentous bacteria are commensal bacteria that induce IL-17 [13]. Murine models lacking segmented filamentous bacteria have reduced levels of IL-17 and a subsequent increased susceptibility to infection, particularly with the pathobiont *Citrobacter* spp. [13]. However, restoration of segmented filamentous bacteria in these murine models is associated with a corresponding increase in the intestinal production of IL-17 and a heightened resistance to infection [71]. Interestingly, the levels of Paneth cell-derived DEFA-5 have been demonstrated to be increased in the terminal ileum of AS patients with acute intestinal inflammation [72]. Thus, one may postulate that activation of Paneth cells by intestinal pathobionts precipitates the activation and release of antimicrobial peptides, such as DEFA-5, with subsequent further alteration of the intestinal microbiota resulting in immune system activation, implication of the type 17 immune response and development of AS.

An alternative hypothesis involves the microbiota-induced activation of mucosa-associated invariant T (MAIT) cells. IL-17 produced by Paneth cells in the gut has been shown to activate MAIT cells in those with AS [73]. MAIT cells are innate-like lymphocytes with antibacterial potential, which, when activated, induce a rapid immunological response with the production of pro-inflammatory cytokines, including both IL-17 and TNF-α [74]. Notably, germ-free mice display an absence of MAIT cells [75], and riboflavin metabolites of bacteria and fungi have been shown to activate MAIT cells [49]. Furthermore, MAIT cells have been demonstrated to be elevated in the serum of AS patients [73]. Thus, one may postulate that MAIT cells, released secondary to dysbiosis, stimulate an aberrant immunological response and thus AS pathogenesis. However, evidence for both these theories remains circumstantial, and significant additional research is required to elucidate the precise role of the intestinal microbiome–immune axis in AS pathogenesis.

Microbiota metabolic function

Intestinal microbial metabolites are essential for host homeostasis, and intestinal dysbiosis is associated with significant alteration in the gut metabolic profile [76]. Such dysregulation of the gut metabolome has been implicated in the pathogenesis of AS, with HLA-B27 expression in murine models shown to alter the intestinal metabolic profile dramatically [77].

One such association is the finding of increased levels of sulphate-reducing bacteria in the faecal samples of those with AS [78]. Sulphate-reducing bacteria catalyse the reduction of inorganic sulphate to hydrogen sulphide [79], and increased levels of this metabolic product have been demonstrated in the intestinal lumen of those with AS [78]. Although a convincing association has been drawn, undeniably further studies are required to establish a causative relationship, in addition to identifying the precise pathogenic mechanism that these sulphate-reducing bacteria play in disease development.

Butyrate, a short-chain fatty acid (SCFA) intestinal metabolite, has also been implicated in AS pathogenesis [80]. It is normally found in high concentrations in the intestinal tract, where it is the end product of microbial fermentation of indigestible polysaccharides [81]. Short-chain fatty acids play a crucial role in defence against infection and inflammation via recruitment and maturation of various subsets of immune cells, in addition to mediating host–microbe interactions [82]. One method by which they exert their function is via the G-coupled protein receptors (GPRs), namely GPR-41 and GPR-43, thus inhibiting histone deacetylases, modulating host gene expression and inducing autophagy [83]. Interestingly, mouse models deficient in GPR-43 display

exacerbated or unresolving inflammation in models of colitis and arthritis, but demonstrate full resolution of their inflammatory response, with increased production of inflammatory mediators and immune cell recruitment, upon activation of GPR-43 by short-chain fatty acids [84]. Reduced levels of butyrate metabolism have recently been identified in AS gut microbiota [38] and, correspondingly, reduced levels of several species capable of producing short-chain fatty acids, such as *Eubacterium halli* and *Faecalibacterium prausnitzii*, were also demonstrated in the microbiota of AS patients [38]. This provides further evidence for the potential role of altered intestinal microbial metabolites in the pathogenesis of AS; however, further mechanistic studies are required.

Potential therapeutic strategies

The established link between the intestinal microbiome in AS disease pathogenesis has catalysed much research in the area of microbiota modulation as a therapeutic target in AS management.

Antimicrobials

Antimicrobial use is associated with a significantly altered taxonomic, genomic and functional ability of intestinal microbiota [85]. Exploitation of this common side effect, by the therapeutic application of antimicrobials to alter intestinal luminal microbiota composition, was thus explored.

SSZ is a DMARD composed of an antimicrobial, sulfapyridine, in addition to salicylate [86]. One of its mechanistic properties includes alteration of the intestinal microbial flora [87], with one study demonstrating decreased numbers of non-sporing anaerobes associated with its use [88]. In the management of AS, it is associated with improved early morning stiffness and reduced ESR, in addition to efficacy in the management of peripheral, but not axial disease [89, 90].

An alternative antimicrobial agent demonstrating promise in the management of AS is moxifloxacin, a fluoroquinolone antibiotic, with action on both Gram-positive and -negative bacteria [91]. Its use was shown to be associated with a marked sustained reduction in ESR and CRP in those with AS [91]. Furthermore, in mouse models, the antimicrobial rifaximin was also effective in halting AS progression and modulating intestinal microbial composition [92].

Undoubtedly, evidence of the application of these antimicrobial agents provides promise for the potential role of antimicrobials in targeting the intestinal microbiome in those with AS.

Diet, probiotics and prebiotics

Dietary intake has the potential to change the composition of intestinal microbiota, thus altering immune homeostasis [93]. Although still an area of evolving research, dietary modifications, such as a low-starch diet, are associated with symptomatic benefit in those with AS, in addition to reduction in the requirement for pharmacological therapy [94]. Intestinal microbiota rely on dietary starch for growth [94]; hence, by inference, reduction in starch intake might modulate the intestinal microbiome, with potential benefit in AS.

In recent years, there has been an explosion of research analysing the application of probiotics and prebiotics in a myriad of inflammatory and autoimmune disorders, including AS. Probiotics are combinations of beneficial live microorganisms, whereas prebiotics work to alter the structure and metabolism of beneficial commensals already present in the intestinal microbiota [95]. Both pro- and prebiotics strive to improve intestinal microbial health, strengthening the epithelial barrier and modulating immune responses [95]. Promising results were observed in HLA-B27 transgenic rat models, with *Lactobacillus rhamnosus* showing benefit in preventing colitis [96], in addition to prebiotic treatment demonstrating efficacy in reducing colitis [97]. However, human studies have been less successful, with two randomized trials demonstrating no significant difference between probiotic use *vs* placebo in those with SpA [98, 99]. Further studies are warranted, to identify the optimal pro-/prebiotic combination capable of modulating the intestinal microflora in those with AS.

Faecal microbiota transplantation

Faecal microbiota transplantation (FMT) involves the transfer of stool from a healthy donor, with a relatively stable intestinal microbiota composition, to the intestine of the recipient [100]. The proposed role of this technique is to restore a normal intestinal microbiome to the recipient, with subsequent modulation of immune homeostasis [100]. It has demonstrated therapeutic benefit in the management of refractory *Clostridium difficile* infections [101] and in IBD [102]. However, its application has yet to be elucidated in AS. Notably, FLORA (NCT03058900), an ongoing double-blind, placebo-controlled randomized control trial is evaluating the application of faecal microbiota transplantation in the treatment of peripheral PsA [103]. This should provide valuable information for the potential use of the technique in intestinal microbial manipulation in patients with SpA, including AS. Additionally, further research is required to characterize the safety of faecal microbiota transplantation fully, in addition to optimal methods of delivery [12]. Although in its infancy, faecal microbiota transplantation is an exciting potential therapeutic avenue in the management of AS associated intestinal microbiome dysregulation.

Conclusion

Despite the declaration by Hippocrates >2000 years ago that ‘all disease begins in the gut’, understanding the elaborate, highly intricate and dynamic relationship between the intestinal microbiota, health and disease pathogenesis is only in its infancy. The intestinal microbiome plays a crucial role in gut homeostasis and, as outlined

throughout, dysregulation of this has significant implications for immune system modulation and AS disease pathogenesis. Although alteration of the microbiota in those with AS is established, studies to date have, unfortunately, failed to identify a consistent uniform alteration. This could be accounted for by study heterogeneity, including the type of sample analysed, the method of analysis, the diversity in severity of AS, and the influence of pharmacological treatment, in addition to potential bias from external factors altering the microbiota, such as age, sex, ethnicity, diet and BMI. The significant advancements in analytical methods, in addition to accounting for biases such as those outlined above, could provide more reproducible data to establish a microbial signature unique to AS. Although we explored potential biological mechanisms by which the intestinal microbiome might participate in disease pathogenesis, the evidence remains circumstantial, and further studies are required to establish the precise pathogenic contribution of dysbiosis to the development of AS. It is an extremely exciting time in intestinal microbiome research, and the potential for exploitation of its dysregulation as a therapeutic target provides substantial impetus for further animal and human studies.

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Data availability statement

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