



The gut-joint axis in spondyloarthritis: immunological, microbial, and clinical insights

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Abstract

The strong genetic and clinical overlaps between spondyloarthritis (SpA) and inflammatory bowel disease (IBD) have placed much needed focus on the gut-joint axis of inflammation in SpA, leading to three key hypotheses that attempt to unravel this complex relationship. The *arthritogenic peptide hypothesis* and the *aberrant cellular trafficking hypothesis* have been put forth to rationalize the manner by which the innate and adaptive immune systems cooperate and converge during SpA pathogenesis. The *bacterial dysbiosis hypothesis* discusses how changes in the microbiome lead to architectural and immunological consequences in SpA. These theories are not mutually exclusive, but can provide an explanation as to why subclinical gut inflammation may sometimes precede joint inflammation in SpA patients, thereby implying a causal relationship. Such investigations will be important in informing therapeutic decisions which may be common to both SpA and IBD. However, these hypotheses can also offer insights for a coincident inflammatory relationship between the gut and the joint, particularly when assessing the immunological players involved. Insights from understanding how these systems might affect the gut and joint differently will be equally imperative to address where the therapeutic differences lie between the two diseases. Collectively, this knowledge has practical implications in predicting the likelihood of IBD development in SpA or presence of coincident SpA-IBD, uncovering novel therapeutic targets, and redesigning currently approved treatments. It is evident that a multidisciplinary approach between the rheumatology and gastroenterology fields cannot be ignored, when it comes to the care of SpA patients at risk of IBD or vice versa.

Keywords Gut-joint inflammation; · Innate immunity; · Adaptive immunity; · Mucosal immunity; · Microbiome; · Spondyloarthritis

Introduction

Spondyloarthritis (SpA) encompasses the family of inflammatory joint disorders that are typified by inflammation in the axial skeleton, impacting the spine, pelvis and thoracic cage, and peripheral joints, impacting the lower extremities [1]. This

inflammation is systemic as other organs, such as the skin or the eye, can be affected as well. Current classification criteria, according to the Assessment of SpondyloArthritis international Society (ASAS), divide this family into axial spondyloarthritis (axSpA), peripheral arthritis, bacterial gut infection-triggered SpA called reactive arthritis (ReA), some juvenile chronic arthritis, arthritis-associated inflammatory bowel disease (IBD), and some variations of psoriatic arthritis (PsA) [2]. AxSpA involves radiographic or non-radiographic sacroiliitis, with the former historically classified as ankylosing spondylitis (AS) [1]. HLA-B27, a major histocompatibility complex (MHC) class I molecule, confers the strongest genetic risk for AS and is positive in 80–90% of AS patients [1]. Clinically, there has been a well-documented overlap between gut and joint inflammation. In fact, as many as 60% of axSpA patients have evidence of subclinical gut inflammation, and 5–10% develop diagnosed inflammatory bowel disease (IBD) [3]. Numerous analyses, including genome-wide

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association studies (GWAS), have also confirmed the existence of a strong genetic overlap between SpA and IBD [4–7], with shared polymorphisms in *IL-23R*, *IL-12B*, *STAT3*, *PTGER4*, *IL-6R*, and *CARD9* [8, 9]. This association of joint symptoms and gut inflammation has led the gut-joint axis of inflammation becoming an emerging area of research in SpA [10]. Three key hypotheses have been proposed to decipher the links in the immune systems of the gut mucosa and the periphery: (i) the “arthritogenic peptide theory” that suggests HLA-B27-presented peptides might drive pathogenesis; (ii) recruitment of mucosal-derived cells to the joints, and (iii) gut bacteria as drivers of joint inflammation [11]. Much work is still needed to prove or disprove these hypotheses, which is why it is important to highlight recent evidence investigating the following themes: immunologic evidence for the gut and joint inflammatory axis, the microbial landscape in the immunopathogenesis of gut inflammation in SpA, and the clinical relevance of subclinical gut inflammation in SpA with insights for drug treatment.

Immunopathogenesis of the gut-joint axis of inflammation: innate and adaptive immunity

Several classical members of the innate and adaptive immune system (Fig. 1) have been implicated in SpA pathogenesis, such as dendritic cells (DCs), macrophages, and CD4+ and CD8+ T cells [12]. In accordance with the shared genetics between SpA and IBD, it is not surprising to observe that the IL-17 pathway and the recently discovered group 3 innate lymphoid cells (ILCs) also share important roles in intestinal and joint inflammation in both conditions [10, 13–16]. These genetic and immunological factors suggest a role for the gut in driving joint inflammation in SpA. Importantly, whether gut inflammation precedes joint inflammation during disease pathogenesis, or inflammation at both these sites manifest concurrently still remains to be resolved mechanistically [10]. Nonetheless, it appears that a convergence of coordinated innate and adaptive immunological processes in the gut and joint occurs during the disease progression of SpA.

Innate immune cells

Cells that comprise the innate immune system confer important first-line protection against pathogens, specifically at barrier surfaces. Perturbations in innate responses have been observed in SpA animal models and SpA patient blood, synovial fluid (SF), and tissues. For instance, synovial tissues obtained from the periphery in SpA are characterized by increased frequencies of CD163+ macrophages, neutrophils and mast cells [17, 18]. These infiltrating macrophages have been reported to be correlated with global disease activity [19], produce TNF α

and coincide with reduced CD69+ lymphocyte numbers, which suggests a decreased activation capacity [20]. This suggests a dual capacity of the CD163+ macrophages in promoting global inflammation and impairing T cell activation [20]. Moreover, the soluble form of CD163, sCD163, has been observed in the SpA synovial fluid (SF) [20]. Comparison of the immunopathology of synovial and intestinal tissues reveals important similarities. For instance, increased frequency of CD163+ macrophage is also found in the gut of SpA patients as well as the joints [21, 22]. Similarly, CD163+ macrophages are part of the cellular infiltrate in the colonic mucosa of Crohn’s disease (CD) patients [22, 23]. A more recent study has shown that the expansion of intestinal CD163+ macrophages was significantly correlated with IL-33 mRNA expression in chronically inflamed axSpA patients, but not in CD [24]. These observations suggest a disease-specific functionality of IL-33, which is an inducer of M2 (alternative/anti-inflammatory) macrophage polarization [25]. In other inflammatory conditions such as systemic lupus erythematosus (SLE) or multiple sclerosis (MS), sCD163 has been observed to be elevated in patient serum, compared to healthy controls (HC) [26, 27]. Additionally, sCD163 was shown to be a potential marker for macrophage activation in newly diagnosed MS patients [28]. Such results have led to the consideration of sCD163 as a biomarker for various autoimmune diseases; however, a recent study suggested that the diagnostic and prognostic potential for sCD163 remains weak in SpA [29]. The phenotypic and functional profiles of CD163+ macrophages in synovial or intestinal tissue from experimental SpA models still remain to be studied.

The shared genetic profile between SpA and IBD implicates a shared aberrant IL-23/IL-17 axis. Cells of the innate (neutrophils, mast cells, group 3 ILCs) and adaptive (lymphocytes) immune systems produce IL-17 or related cytokines such as IL-22 [13], when induced by IL-23 or IL-7 [10, 14, 30]. Such cells generally express the ROR γ T transcription factor and collectively promote these IL-17 producing type 3 immune responses in epithelial barrier surfaces which protect against extracellular bacteria and fungi. This occurs through the ability of IL-17 or IL-22 to recruit neutrophils and mononuclear phagocytes to tissues and promote antimicrobial peptide production by epithelial cells [13]. Other key roles played by type 3 immunity include epithelial proliferation and formation of tight junctions, which in turn promote maintenance of barrier surfaces [31–34]. However, experimental models have indicated that IL-17 in the gut has both protective as well as pathological roles [35, 36].

Neutrophils have been suggested to play a role in amplification of inflammation in SpA through the production of IL-17A. Support for this deduction has stemmed from research illustrating IL-17A+ neutrophils in psoriatic skin, PsA synovium and axSpA facet joints [37]. However, emerging consensus reports that that these neutrophils lack the

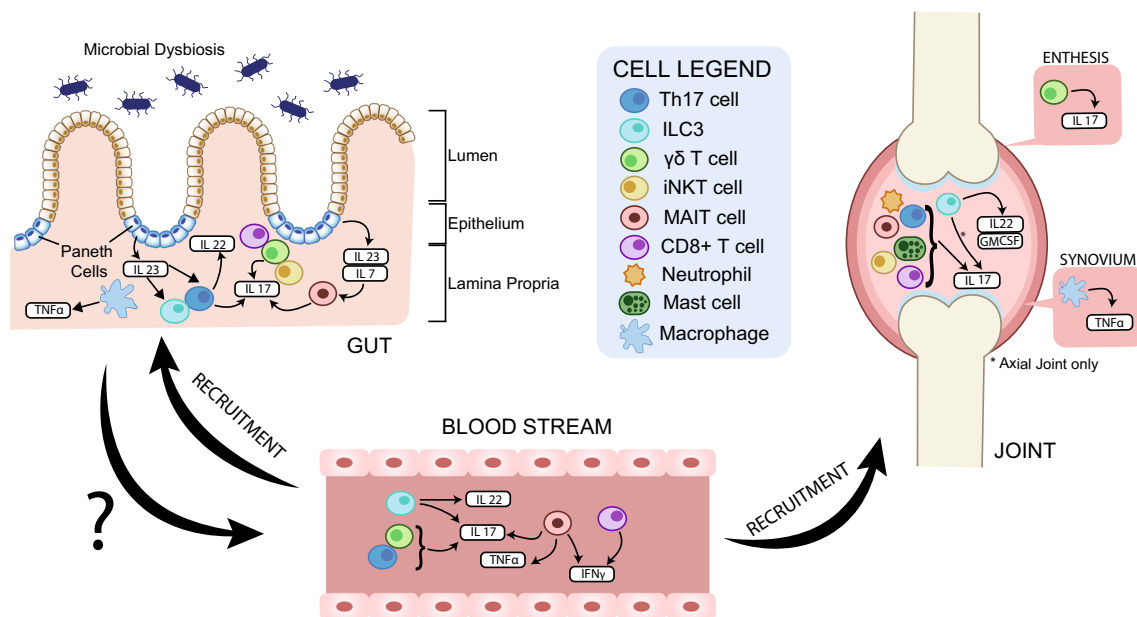


Fig. 1 Innate and adaptive immune cells implicated during the immunopathogenesis of SpA. In the intestine, a cascade of immunologic changes takes place as a result of subclinical inflammation. An elevated TNF α signature is promoted by the recruitment of macrophages. Activated Paneth cells have the propensity to produce IL-23 and IL-7 upon recognition of an altered microbiome. IL-23 aids in the differentiation of T_H17, ILC3 cells and MAIT cells, all of which promote the production of elevated amounts of IL-17 beyond homeostasis. IL-7 has also been recently shown to prime MAIT cells. $\gamma\delta$ T cells and iNKT cells are able to recognize microbial antigens and release IL-17. A role for CD8+ T cells has yet to be observed in the SpA gut, but some studies provide evidence of an IL-17 signature. Cells can be recruited to the intestine from the bloodstream but whether they undergo

systemic migration from the gut to the bloodstream and beyond remains to be resolved. Nevertheless, a combination of cytokine responses is initiated in the blood. T_H17 cells, $\gamma\delta$ T cells, ILC3 cells, and MAIT cells are primed to produce IL-17. T_H17 cells also produce IL-22, while MAIT cells contribute towards TNF α and IFN γ production as well. An IFN γ -producing CD8+ T cell profile is also observed. From the bloodstream, cells can be recruited to other inflammatory sites such as the axial/peripheral joints, where immunological perturbations can also occur. In the peripheral joints, neutrophils, mast cells, T_H17 cells, CD8+ T cells, MAIT cells, and iNKT cells all act towards the production of IL-17. ILC3 cells produce IL-22 and GM-CSF, and IL-17 is produced in the axial joint only. $\gamma\delta$ T cells promote enthesitis through elevated IL-17, and macrophages induce TNF α in the synovium

production of IL-17A at the mRNA or protein level, despite stimulation with various cytokines. On a similar note, even though IL-17A+ mast cells have been highlighted in SpA synovial tissue, it appears that these cells exogenously capture and release IL-17A, instead of synthesizing the cytokine. This has been a controversial topic since initial reports of *IL-17A*, *IL-17F*, *IL-23R*, and *RORc* knock-in reporter mice indicated mast cells and neutrophils did not express the promoter of interest. However, a newer study showed ROR- γ T and IL-17A expression in bone marrow neutrophils from knock-in reporter mice. This mechanistic concept extended to isolated human peripheral blood neutrophils which showed that stimulation using high IL-6 combined with IL-23 was able to induce IL-17A production dependent on RORC. Another study has come to the opposite conclusion from the observation that neither IL-17A mRNA transcripts nor protein was detected from human peripheral blood neutrophils. Mast cell production of IL-17A is equally controversial. One report suggested mast cells derived from human cord-blood had the ability to produce IL-17A in a RORC-dependent fashion. Yet another study revealed no IL-17A mRNA transcript in synovial tissue mast cells [38]. Furthermore, in humans, upon IL-17A blocking using secukinumab, IL-17A levels were higher

in joint-resident mast cells contributing to the notion that this cytokine is stored under normal tissue homeostasis and released during the inflammatory process [37, 39]. As such, tissue-resident mast cells have been described as IL-17A-loaded sentinel cells that serve to amplify tissue inflammation. In SpA-related conditions, total mast cells and IL-17A+ mast cells are observed in high frequencies in the psoriatic skin and IBD gut [39]. Further research is needed to specifically examine the neutrophilic and mast cell profile in SpA gut. Owing to their crucial function as first-line defences against various pathogens, a better understanding of the role of IL-17+ neutrophils and mast cells overall is mandated.

ILCs constitute a rare population of innate type 3 immune cells that lack typical T cell, B cell, myeloid cell, or granulocyte markers, resulting in their classification as “lineage-negative.” ILCs are enriched at skin, lung, and intestinal barrier surfaces, which enable them to respond to early immune challenges [13, 40]. Currently, ILCs are classified into three subsets termed as group 1, 2, or 3 ILCs. Group 3 ILCs (ILC3s) are characterized by the expression of ROR γ T transcription factor, IL-7R, IL-17, and IL-22, along with a number of TNF factors such as LT α 1 β 2 [13]. A prominent study characterized group 1, 2 and 3 ILCs in the peripheral blood, gut, SF and

bone marrow of AS patients. Here, ILC3s denoted as $\text{Lyn}^{\text{ROR}\gamma\text{T}}\text{Tbet}^{\text{+}}\text{NKp44}^{\text{+}}$, were found to be expanded in all these sites and secreted IL-17 and IL-22 [41]. Given the lack of ROR γ T expression, it is yet to be determined whether these cells are firmly classified as ILC3s and if defined as such, whether they should be considered exILC3s [40]. In PsA blood, elevated ILC3s were shown to possess an immature phenotype, lack of NKp44 expression, and only produced moderate levels of IL-17/IL-22. This could potentially imply that final maturation might occur directly at the inflammation site, presumably the joints [42]. Besides this, there are reports of an abundance of NKp44+CCR6+IL-17- [43] or GM-CSF-producing ILC3s [44] in SpA SF. Here the circulating NKp44+ILC3 cells were inversely associated with disease activity [43], which could have been as a result of the migration of circulating, immature ILC3s to target sites. Assessment of facet joints in AS through immunohistochemistry has revealed significant expression of IL-17 in myeloid cells [45]. Furthermore, axial joint enthesis housed tissue-resident ILC3 populations that secreted IL-17 as a result of IL-23 induction. This enthesal ILC3 profile was transcriptionally similar to that observed in SF [46]. A more recent study reported the expansion of IL-22- and GM-CSF- (but not IL-17A) expressing ILC3 in the inflamed SpA joint [47], which illustrates the heterogeneous profile of these cells. In fact, in IBD patients, the frequency of ILC3s is still debated, as some studies suggest both reduced as well as expanded numbers [48, 49]. There was even a study that reported higher levels of IL-17-producing ILC3s in enteropathic SpA blood, as compared to IBD patients and HCs. Furthermore, IL-22-producing NKp44+ ILCs were found to be predominant in the axSpA gut. This is in contrast to CD, where intestinal CD4+ T cells represent the main source of local IL-22 [42]. This points to the differences in the pathogenesis of IBD and SpA-associated intestinal inflammation and the complexity of the roles played by ILC3s. Taken together, it is evident that the precise role of ILC3s in SpA is yet to be determined. Future studies should take into account the heterogeneity and probable lineage plasticity of this subset.

Adaptive immune cells

Adaptive immune cells are likely responsible for driving chronicity in SpA and for contributing towards a major source of IL-17. Lymphocytes that elicit these type 3 immune responses include CD4+ ($\text{T}_{\text{H}}17$) and CD8+ ($\text{T}_{\text{C}}17$) cells, which have been implicated in SpA and IBD pathogenesis through clinical and experimental models. Animal studies using the curdlan-injected ZAP-70-mutated SKG mouse and HLA-B27 transgenic rat models have revealed the involvement of $\text{T}_{\text{H}}17$ and $\text{T}_{\text{C}}17$ cells in SpA pathogenesis [10]. In humans, $\text{T}_{\text{H}}17$ cells are reported to be elevated in SpA patient blood [50, 51], with a particular male sex-biased increase in axSpA [52]. Their

high levels are also correlated with disease activity in axSpA [37]. Additionally, $\text{T}_{\text{H}}17$ cells are found to be numerous in the SF, consistent with earlier research that demonstrated increased IL-17 in serum and SF from SpA patients, and also found enriched in skin inflammatory lesions in psoriasis [53]. There is also evidence of an enrichment of killer immunoglobulin receptor (KIR) KIR3DL2+ CD4+ $\text{T}_{\text{H}}17$ cells in axSpA blood and SF. This is interesting since KIR3DL2+ cells account for approximately 15% of CD4+ T cells in peripheral blood, yet contribute to about 70% of the increase in $\text{T}_{\text{H}}17$ cells in patients [54]. Outside of the circulation, $\text{T}_{\text{H}}17$ cells have been identified in tissues from axSpA facet joints [53], and reports suggest they may contribute to IL-17A-induced bone erosion [37]. Besides the skin and joints, $\text{T}_{\text{H}}17$ cells are prominent in the intestinal mucosa, where they contribute towards tissue homeostasis and protection against microorganisms [36]. These cells are also numerous in IBD gut inflammatory lesions [53], where there is an association between the number of intestinal $\text{T}_{\text{H}}17$ cells or the expressions of $\text{T}_{\text{H}}17$ -related cytokines with disease activity index, endoscopic and histological grading and CRP levels [55]. Interestingly, in the terminal ileum of axSpA patients, high concentrations of IL-23 and IL-22, but not IL-17 were found, which contrasts with the findings in serum of high IL-23 and IL-17 [35], indicating tissue and disease-specific roles for IL-17. In order to attempt to negatively regulate these increased $\text{T}_{\text{H}}17$ cells in SpA, miR-10b-5p and IL-10-producing B cells have been recently proposed. It has been proposed that this is because regulatory T (T_{reg}) cells are unsuccessful in suppressing autoinflammation, indicating a functional defect, despite their increased numbers in the gut, blood and SF [42]. It is now apparent that different $\text{T}_{\text{H}}17$ subtypes can emerge depending on the particular cytokine stimulus. Furthermore, the mucosal microenvironment is revealed to be physiologically important for the differentiation and regulation of $\text{T}_{\text{H}}17$ cells.

A mechanistic role for the cytotoxic CD8+ T cells is yet to be determined in SpA, even with HLA-B27 being such a strong risk factor for AS. Currently, illuminating the precise roles of $\text{T}_{\text{C}}17$ cells in SpA also remains an active area of research; however some important observations have been made. For instance, $\text{T}_{\text{C}}17$ cells are present in the SF of inflamed joints in axSpA and PsA patients, and their levels correlate with disease activity [56–58]. These cells are also found in IBD gut, PsA synovium and psoriasis skin lesions. In healthy tissues, tissue-resident memory T cells (T_{RM}) represent about 50–70% of the resident T cell pool. It appears that $\text{T}_{\text{C}}17$ cells may have hallmarks of T_{RM} cells in PsA SF [59]. Furthermore, unaffected/clinically resolved skin lesions from psoriasis patients feature these IL-17-producing CD8+ T_{RM} cells, which may be involved in recurrence of psoriasis at sites of prior resolution [37]. Ongoing efforts seek to not only resolve the role of T_{RM} cells in other SpA tissues, but also investigate the interplay between $\text{T}_{\text{C}}17$ and T_{RM} cells. An

in-depth review on the biology of CD8+ T cells in SpA has been provided in the *Recent advances on the role of cytotoxic T lymphocytes in the pathogenesis of spondyloarthritis* chapter of this issue.

Innate-like T cells such as mucosal-associated invariant T (MAIT) cells, $\gamma\delta$ T cells and invariant natural killer T (iNKT) cells have also been implicated in SpA pathogenesis through their ability to promote type 3 immune responses [37]. MAIT cells function right at the intersection of innate and adaptive immunity and the fact that they mature and reside in the gut epithelium and lamina propria makes them uniquely positioned to be part of the host-microbe interplay. This occurs through their ability to recognize non-peptide antigens, such as vitamin B₂ metabolites specific to *Salmonella enterica*, presented by MR1 which is a non-classical class I antigen-presenting molecule. Besides this T cell receptor (TCR)-dependent activation, MAIT cells are also able to activate in a TCR-independent fashion through direct or indirect induction of cytokines; the latter occurs subsequent to innate immune cell activation through their toll-like receptors (TLRs). Collectively, these signals allow MAIT cells to produce pro-inflammatory cytokines such as interferon-gamma (IFN γ) and IL-17A, which enable them to promote both T_{H1} and T_{H17}-type responses [60]. In the context of SpA-related diseases, IL-17-producing MAIT cells have been observed in the psoriatic blood and skin lesions [37]. In IBD, MAIT cells expressed higher activation markers, such as CD69, and they produced more IL-17 [61]. In axSpA, these cells are enriched in blood compared to HCs, where they produce IL-17A, together with a lower IFN γ production, upon IL-7 priming. Interestingly, this increased proportion of IL-17+ MAIT cells was restricted to male axSpA patients. Additionally, MAIT cells in the SF showed higher IL-17 levels than in blood. Furthermore, these observations were not reflected in RA, indicating disease-specific functions [30]. Results from this axSpA study suggest that TCR-independent mechanisms might be more crucial in driving disease than when MAIT cells are directly activated through their TCR [30, 60]. In another axSpA study, MAIT cell activation status positively correlated with patient disease activity measures [61]. Disentangling a role for MAIT cells has been possible through MR1-deficient mice, using collagen-induced arthritis (CIA) and collagen antibody-induced arthritis (CAIA), where they show effector activities. In particular, MR1 deficiency led to a reduction in arthritis, and adoptive transfer of MAIT cells to MR1-deficient mice exacerbated arthritis in CAIA. The role of MAIT cells in IBD mouse models is less clear, with a TNBS-induced colitis mouse model reporting milder disease upon MAIT cell adoptive transfer [61]. Taken together, MAIT cell residence and functionality highlight their particular importance in the gut-joint axis of inflammation.

Ever since their discovery, a link between $\gamma\delta$ T cells and SpA has been established. The ability and manner in which $\gamma\delta$

T cells are suggested to drive joint pathology is yet to be fully understood. Nonetheless, numerous murine models have illustrated that $\gamma\delta$ T cells are the main cell type producing IL-17, particularly in the context of non-autoimmune arthritis and play a key role in promoting joint inflammation. The contribution of these IL-17-producing $\gamma\delta$ T cells to the pathogenesis of IL-23-dependent SpA mouse models, such as HLA-B27 transgenic mice and rats or the curdlan-injected SKG mice, is yet to be explored [62]. Nevertheless, it was found that they were responsible for promoting enthesitis using an IL-23 minicircle overexpression mouse model [38], which is a model that revolves around the activation of tissue-resident $\gamma\delta$ T cells [10]. This murine work is supported by the observation in humans that $\gamma\delta$ T cells reside in the entheses, where they have the potential to produce IL-17A, which in turn drives enthesitis. Further, it is suggested that $\gamma\delta$ T cells may be involved in IL-23-independent production of IL-17A in spinal entheses [37, 63]. Contrastingly, $\gamma\delta$ T cells are rare in the inflamed synovial tissue [38], but may contribute to bone formation or erosion induced by IL-17A and RANKL respectively [37, 64]. In the context of peripheral blood, IL-23R+ IL-17-producing $\gamma\delta$ T cells are found enriched in SpA compared to RA and HCs [10, 37], suggesting disease specificity, and have been shown to produce IL-17A *ex vivo* [38]. There is also an increase in SF IL-17-producing $\gamma\delta$ T cells from ReA, undifferentiated SpA and juvenile idiopathic arthritis (JIA) [42]. Apart from IL-23, IL-9-driven expansion of IL-17-producing $\gamma\delta$ T cells in PsA SF has been recently observed [42]. The importance and impact of $\gamma\delta$ T cells towards gut pathology is clearer in comparison to the joint, owing to their IL-17-producing capacity. Intestinal IL-17-producing $\gamma\delta$ T cells are responsible for promoting tissue repair and maintenance of barrier integrity through regulation of the tight junction protein, occludin [62]. It is not surprising that there is a link between $\gamma\delta$ T cells and underlying gut inflammation in SpA, which is thought to be an IL-17 driven disease. Indeed, there has been an established body of literature that highlights a $\gamma\delta$ T cell expansion in the IBD gut as well as in the AS gut with concurrent colitis [65]. In animal studies however, there appears to be divergent roles for $\gamma\delta$ T cells, suggesting both a promotion or amelioration of IBD [42]. Collectively, these reports are suggestive of a $\gamma\delta$ T cell contribution to the IL-17 imbalance at inflamed sites in SpA. However, the manner in which $\gamma\delta$ T cells interact with the gut microbiome and the precise role of IL-17-producing $\gamma\delta$ T cells in SpA remains elusive.

iNKT cells are CD1d-restricted T cells which express a semi-invariant MHC class I-like TCR capable of recognizing only glycolipid antigens. The most well-known and potent antigen is α -galactosylceramide (α -GalCer), which is non-mammalian in nature; however, iNKT cells are also able to recognize microbe-derived and endogenous lipid ligands. iNKT cells are another cell type that act at the intersection of

innate and adaptive immune responses through their ability to rapidly secrete copious amounts of cytokines and chemokines subsequent to TCR dependent or independent activation. In particular, they produce IL-4 and IL-13, but a subset of iNKT cells are ROR γ T-expressing which enables them to produce IL-17. Upon thymic education, all iNKT subsets are known to populate the peripheral organs, expressing tissue-specificity. In fact, it has been reported, using germ-free mice, that further maturation occurs at mucosal surfaces, such as the lung and gut, and they may also further functionally differentiate under inflammatory conditions [61]. There has been conflicting evidence regarding their ability to modulate autoimmune diseases, with some showing iNKT cell activation being protective against joint inflammation, and some highlight disease exacerbation [61]. For instance, in the TNF-overexpressing (TNF Δ ARE) model for SpA-like gut and joint inflammation, the TNF-driven CD1d^{high} DCs activated iNKT cells lead to immunomodulatory cytokine production, thereby ameliorating arthritis and colitis. The frequency of these DCs was also enhanced in the SpA SF [66]. Coupled with results from an in vivo iNKT-dependent infectious disease model, this suggests that microbial antigens can be picked up by inflammatory DCs at intestinal draining sites and subsequently activate iNKT cells. Furthermore, this DC-iNKT crosstalk appears to be TNF-mediated [61]. Other studies have reported an increase in IL-17+ iNKT cells in SpA, with IL-17-expression upregulated in pathogenic subsets in the SF [37]. Similarly, contradictory evidence has been presented in the case of IBD. A dextran sodium sulfate-induced (DSS) colitis model revealed protective effects of iNKT activation by α -GalCer. This is in contrast to oxazolone-induced colitis that showed an exacerbation of inflammation. In humans, iNKT cell frequency was reduced in IBD blood vs HCs, with an enrichment in the intestinal lamina propria where they produced copious amounts of IL-13 [61]. It is evident that iNKT cells may be important in a dual capacity in gut and joint disease, but the nature of their diversity and related functionality in SpA pathogenesis warrants further investigation.

How immune cells converge and cooperate in SpA pathogenesis

There have been several mechanisms proposed that describe the manner by which some of the aforementioned cellular contributors in axSpA might act in a coordinated fashion to promote gut-joint pathology. The most compelling hypotheses include the *arthritogenic peptide hypothesis* and the *aberrant cellular trafficking hypothesis* (Fig. 2). Apart from these theories, the observation of stromal cells in gut and joints responding to pathologic levels of tumour necrosis factor (TNF) led to the consideration of an alternate mechanism of action, whereby coincident gut and joint pathologies occur. In fact, a TNF-dependent pathogenesis may be possible owing to

increased TNF transcripts at both sites in mice [67]. The most compelling murine model to support this theory is the TNF Δ ARE model, which leads to simultaneous occurrence of Crohn's-like IBD and articular disease combining peripheral synovitis and sacroiliitis. Indeed, through bone marrow grafting experiments, it appears that under chronic TNF overexposure, TNFR1 signaling in synovial fibroblasts and intestinal myofibroblasts is sufficient for the development of combined gut and joint pathologies [2].

The arthritogenic peptide hypothesis

The *arthritogenic peptide hypothesis* stems from the fact that the HLA-B27 association with SpA is one of the strongest that has been documented for any autoimmune disease. It revolves around the role of HLA-B27 specific MHC class I-restricted cytotoxic CD8+ T cells, commonly known as cytotoxic T lymphocytes (CTLs), in activating an immune response upon recognition of an arthritogenic peptide [11]. Specifically, after this recognition, CTLs could cross-react with structurally similar self-peptides (*molecular mimicry*). It is this cross-reactive CTL response that is proposed to cause a breakdown in self-tolerance and to initiate tissue damage [68]. An historical study reported that HLA-B27 may present self or bacterial arthritogenic peptides to CD8+ T cells in the context of axSpA or ReA SF [69]. It is now known that a number of HLA-B27 self-peptides are similar to pathogenic bacterial-derived peptides; however, it is still unclear whether these bacterial peptides are generated in vivo or are presented directly by HLA-B27 [68].

This theory has garnered support by the observation that SpA disease onset is often preceded by enteric bacterial infection. It is important to note however that the capacity for arthritogenic peptide presentation to directly link infection and autoimmunity still lacks rigorous proof. Nevertheless, the most compelling evidence for this hypothesis lies in the discovery of closely-related HLA-B27 allelic variants whose prevalence varies by population and either confer protection or susceptibility to SpA [68]. Thus, research has focussed on defining these HLA-B27 subtype-bound peptide repertoires that may shed insights into disease-associated allotypes. In fact, significant advances in the discovery of ~6000 novel endogenous peptides have been made, some which might be key to SpA onset [68]. However, in order to discover the arthritogenic peptides, it may be prudent to supplement traditional analyses with precise quantification of individual peptides throughout disease-associated HLA-B27 allotypes. It is equally imperative to understand that a coordinated contribution of factors, such as enteric bacterial infection, misfolding of HLA-B27 heavy chains, or formation of HLA-B27 homodimers, may be at play in affecting disease initiation and progression [68]. Certainly, the impact of these factors on peptide repertoire remains a key area of research.

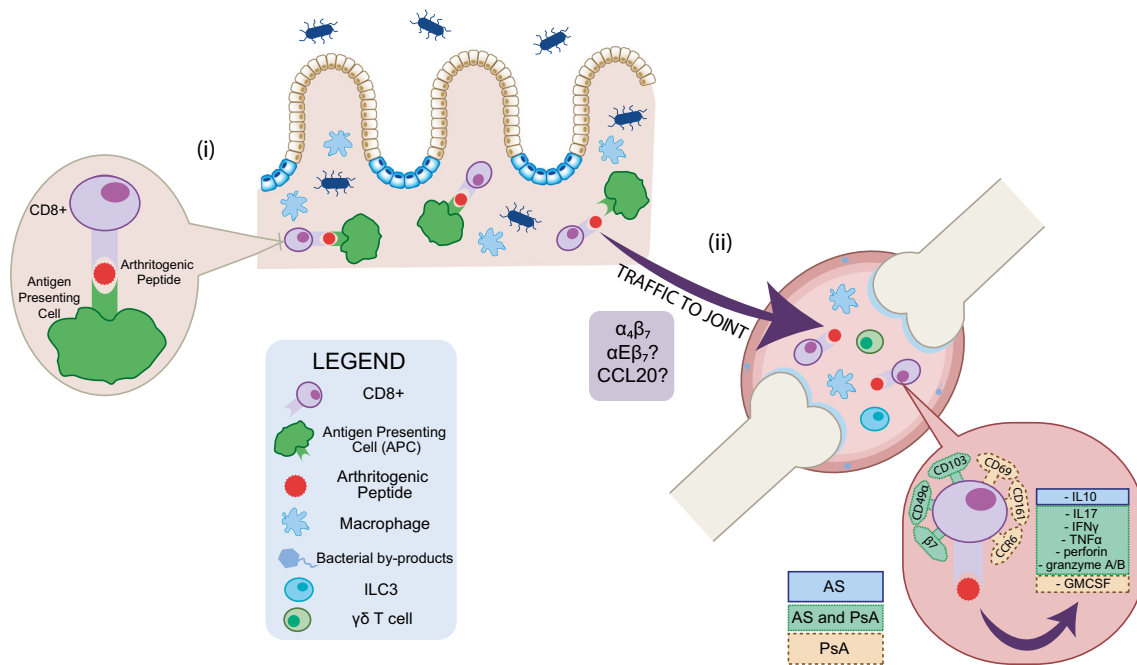


Fig. 2 Two hypotheses explaining the convergence of immune cells in SpA. (i) The *arthritogenic peptide hypothesis* is supported by the observation that enteric bacterial infection precedes SpA disease onset. It proposes that CD8⁺ T cells, which are HLA-B27 specific MHC class I-restricted, are able to active an immune response upon recognition of an arthritogenic peptide. Through molecular mimicry, homologous self-peptides could also induce activation of these cytotoxic CD8⁺ T cells. (ii) The *aberrant cellular trafficking hypothesis* centers around the concept that after antigen recognition, proliferation, and differentiation, immune cells have the propensity to upregulate expression of homing markers such as $\alpha_4\beta_7$ and $\alpha_E\beta_7$ that allow them to recirculate to other

A corollary to the *arthritogenic peptide hypothesis* is the examination of identical clonally expanded T cells at inflammatory sites. This stems from the fact that the conjugate partner of the HLA-B27/arthritogenic peptide is the $\alpha\beta$ TCR heterodimer. Moreover, the *arthritogenic peptide hypothesis* presupposes the T cell repertoire is restricted, given the idea that both the HLA-B27 and common putative peptides could potentially be shared amongst patients [70]. The observation of T cell clones, specific for enterobacterial antigens in the SF or synovial tissues of ReA patients, supports this notion [71, 72]. Added to that, a prominent study highlighted the existence of clonally expanded T cells in the colonic mucosa and synovium of a patient diagnosed with enterogenic SpA [73]. Similarly, HLA-B27-restricted CD8⁺ T cell clones have been identified in the SF of axSpA or ReA patients, which specifically recognize ReA-associated enterobacteria, *Y. enterocolitica* and *S. typhimurium* [69]. Direct comparison between axSpA and ReA revealed the existence of shared TCRBV (V region of the β chain) expansions between the two diseases [70]. Furthermore, a recent study confirmed results of previous research and demonstrated that dominant TCR β motifs in blood were shared among HLA-B27+ axSpA patients, suggesting the presence of a common antigen

inflammatory sites, such as the joints. Besides integrins, IL-17-induced CCL20 production also may be responsible for altered cellular migration. Tc17 cells in PsA SF were found to express various gut-homing receptors and T_{RM} features, and released IL-17A, IFN γ , TNF α , GM-CSF, perforin, granzyme A and granzyme B. In AS SF, CD8⁺ T cells were observed to also express gut-homing markers and produced IL-17A, IFN γ , TNF α , perforin, granzyme A and granzyme B, in addition to IL-10. Besides CD8⁺ T cells, $\gamma\delta$ T cells and ILC3 cells might also have the capacity for aberrant migration to the joint. Finally, altered trafficking of antigen-presenting cells, such as macrophages, might explain the translocation of bacterial products to the joints

[74]. It may yet be plausible that the SpA intestinal mucosa is the optimal site for identification of the arthritogenic peptides by HLA-B27-expressing antigen presenting cells (APCs), which in turn clonally expands the conjugate $\alpha\beta$ TCR heterodimer resulting in a restricted T cell repertoire. The potential “aberrant” migration of these intestinal clonal T cells to sites of inflammation and eventual cytotoxicity may tie the two hypotheses together. The joint tropism in such a scenario is yet to be defined. Certainly, the discovery of identical clonally expanded T cells in both the intestine and joint would support this notion [2].

The aberrant cellular trafficking hypothesis

The *aberrant cellular trafficking hypothesis* centers around the idea that naïve lymphocytes, upon egression from the bone marrow and thymus, recirculate between different lymphoid organs until a specific antigen is encountered. Subsequent to antigen recognition, lymphocytes proliferate and differentiate. Moreover, they upregulate the expression of adhesion molecules that allow them to migrate to other sites that may be similar to where antigen recognition first occurred [2]. Types of adhesion molecules that orchestrate lymphocyte exit from

the circulation and retention in tissue include integrins and chemokine receptors. Such trafficking molecules have been found shared between the gut and joint tissue. Early studies showed that intestinal immunoblasts from IBD patients have the capacity to bind to inflamed synovial vessels in vitro using distinct adhesion receptors [2]. In fact, there is also evidence that $\alpha_4\beta_7$, the prototypic gut homing integrin, and $\alpha_E\beta_7$, which is constitutively expressed by intraepithelial T cells, are expressed by activated T cells in the inflamed synovial tissue from early SpA patients [2]. Early studies have shown that small intestinal lymphocytes had the ability to adhere to synovial vessels using $\alpha_4\beta_7$, which binds weakly to VCAM-1 expressed on the endothelium [75]. Additionally, the primary $\alpha_4\beta_7$ ligand, MAdCAM-1, is also found expressed on various endothelial cells from the joints, gut, eyes, skin and liver [76]. However, with respect to β_7 integrin, it is entirely possible that its altered expression in the synovium is circumstantial, owing to its typical modulation during T cell activation or upregulation by certain cytokines like TGF- β [2].

It is suggested that type 3 immunity plays a role in this altered trafficking hypothesis. IL-17 has been shown to induce the production of CCL20 chemokine, which in turn may be responsible for aberrant trafficking of immune cells from the gut to the joint, involving antimicrobial peptides such as β -defensins and lipocalin2, as well as pro-inflammatory cytokines [35]. A couple of recent studies have reported on the presence of gut-homing integrins on T_C17 cells found in PsA SF [59], and on “InEx” cells in AS SF, which confirm the potential for shared tropism between the gut and joint [77]. Further details on these studies, especially about these cells exhibiting T_{RM}-like features, have been provided in the *Recent advances on the role of cytotoxic T lymphocytes in the pathogenesis of spondyloarthritis* chapter in this issue. Another study revealed that the integrins defined in InEx cells were most predominant in the human colon and to lesser degrees in the skin, lung, liver, tonsil, and spleen, lending support to preferential tissue distribution [78]. These PsA and AS SF reports also shed novel insights to our understanding of T_{RM} cells. In a new study, an “outside-in” concept has been proposed, which proposes a model whereby T_{RM} cells have the potential to exit their tissue of residence following activation. After tissue exit, they maintain their T_{RM} phenotype and can migrate back to tissue of origin or can transdifferentiate into central memory or effector memory phenotypes. This introduces the notion that these ex-T_{RM} cells are not terminally differentiated, but can migrate and retain some developmental plasticity, since intestinal T_{RM} cells were shown to populate the spleen following adoptive transfer. Whether these features extend to all T_{RM} cells or are specific to a subpopulation remains to be explored [79, 80]. Nonetheless, in the context of SpA, the “gut iteropathy concept” was proposed, which hypothesised that intestinal T cells activated by antigen migrate to joints and induce synovial inflammation

[81]. It might then be plausible to build on this concept and speculate that intestinal T_{RM} cells are activated by arthritogenic peptides, clonally expand, egress from the tissue and migrate to various inflammatory sites where they have the potential to exert effector functions. This is probably why T_C17 cells in the SpA SF might be ex-T_{RM} cells that have a mucosal origin. Further research to investigate this scenario needs to be conducted.

Models about circulating and/or tissue-resident $\gamma\delta$ T cells leaving their origins and migrating into inflamed sites, such as the joints, have been presented. In support of this, blood isopentenyl pyrophosphate-responsive V γ 9+V δ 2+ or $\alpha_4\beta_7$ + mucosal $\gamma\delta$ T cells have been suggested to preferentially accumulate in JIA joints during an acute flare or a low acute-phase response, respectively [42]. Intriguingly, the appearance of CD103+ $\alpha_4\beta_7^{\text{high}}$ $\gamma\delta$ T cells in the gut-draining mesenteric lymph nodes (MLNs) preceded colitis development, and their adoptive transfer was shown to enhance the accumulation of IL-17 or IFN γ -producing effector T cells and further exacerbate colonic inflammation [82]. Similarly, CCR2+V γ 6+ IL-17-producing $\gamma\delta$ T cells were shown to be recruited to joints by CCL2-inducing CD4+ T cells using *IL-1rm^{-/-}* mice [42]. This is interesting because V γ 6+ IL-17-producing $\gamma\delta$ T cells are typically predominant in the murine gut lamina propria [83]. Overall, migration of cells $\gamma\delta$ T cells alone does not fully explain the enthesal inflammation that occurs in previously described murine models. Nevertheless, it appears that this aberrant recruitment plays a small role, though research into this concept using IL-23-dependent SpA models is required.

ILCs have been proposed to utilize this aberrant migration in axSpA pathogenesis. The ILC3 cells found expanded in the AS gut, SF and bone marrow, also expressed the gut-homing $\alpha_4\beta_7$ marker, with its counter-receptor MAdCAM1 upregulated in the high endothelial venules of the gut and the bone marrow [41]. Photoconvertible murine models illustrate that CCR7-dependent ILC3 trafficking from the gut to the MLNs is possible, lending support to the notion that intestinal ILCs have the potential to migrate to distant sites [42]. These results provide evidence for the “shuttle” hypothesis, which proposes that certain pro-inflammatory cells differentiate in the gut and have the potential to “transfer disease” to extra-intestinal sites such as joints. This would be consistent with the established gut-joint axis in SpA [40, 84, 85]. However, further research is needed to investigate the potential migration of gut-derived ILC3s to the joints in SpA.

It is worthwhile to note the evidence of bacterial migration from the mucosa to joints in ReA [86] and the presence of bacterial fragments in the synovial tissue and fluid in ReA and other forms of SpA [67]. In fact, the synovium is often colonized by commensal bacteria in chronic inflammatory rheumatism [87]. As such, the trafficking of lymphocytes alone does not explain the presence of such bacterial components in

SpA SF or tissue during disease onset. It may well be that immune dysregulation within the joints or entheses is triggered by microbial antigens within immune cells [86]. Given that there is an increase of lymphoid follicles in the SpA ileum and colon [88], it was proposed that these antigens make their way from the gut to MLN to joints and might be responsible for the initial disease flare [86]. It makes sense that since SpA-associated intracellular pathogens found in the gut are primarily taken up by macrophages or other APCs, the trafficking of such mononuclear cells would also be a critical component in linking gut and joint inflammation. In support of this, human intestinal macrophages isolated from the lamina propria have been found to bind in vitro to vessels from the inflamed human synovial tissue, and this adhesion to the synovial endothelium was primarily P-selectin-dependent [2]. It may well be that there are different trafficking routes employed by infected leukocytes leaving the gut mucosa between axial and peripheral SpA. This may ultimately have a partial impact on the specific sacroiliac joints and spine involved in SpA.

The precise manner by which cellular or bacterial trafficking occurs has been a topic of controversy, as the mechanism could depend on the host or the triggering pathogen. Increased focus on lymphatic vessels as lymph node conduits and as a countercurrent system to the blood vasculature has led researchers to speculate on such alternative pathways [86]. It is suggested that gut bacteria have the potential to invade the host system via blood or lymphatic routes [89]. However, the few gut bacteria that have the capacity to enter the bloodstream in the gut via DCs are commensal in nature and end up getting captured in the liver vasculature. Eventually, some egress from the liver and traffic in the bloodstream within infected myeloid dendritic cells (mDCs). These peripheral blood mDCs are highly sensitized to chronic infections and are good candidates for the detection of bacterial components during SpA disease onset or flares. In the lymphatic system, MLNs provide a barrier mechanism that prevents the systemic spread of bacterial-triggered infection. The bacteria sampled from the gut lumen by DCs are commensal as well and these ‘infected’ DCs typically migrate towards the draining MLN, but no further. This occurs in the case of ReA, where DCs loaded with pathogenic bacteria are arrested at the MLN and exposed to antibacterial products from the commensal microbiota. Interestingly, in CD, a large number of non-commensal bacteria, such as *Escherichia*, *Shigella*, *Helicobacter*, and *Yersinia*, are commonly found in MLNs, indicating their ability to populate lymphatic vessels. The submucosal lymphoid tissue from patients suffering from chronic ileitis and arthritis has also been found to contain macrophages loaded with virulent *Yersinia enterocolitica*. As expected, a high influx of pathogens can overwhelm LNs, which promotes their egress into the thoracic duct and then systemically. This local obstruction of lymphatics is seen in CD. A similar phenomenon has been observed in ReA and some other forms of SpA,

where DCs carrying pathogens entering the bloodstream from gut through the lymphatic system and thoracic duct, thus becoming silent contributors to peripheral arthritis and enthesitis [86].

It is evident that immune responses tying the gut-joint axis of inflammation are more convoluted than previously thought, given their ability to utilize multiple mechanisms of pathogenesis. SpA animal models that consider experimental designs using blood as well as the lymphatic system could shed key insights into the cellular and bacterial translocation from gut to sacroiliac joints and spine.

Immunopathogenesis of gut inflammation in SpA

The gut microbiome in SpA has long been shown to important drivers of the disease course. This collection of microorganisms is considered to be in state of dysbiosis, since their composition deviates from normal, a phenomenon seen in RA and IBD as well. It is unclear whether dysbiosis arises as a consequence of the inflammatory process, or it helps mediate the involvement of the epithelium during the pathogenesis [10]. Nevertheless, the gut microbiota has been associated with profound alterations of both the gut-vascular barrier (GVB) and the gut-epithelial barrier, and also noted to be responsible for immune changes in SpA. This has led to the *bacterial dysbiosis hypothesis* [11] (Fig. 3).

The microbiome in SpA

Studies examining the microbial composition in SpA have primarily used 16S ribosomal RNA techniques. This has led to the observation that the AS microbiome is very different than that of HCs. For instance, in a small study, AS terminal ileal biopsies were found to be comprised of higher abundance of *Bacteroidaceae*, *Lachnospiraceae*, *Porphyromonadaceae*, *Rikenellaceae* and *Ruminococcaceae*, with lowered *Prevotellaceae* and *Veillonellaceae*. The healthy samples were dominated by *Actinomycetaceae*, *Gemellaceae*, and *Streptococcaceae* [90]. A more recent study using fecal metagenomic shotgun sequencing revealed an enrichment of the following species in 85 untreated AS patients compared to 62 controls: *Bacteroides coprophilus*, *Parabacteroides distasonis*, *Eubacterium siraeum*, *Acidaminococcus fermentans*, and *Prevotella copri* [91]. Yet another recent study employing shotgun metagenomics using 127 AS patients compared with 123 controls also highlighted an altered stool microbiome, with elevated *Clostridiales bacterium 17 47FAA*, *Clostridium boltae*, and *Clostridium hathewayi*. Interestingly, in this study, *Prevotella copri* was reduced in AS vs controls, while *Dialister invisus* was enriched in AS only in the discovery cohort [92]. In fact, in a different study, it

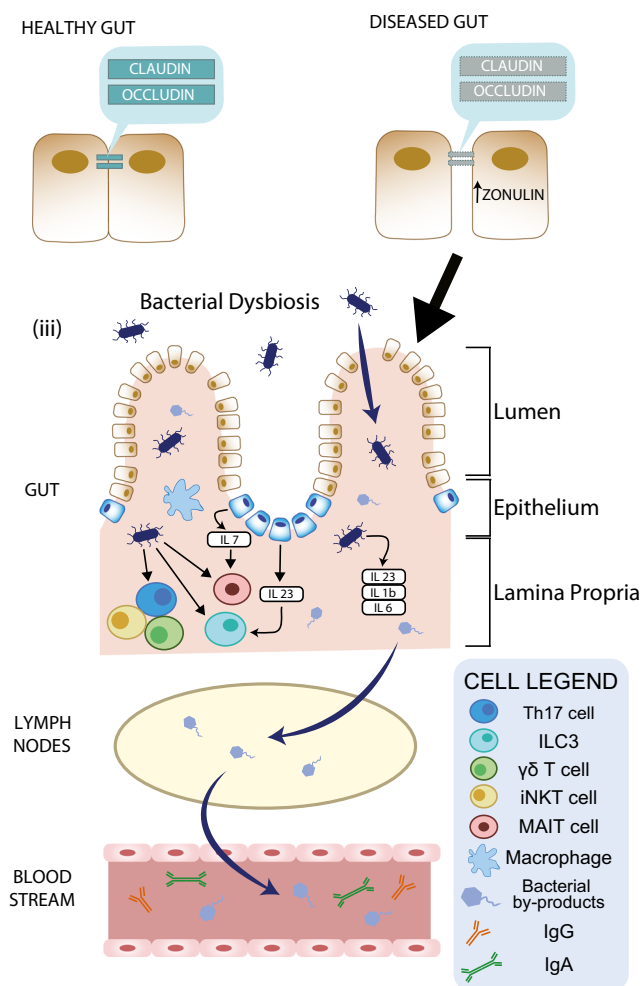


Fig. 3 Altered intestinal microbiome in SpA and its structural and immune consequences. Perturbations in the SpA bacterial composition, according to the *bacterial dysbiosis hypothesis*, have an impact on the intestinal epithelial layer, where tight junction proteins claudin and occludin expression is decreased with a subsequent elevation of zonulin. This affects gut permeability and epithelial cells also detach from the basement membrane leading to a compromised architecture. Microbial infiltration occurs as a result and cells like MAIT cells, ILC3 cells, $\gamma\delta$ T cells, and iNKT cells can respond to microbial products and trigger a downstream cascade of signaling events. Other bacterial signals, such as flagellin and lipopolysaccharide (LPS), facilitate the release of IL-1 β , IL-6, and IL-23, thereby creating a microenvironment which is favourable to T_H17 differentiation. Activated Paneth cells respond to the perturbed microbiome and release IL-23 and IL-7 which also induce differentiation of ILC3 and MAIT cells. Further, translocation of bacterial products to the lymphatic system, and eventual movement in the bloodstream and beyond is also possible, given the abilities of antigen presenting cells like macrophages and dendritic cells. This might explain why there is potential anti-microbial activity through elevated IgA and IgG antibodies in the SpA serum. Additionally, increases in lymphoid follicles in the SpA ileum have also been observed, which may implicate a gut origin of elevated serum antibodies

was observed that there was a significant difference even in the microbial composition of SpA patients with or without bowel inflammation. There was also a suggestion that the amount of *Dialister* could be a potential microbial marker,

owing to its strong correlation with clinical disease activity [93]. Another fecal microbiota study was able to demonstrate a reduced bacterial diversity in SpA patients as compared to HCs, where a two- to threefold increase in *Ruminococcus gnavus* was the most notable difference and this correlated with disease activity as well [94]. Specifically, a decreased microbial diversity is seen in axSpA, AS, PsA, but not ReA [10]. There is evidence to suggest that host genetics, specifically HLA-B27 positivity, may be one of the potential drivers of changes in intestinal microbiota, which may ultimately affect the risk of AS development through effects on the gut microbiome. This was reported using 16S rRNA profiling of stool and biopsy samples from HCs, which showed a significant difference in microbial composition based on HLA-B27 status, although the overall diversity of the bacterial communities was not statistically different in HLA-B27+ vs HLA-B27- samples [95].

The bacterial dysbiosis hypothesis

The *bacterial dysbiosis hypothesis* proposes that the expression of similar receptors between the gut epithelium and the synovial membrane may be the reason for joint inflammation, and this could be linked to a reduction in HLA-B27-mediated clearance of intracellular bacteria. Supporting this notion, a recent study observed an enrichment of bacterial peptides which were homologous to those presented by HLA-B27 in the AS fecal matter, suggesting a failure of HLA-B27 in clearing these peptides or that they are involved in promoting HLA-B27-related immune responses [92]. Prospective studies in ReA showed a clear correlation between the disease development and enterobacteria, *Yersinia enterocolitica* and *Salmonella typhimurium*, and the recognition of these bacteria by HLA-B27-restricted CD8+ T cells has been established. Even though there are reports suggesting a link between the HLA-B27 gene and gram-negative bacteria, the associated pathophysiological processes are far from clear. Through early studies, the HLA-B27 protein has been shown to elicit cytotoxic responses against *Klebsiella*; there may even be shared amino-acid sequences between HLA-B27 and bacteria, as evidenced by the ability of anti-HLA-B27 monoclonal antibodies to also recognize *Shigella flexneri*, *Y. enterocolitica* and *Klebsiella* [11]. Mechanistically, a well-defined link between dysbiosis and arthritis in SpA is still elusive. However, animal models, such as the HLA-B27/ β_2 -microglobulin transgenic rats, highlight that bowel or musculoskeletal inflammation is absent under germ-free conditions. Furthermore, the microbiome of these animals also varies from their littermate controls, even in the absence of gut inflammation. These results might hint towards a susceptibility in HLA-B27-positive SpA patients [90]. A prominent study demonstrated that these alterations in the gut microbiome occurred early in life, particularly affecting the microbial amino acid, carbohydrate,

medium-chain fatty acid, and xenobiotic metabolites [96]. Curiously, these changes were subsequent to an inflammatory T_H17 and T_H1 cytokine signature which ultimately resulted in dysbiosis and gut inflammation [97]. Further evidence needs to emerge that can fully explain how dysbiosis is initiated: are there possible genetic (HLA-B27) and immunological changes (T_H17/T_H1) that lead to dysbiosis or does dysbiosis itself drives the inflammatory process?

Structural consequences of microbial dysbiosis: gut permeability and integrity

In health, the GVB and gut-epithelial barrier typically ensure compartmentalization between gut-resident microbes and the underlying stromal and immune cells of the lamina propria [67]. This allows regulation of the movement of antigens into the blood stream, and also prevention of bacterial translocation. These barriers are composed of a network of protein-protein interactions, including tight junctions, desmosomes and adherens junctions that line and link intestinal epithelial cells [98]. Intestinal short-chain fatty acids (SCFAs) usually promote anti-inflammatory responses by reducing pro-inflammatory cytokines and increasing T_{reg} cell responses [10]. In particular butyrate, which is a SCFA, decreases bacterial translocation and strengthens the overall intestinal barrier function. Microbial dysbiosis has been shown to impact SCFA levels [99]. For instance, *Akkermansia muciniphila*, which is typically responsible for the conversion of mucin into SCFAs, was reportedly absent in a PsA cohort and decreased in IBD compared to HCs [10]. Breakdown of the intestinal barrier can also occur either by apoptosis of epithelial cells or by compromise of the tight junctions in response to microbial infection [99]. In the SpA gut, there was a strong correlation between the presence of adherent and invading bacteria and the decrease in expression of tight junction proteins claudin 4 and occludin. This was further accompanied by an upregulation of the zonulin and PV1 proteins; the former modulates the permeability of tight junctions and the latter marks endothelial cell permeability in chronic gut inflammatory conditions. In vitro studies showed that isolated ileal bacteria from SpA patients were able to induce zonulin upregulation on Caco-2 cells. *Ex vivo* studies also highlighted the observation that bacterial products and zonulin induced monocytic changes to an M2 phenotype. These findings of adherent bacterial presence and downregulated occludin were also observed in HLA-B27 transgenic rats, and such alterations were restored by antibiotic treatment [98, 100]. A more recent study using an RA murine model showed that the intestinal barrier function is impaired prior to clinical onset of arthritis. Furthermore, zonulin was identified as key to triggering disease onset and controlling transmigration of immune cells from the gut to joints. Importantly, targeting zonulin using an antagonist attenuated arthritis and inhibited migration of gut-primed

immune cells to the synovial tissues [99]. Other changes to the gut architecture include the detachment of epithelial cells from the basal membrane and vasculitic lesions [101]. Together, these reports lend support to the notion that dysbiosis has a role in modifying gut permeability and integrity in SpA.

Immune consequences of microbial dysbiosis

An inflammatory cascade of events can occur as a result of dysbiosis in SpA, which induces cell differentiation and modulation of cytokine responses. Paneth cells in the terminal ileum have the capacity for activating and amplifying innate immune responses. For instance, they have been known to be activated as a result of an altered gut microbiome and not only produce high amounts of anti-microbial peptides but also release IL-23 and IL-7 cytokines [101]. In HLA-B27 transgenic rats, IL-23 expression tends to normalize upon antibiotics treatment, suggesting that dysbiosis has the potential to drive systemic inflammation and modulate IL-23 activity. However, the precise manner in which this modulation occurs is unclear, but different innate immune players may be implicated [98]. In turn, IL-23 and IL-7 lead to the production of cryptopatches, which are lymphoid tissue inducer (LTi) cell aggregates. Interestingly, it was observed that coculture of LTi with epithelial cells isolated from AS patients resulted in ILC3 differentiation and IL-17 and IL-22 expression. Together, IL-17 and IL-22 may be responsible for tissue inflammation [101] and downstream ILC3 activity. It is known that NKp44 ligands typically include microbial products, which supports the possibility that there is direct interaction of ILCs with the gut microbiota [67]. Curiously, a breakdown in the ILC/IL-22 axis may result in the growth of commensal bacteria, and ILCs themselves may limit commensal reactive T_H17 responses thereby favouring T_{reg} cell induction. Therefore, it is conceivable that intestinal IL-22+ NKp44 cell upregulation may be an attempt at restoring homeostasis [67].

Intestinal macrophages normally do not respond to bacterial stimuli; however in CD, this anergy is lost and CD14+ macrophages accumulate in the inflamed lamina propria to release proinflammatory cytokines and induce T_H1 and T_H17 responses. In the AS terminal ileum, however, CD14+ macrophages are virtually absent [101]. A recently described macrophage subset, the resolution phase macrophages (rMs), have both M1 and M2 capabilities and are also found expanded in the AS gut compared with CD [24]. As mentioned previously, there is an upregulation of M2 macrophages, and it is hypothesized that the presence of rMs and M2 macrophages, that are unable to eradicate pathogenic bacteria, may be the reason why intestinal dysbiosis persists in the AS ileum [101]. Besides these observations, HLA-B27 transgenic rat models have also illustrated that microbiota-dependent intestinal inflammation directly drives the bone-erosive potential of

monocytes in the presence of TNF through in vitro studies [102].

The microbiome has an impact on adaptive immune cells in the pathogenesis of SpA and serves to promote a balanced homeostatic environment. Microbial signals, such as flagellin and lipopolysaccharide (LPS), facilitate the release of IL-1 β , IL-6 and IL-23, thereby creating a microenvironment which is favourable to T_H17 differentiation. This may be potentiated upon encounter of pathogenic microbes. In animal infection models, *Salmonella enteritidis* induces local T_H17 responses resulting in an ReA-like disease. Segmented filamentous bacteria (SFB) are also known to induce T_H17 generation in the intestinal lamina propria during homeostasis. Using the K/BxN murine model, SFB were found sufficient for inducing arthritogenic T cells, highlighting an association between bacteria and extraintestinal disease. Interestingly, another study utilizing a transgenic reporter system in K/BxN mice, demonstrated that intestinal T_H17 cells migrated to the spleen, coincident with arthritis development [67].

It is important to reiterate that few IL-17-producing T cell expansions have been observed in the inflamed AS gut, which is why consideration of alternative sources of IL-17 such as ILCs and innate-like cells like MAITs, iNKTs and $\gamma\delta$ T cells is crucial in SpA mucosal immunity. Particularly, these cells have the capacity to recognize microbial antigens, thereby sensing alterations in the gut microbiome [67]. Keeping in mind the potential regulatory and effector abilities of ILCs, iNKTs and $\gamma\delta$ T cells, further studies assessing precise dysbiosis changes would be required to see how this balancing act shifts.

Clinical relevance of subclinical gut inflammation in SpA

Delving deep into the immunopathogenesis of the gut-joint axis is important in order to design new therapies and ultimately be able to predict the presence of SpA-IBD or the likelihood of IBD development in SpA patients (Fig. 4a). Male AS patients were found to be diagnosed with IBD more often than females, and IBD frequency reduced with age, according to one study [103]. A multi-center study revealed no differences between baseline patients who had an IBD history or not. However, IBD development (at the time of diagnosis) was linked with disease activity, baseline spinal pain scores, worse physical function and patient well-being [104]. In another analysis using the GIANT cohort, there appeared to be a correlation between gut inflammation and bone marrow edema in the sacroiliac joints. Furthermore, SPARCC (Spondyloarthritis Research Consortium of Canada) scores that measure MRI-defined sacroiliitis were higher in patients with chronic gut inflammation, as compared to those without [105]. Overall, several features in axSpA are linked to

microscopic gut inflammation: young age, male sex, progressive disease, early disease onset, radiologic sacroiliitis, high disease activity, and restricted spinal mobility. No correlation was identified with HLA-B27 status [106].

Looking at the other side of the equation, 10–39% of IBD patients have coincident SpA, with CD patients experiencing SpA more than those with ulcerative colitis (UC). Interestingly, arthritic symptoms typically follow IBD diagnosis; however, in 20%, the reverse is true when considering axial disease. In general, it is estimated that AS and sacroiliitis is expected to manifest in 2–16% and 12–46% of IBD patients, respectively [106]. Specifically based on computed tomography (CT) scans, sacroiliitis was observed in 15% of CD and 16.9% of UC patients, with an overall 3-fold higher prevalence of sacroiliitis in IBD compared with controls. Curiously, according to referral strategies, many IBD patients with sacroiliitis were not referred to a rheumatologist, despite growing awareness of this increased prevalence [107]. In fact, subclinical sacroiliitis in IBD is often underdiagnosed and has also been linked to male sex, arthritis and inflammatory CD [108]. Examination of CD patients with or without AS revealed no differences according to a small single center study, and CD disease activity correlated strongly with AS disease activity and AS functional disability. Finally, 2–578% of IBD-coincident AS patients are HLA-B27+, as compared to 7–15% of patients with IBD-coincident sacroiliitis [106].

Apart from clinical characteristics that overlap gut and joint inflammation, recent studies have highlighted calprotectin as a potential biomarker that connects the two sites. Calprotectin is a protein released upon monocytic infiltration into inflamed sites. It has been linked to structural joint damage in PsA and RA. In axSpA, baseline serum calprotectin appeared to be a predictor of worse modified Stoke Ankylosing Spondylitis Spine Scores (mSASSS) and syndesmophyte formation/progression [109]. Specifically, 64% of those with increased C-reactive protein (CRP) and calprotectin had bowel inflammation, compared to 25% patients with lower levels of these proteins [106]. High fecal calprotectin levels marked subclinical bowel inflammation in SpA patients and at baseline, those with elevated calprotectin had more sacroiliac joint severity and inflammation [110]. Worse disease activity and physical function were also observed in axSpA patients with increased fecal calprotectin [111]. A treatment response can be predicted based on the return to almost normalized levels of fecal calprotectin upon adalimumab therapy [110]. Interestingly, using nonsteroidal anti-inflammatory drugs (NSAIDs) was linked with increased fecal calprotectin in AS patients [112].

Given Paneth cells' ability to produce anti-microbial peptides, it has been speculated that anti-microbial reactivity may ensue over the disease course. Elevated IgA and IgG levels in the serum have been well documented in SpA [113, 114], despite the lack of B cells playing a central role in the pathogenesis based on the absence of B cell-related risk genes in AS

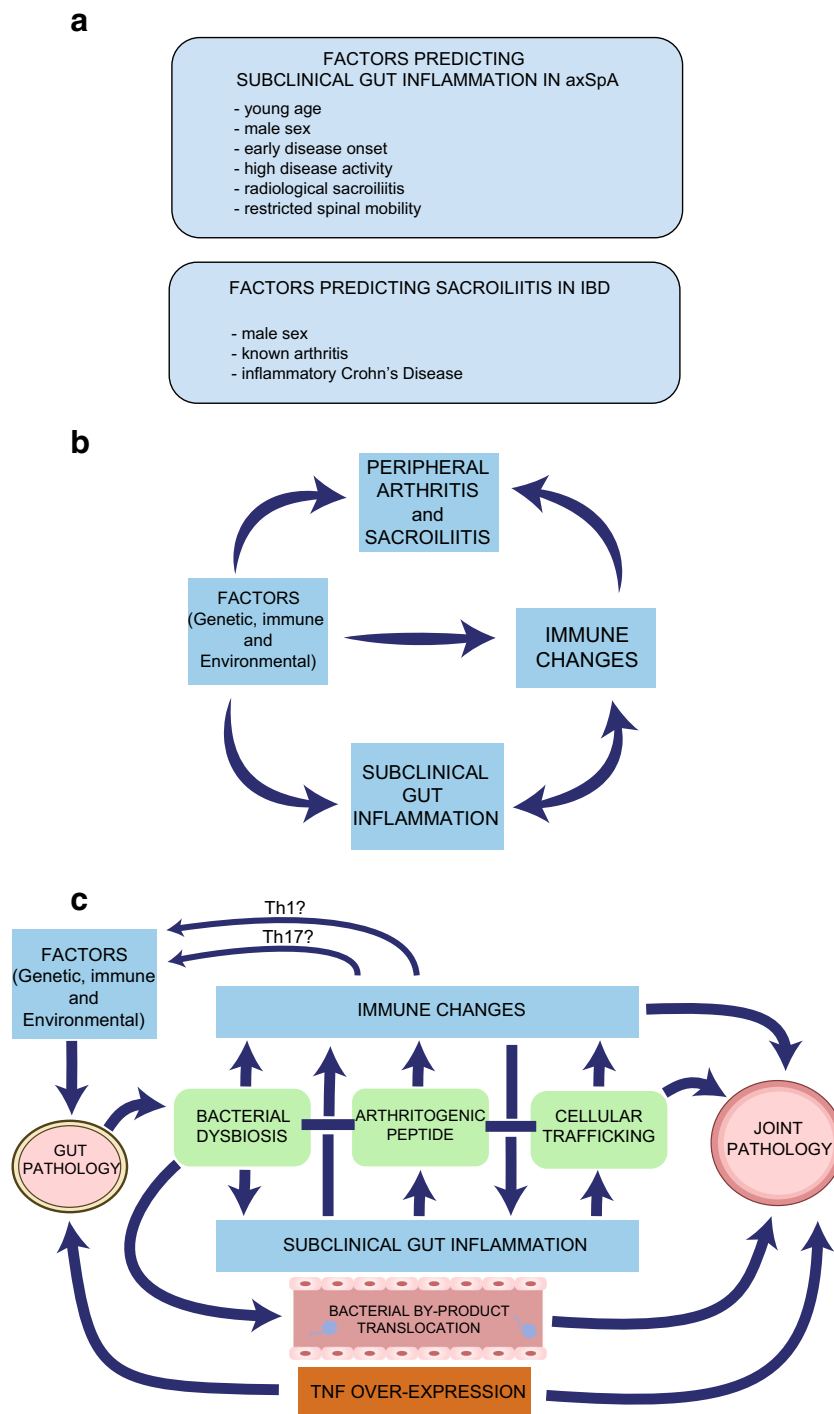


Fig. 4 The clinical and hypothetical relationships between gut and joint pathologies in SpA. **a** Clinical features associated with subclinical gut inflammation in axSpA and with sacroiliitis in IBD. **b** Genetic, immune and environmental (dysbiosis) factors may lead to coincident existence of gut and joint inflammation in SpA. These factors may directly influence immunologic changes, which in turn induce subclinical gut inflammation, peripheral arthritis and sacroiliitis. This conceptual framework would help explain why some SpA patients are do not develop intestinal symptoms yet can be predisposed to experiencing immunologic changes resulting in arthritis. **c** A causal relationship between gut and joint pathologies might ensue when genetic, immune and environmental features influence gut pathology first, resulting in bacterial dysbiosis,

recognition of arthritogenic peptide(s), clonal expansion, eventual migration to other inflammatory sites, such as the joints, and occurrence of arthritic symptoms. During this process, subclinical gut inflammation develops and immunologic perturbations perpetuate the inflammatory cascade. T_H1 and T_H17 responses may feed back into this entire process and influence gut pathology. Furthermore, translocation of bacterial products into peripheral sites can also link the gut and joint pathologies. Finally, the $TNF\alpha$ signaling cascade that is initiated could also independently link gut and joint inflammation by activating stromal cells in these sites leading to more perturbations. This appears to be the current dogma in understanding why the majority of axSpA patients experience subclinical gut inflammation

[9] and inconclusive therapeutic responses when targeting B cells [115]. As mentioned previously, there is an increase in lymphoid follicles in the SpA ileum and colon, which may implicate a gut origin of elevated serum antibodies. Supporting this notion are the observations of high serum anti-flagellin (anti-CBir1) antibodies in AS patients that correlated with elevated acute phase reactants [116]. Compared to HCs, increased prevalence of IgA anti-*Saccharomyces cerevisiae* antibodies (ASCA) were found in the AS serum [117]; and this has also been reported to be true for patients with AS-IBD compared to AS alone, except these patients had high IgG ASCA in their serum [116]. AS-IBD patients also had increased serum antibody responses against *E. coli* outer membrane porin C (anti-OmpC), compared to AS only [116]. This increased reactivity against *E. coli* was recently observed in CD-SpA compared to CD alone, where IgA-seq revealed a selective enrichment in IgA-coated *E. coli* in the former group. Additionally, a T_H17 response was induced when germ-free mice were colonized with CD-SpA *E. coli* isolates. Furthermore, this study was able to demonstrate increased severity of colitis or arthritis when CD-SpA *E. coli* colonized IL-10 deficient or K/BxN mice, respectively. The mucosal and systemic T_H17 immune responses typically observed in CD-SpA could be modelled this way [118]. Antibodies against other microbes, such as *Yersinia* and *Campylobacter*, were also found in SpA sera in a small, proof-of-concept study [114]. These reports are suggestive of subclinical bowel inflammation driving the production of enhanced serum antibody responses, which may ultimately impact T_H17 immunity. It is still unclear whether this phenomenon occurs early during the disease course or much later, and there may even be a strong possibility that subclinical gut inflammation is simply coincident to joint inflammation.

Insights for drug treatments

It is clear that there are similarities in the etiology and pathophysiology between SpA and IBD which can help inform therapeutic decisions. It is imperative to understand where the differences lie in order to rationalize the bacterial and immunological implications of certain treatments and why some drugs do not have strong efficacy.

Anti-TNF agents are the most well-studied gold standard in therapies when it comes to treating SpA-IBD patients. Some that have been approved for axSpA, such as infliximab and adalimumab, have indication for CD and UC as well. Interestingly, the TNF α and TNF β -blocking etanercept has not been efficacious in IBD [106]. A number of theories have attempted to explain the reasons why; for instance, there appears to be an inability to induce T cell apoptosis in the lamina propria [119], and intestinal TNF β has been reported to be an upstream regulator of T cell-dependent IgA production which

is important for microbiota composition [120, 121]. In the case of etanercept, there are conflicting reports suggesting the development of new onset IBD in the context of AS, with the symptoms resembling CD more than UC. However, a clear association between anti-TNF agents and IBD development is yet to be defined [106].

There is no debate about the importance of the IL-23/IL-17 axis in the immunopathogenesis of SpA and IBD and while therapies targeting these cytokines have largely been effective, a number of discrepancies in efficacy between the two conditions need to be noted. Specifically, the IL-12 and IL-23 p40 subunit targeting ustekinumab was effective in CD and PsA but not in AS. Phase II trials using the IL-23 p19 inhibitor risankizumab were terminated due to lack of clinical and radiological efficacy in AS [106]. This contrasts risankizumab's efficacy in increasing clinical response and remission rates in moderate to severe CD, according to a phase II induction study [122]. On the other hand, the IL-17 blocking secukinumab has recently obtained indications for AS. However, phase II results show worsening of disease upon secukinumab induction in CD patients when compared to placebo [106]. Since IL-17 plays an anti-fungal role, an increase in *Candida albicans* has been speculated as an explanation for CD disease exacerbation [123]. It may even be that IL-17 suppression might interfere with its gut protective function and worsen disease [36]. New onset IBD in axSpA patients was infrequent upon secukinumab treatment according to a meta-analysis [124], although there are two cases suggesting the opposite [125]. Real-world evidence also highlights 19 newly diagnosed IBD cases among SpA patients treated with secukinumab. Interestingly, there was substantial improvement of symptoms once therapy was terminated [36]. The most glaring question that emerges concerns the reasons why therapies that block both IL-23 or IL-17 do not work similarly in SpA and IBD. Specifically, given that IL-23 is upstream of IL-17 and the prevalence of many IL-23-related SNPs, it is surprising to note the difference in effect of IL-23 blocking agents in AS and PsA. It is clear that at some point the IL-23/IL-17 pathway diverges during the disease pathogenesis and current research is focusing on unraveling this concept. Some theories have emerged: firstly, anatomical differences in spinal versus peripheral entheses may play a role; and secondly, there are several cellular sources of IL-17A production in spinal entheses and emerging evidence highlights IL-23-independent sources of IL-17A such as ILCs, MAIT cells and $\gamma\delta$ T cells [37]. Therefore, blocking IL-23 might leave some basal IL-17 levels. On a similar note, animal models have shown that there is redundancy in the role of IL-23 once adaptive immunity has been primed and there is IL-17 production [126]. Furthermore, the important role that IL-17 plays in maintaining the gut epithelial barrier could be a reason why there are some reports of new onset IBD in SpA patients after IL-17 inhibition.

It is important to note that microbial dysbiosis is inherently a dynamic process and it may well be that there are crucial changes in the gut microbiota composition upon administration of anti-TNF or anti-IL-17 treatments in AS. A recent study in Chinese AS patients revealed that blocking TNF resulted in a normalization of perturbed gut microbiota, such as the decline of *Prevotella copri* levels. Additionally, this therapy was also linked with a reduction of potentially arthritogenic peptides in patients compared to those untreated [91]. Another recent study also confirmed a restoration of the perturbed microbiome in untreated AS patients after TNF therapy, comparable to that of HCs [92]. A proof-of-concept study even brought forth the notion that microbial composition in SpA may predict anti-TNF responses [120]. Microbial taxa alterations upon IL-17 inhibitor treatments in SpA were also observed. In a recent study, *Clostridiales* and *Candida albicans* were expanded compared to those given anti-IL-17 versus anti-TNF blockers. These expansions were correlated with metabolic pathways, related cytokines and fatty acids. Furthermore, development of clinically overt CD in SpA was linked to ILC2 expansions during anti-IL-17 treatment compared to pre-anti-IL-17 levels [127].

The Janus kinase (JAK), signal transducer of activation (STAT) pathway, has been long studied as a crucial signaling pathway employed by a range of cytokines, interferons and growth factors. Owing to the myriad of research implicating this pathway in autoimmunity, it has become an attractive therapeutic target [128]. GWAS have shown an association between single nucleotide polymorphisms in the JAK-STAT pathway and CD [106], and some SNPs have been linked to RA and SLE [128]. This has led to the targeting of JAK-STAT pathway members in phase II trials for IBD and SpA. Interestingly, tofacitinib was not effective in CD, but showed favourable results in AS [129, 130]. This contrasts the newer, more selective JAK inhibitors, such as filgotinib and upadacitinib, which are efficacious in achieving clinical remission in CD [131, 132]. Both have also shown promise in AS in attaining clinical improvement, while being well tolerated [133, 134].

Integrin-blocking therapies that are efficacious in IBD, particularly CD, are natalizumab [135] and vedolizumab [136], which target α_4 and $\alpha_4\beta_7$ integrins, respectively, as a mechanism to block cellular trafficking to the gut. Currently, no randomized controlled trials in SpA using these drugs have been conducted and the ability of these drugs to resolve articular manifestations is controversial according to proof-of-concept studies. A case report using natalizumab in AS showed a reversal of arthritis as well as gut inflammatory symptoms [137]. This is in stark contrast to treatment with vedolizumab, which has been reported to be associated the development of arthritic flares or new onset arthritis in IBD patients [138, 139]. Another study reported that vedolizumab treatment was able to improve peripheral arthropathy

symptoms, although there was a limited sample size and the follow-up period was limited [140, 141]. Of note, a recent post hoc analysis of the “Gemini” trials reported a decreased likelihood of new or worsening arthritis/arthralgia in CD patients upon vedolizumab administration [142]. It is not exactly clear why blocking of $\alpha_4\beta_7$ integrin has paradoxical consequences in the gut and joint, despite it being prevalent along with its ligand at both sites. It may be plausible that this blockade results in fewer T_{reg} cells in the joints, as evidenced by the importance of $\alpha_4\beta_7$ integrin for UC-derived T_{reg} cell homing to the inflamed colon in humanized mice *in vivo* [143] and the fact that T_{reg} cells are known to be expanded in SpA SF [144, 145]. Such studies also need to be conducted for natalizumab in SpA patients to provide a clearer picture.

Conclusions

There are numerous epidemiological, genetic, clinical, microbial, histopathological, and immunological overlaps between SpA and IBD, which has implications for the gut-joint axis of inflammation. Firstly, whether there is a correlative or causal association between gut and joint inflammation remains to be resolved, but the aforementioned bacterial and immunological theories linking the disease pathology at both sites are not mutually exclusive (Fig. 4b and c). Secondly, there is a complex interplay between the innate and adaptive immune system in promoting immunopathogenesis between the gut and joint, such as the induction of type 3 immunity. Thirdly, multiple mechanisms such as bacterial translocation, recognition of arthritogenic peptides and T cell clonal expansion, and aberrant cellular trafficking may be employed when considering how the gut pathology links with joint pathology. Fourthly, bacterial dysbiosis is becoming increasingly important in understanding the interplay between the gut microenvironment and innate/adaptive immunity; there may be potential microbial-related biomarkers that can be uncovered and future studies need to also consider the consequences of microbial composition upon therapeutic interventions. Fifthly, IL-23/IL-17 cytokines have different roles in the various forms of SpA and different cellular sources of IL-17 have been identified, making therapeutic efficacies different between SpA and IBD. Finally, it is conceivable that using anti-integrin therapies in SpA results in a re-direction of trafficking using other integrins or chemokine receptors, ultimately resulting in worsening of arthritic symptoms. Our understanding of many concepts in SpA, such as the T_{RM} profile, contribution of myeloid cells, mechanism of action of various cells and integrin biology, is still in its early stages and further research delving into these ideas would help in unraveling the immunopathogenesis of disease and designing more precise therapeutics. It is becoming more apparent that a multi-disciplinary approach between the rheumatology and

gastroenterology disciplines is needed when providing care to SpA patients that may be at risk of developing IBD or vice versa.

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Declaration

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