SPONDYLOARTHRITIS AND THE INTESTINAL MICROBIOTA

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ABSTRACT

The intestinal microbiota is involved in the pathogenesis of inflammatory bowel disease (IBD) and in the development of inflammation in the extraintestinal tissue sites in spondyloarthritis (SpA). There is genetic, immunological and microbiological overlap between IBD and SpA, which indicates that pathophysiological mechanisms are shared between these diseases and can be linked to the intestinal microbiota. According to recent findings, in autoimmune diseases (such as SpA and IBD) and gut microbiota, there is a decrease in the number of *Firmicutes* (especially *Faecalibacterium prausnitzii* and *Clostridium leptum*) and an increase in the number of bacteria belonging to the *Enterobacteriaceae* family, which could be an important link between SpA and gut inflammation.

Keywords: spondyloarthritis, microbiota, dysbiosis, HLA-B27, inflammatory bowel disease.

REZUMAT

Microbiota intestinală este implicată în patogeneza bolilor inflamatorii intestinale și în dezvoltarea inflamației în situsurile extraintestinale în spondiloartrite. Se evidențiază o suprapunere între mecanismele genetice, imunologice și microbiologice din bolile inflamatorii intestinale (IBD) și spondiloartrite (SpA), care indică existența unei fiziopatologii comune acestor boli, ce pot fi corelate cu dezechilibre ale microbiotei intestinale. Conform descoperirilor recente legate de bolile autoimune și microbiota intestinală, s-a observat o scădere a numărului de bacterii din genul *Firmicutes* (in special *Faecalibacterium prausnitzii* și *Clostridium leptum*) și o creștere a numărului de bacterii din familia *Enterobacteriaceae,* fapt care poate susține legătura între patologiile menționate mai sus și flora intestinală.

Cuvinte-cheie: spondiloartrite, microbiota, disbioza, HLA B27, boli inflamatorii intestinale.

INTRODUCTION

Spondyloarthritis (SpA) includes a group of inflammatory diseases characterized by the involvement of spine and asymmetrical arthritis: ankylosing spondylitis (AS), psoriatic arthritis (PsA), enteropathic arthritis, reactive arthritis (ReA), undifferentiated spondyloarthritis and juvenile SpA [1].

Chronic gut inflammation is most common in SpA patients with axial involvement as compared to those presenting with peripheral involvement. The pathogenesis of gut inflammation in SpA can be explained by two factors: activation of immunological cells and altered gut microbiota [2]. The inflammation of the gut wall can lead to the entry of luminal bacteria into mucosa and submucosal regions and subsequently into systemic circulation. The changes in the gut microbiota can lead to an increase in pathogenic bacteria predisposing to gut inflammation [3].

I. Human Intestinal Microbiota

Gut microbiota represents the entire population of microorganisms that colonizes the gut and includes not just bacteria, but also other microbes such as fungi, archaea, viruses, and protozoans [4] and reflects natural selection at both microbial and host levels promoting cooperation and functional stability of this complex ecosystem.

The gastrointestinal microbiota lays a fundamental role in health and disease. Host-microbe interactions occur along mucosal surfaces. The intestine harbors a diverse bacterial community and is separated from

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the internal milieu by only a single layer of epithelial cells.

Mucosal surfaces serve as a protective barrier and are protected by a first-line defense mediated by immunoglobulin A (IgA) [5]. T helper (Th) 17 cells are abundantly present at the mucosal surface of the intestine and contribute to intestinal homeostasis by regulating intestinal IgA secretion [5]. The chronic activation of Th17 and hyperactive IgA synthesis occurs in many types of inflammatory joint diseases and can cause arthropathies [6].

1. The structure of the intestinal microbiota

The human intestine harbors more than 1,000 different bacterial species [7]. Bacterial density increases in the distal small intestine, and in the large intestine rises to an estimated 10^{11}-10^{12} bacterium per gram of colonic content, which contributes to 60% of the fecal mass [8].

Facultative anaerobic bacteria predominate in the small intestine, whereas the large intestine is heavily populated by obligate anaerobic bacteria [9].

The adult gut microbiota is dominated by five bacterial phyla, in healthy human populations, *Firmicutes* (50-75% including *Ruminococcus*, *Clostridium*, *Lactobacillus*, *Eubacterium*, *Faecalibacterium* and *Roseburia*), *Bacteroidetes* (10–50%, including *Bacteroides*, *Prevotella*, *Porphyromonas* and *Xylanibacter*), *Actinobacteria* (1-10%, including and *Collinsella*), *Proteobacteria* (<1%, including *E. coli*, *Desulfovibrio*), *Verrucomicrobia* (*Akkermansia*) and one phylum including *Archaea* (*Euryarchaeota*). Bacterial groups as *Cyanobacteria*, *Fusobacteria*, *Lentisphaerae*, *Spirochaetes* are present in lower proportions [10-12].

There is individual variation within the adult gut microbiota [13]. Butyrate-producing bacteria, including *Faecalibacterium prausnitzii, Roseburia intestinalis* and *Bacteroides uniformis*, have been identified as key members of the adult gut microbiota [14].

Arumugam *et al*., identified three robust clusters, termed „enterotypes", that were not nation- or continent-specific. Although still controversial, each enterotype would harbour a dominant bacterial genus: enterotype 1 would be dominated by the genus *Bacteroides*, enterotype 2 by *Prevotella*, and enterotype 3 by *Ruminococcus* [15].

Enterotype 1 bacteria (*Bacteroides*, which co-occurs with *Parabacteroides*) are known to obtain energy primarily from carbohydrates and proteins through fermentation [16]. This fact is confirmed by the presence of the genes encoding enzymes involved in the degradation of these substrates (galactosidases, hexosaminidases, proteases). Furthermore, glycolysis and pentose phosphate pathways are present in this enterotype. Enterotype 2 bacteria (*Prevotella* and the co-occurring *Desulfovibrio*) act in synergy to degrade mucin glycoproteins present in the mucosal layer of the gut: *Prevotella* is a known mucin-degrader and *Desulfovibrio* could enhance mucin desulfation step by removing the sulfate [17].

Enterotype 3 is the most frequent one with *Ruminococcus* and *Akkermansia)*, both being able to degrade mucins [18]. The enterotypes employ different routes to generate energy from fermentable substrates available in the colon reminiscent of a potential specialization in ecological niches or guilds. The gut microbiota is also beneficial to the human host by producing vitamins. Enterotypes 1 and 2 are involved in biosynthesis of different vitamins: biotin, riboflavin, pantothenate and ascorbate in the former, and thiamine and folate in the latter.

2. Development and functions of the intestinal microbiota

The main function of bacteria in the intestine is to aid the digestion and absorption of food [19]. Microbiota produces metabolites (10% of all the metabolites), short-chain fatty acids and water-soluble vitamins. Microbiota exerts protective function by preventing adherence of pathogenic bacteria to mucosal layer [20], regulates epithelial cell proliferation and differentiation, maintains immune homeostasis and sustains symbiotic relationship with the host [14]. This mutual relationship offers host nutrients to intestinal commensal bacteria, in return for metabolic and physiological capabilities.

The microbiota has an „ecological memory" that favors a return to previous microbiota after dietary- or antibiotic-induced changes. A myriad of factors influence the early structure

of the microbiota: diet, smoking, long-term antibiotics, obesity, parenteral nutrition, early infection, and antibiotic use in infancy. The microbiota has a complex relationship with the intestinal wall and the products of microbial metabolism may have additional effects on this gut component.

Archaea are known for being involved in producing methane. Sulfate reduction is carried out by Delta *Proteobacteria* (e.g., *Desulfovibrio* spp.) and one clade within the *Firmicutes* (*Desulfotomata* spp.). Fermentation is a phylogenetically widely held skill and the principal energy pathway for members of *Bacteroidetes* and *Firmicutes* (and thus the dominant energy-producing pathway for the microbiota) [14]. *Bacteroides thetaiotaomicron* - a key element of symbiotic microbial ecology (comprises 12% of all *Bacteroidetes* and 6% of all Bacteria) is an anaerobic symbiont in the distal intestine with an unusually large repertoire of genes involved in the acquisition and metabolism of polysaccharides (this includes 163 outer membrane proteins, that bind and import starch, over 200 predicted glycoside hydrolases, and 15 polysaccharide lyases) [21]. This glycobiome enables *B. thetaiotaomicron* to turn to host polysaccharides when dietary polysaccharides become limited. *B. thetaiotaomicron* hydrolyzes host-derived glycans and determines the type of glycans produced by gut epithelial cells [22-23]. The induction of host-derived glycans by *B. thetaiotaomicron* may serve an adaptive function, creating a habitable niche for itself that other glycophiles could exploit, thereby contributing to ecosystem stability and functional diversity. One mechanism by which *B. thetaiotaomicron* may stabilize microbial ecology involves its ability to induce the antimicrobial peptide angiogenin, which kills opportunistic or pathogenic organisms but not *B. thetaiotaomicron* or other commensals. *B. thetaiotaomicron* inhibits proinflammatory gene transcription through peroxisome proliferator activated receptor-γ (PPARγ)-dependent nuclear export of nuclear factor kappa B (NF-κBp65) [24], thereby potentially resisting inflammatory changes that could destabilize the symbiotic microbiota. These unique features have prompted the characterization of B. thetaiotaomicron as a keystone species [25].

F. prausnitzii metabolizes short chain fatty acids (SCFA), useful as a nutrient for enterocytes, so that their reduction can lead to cell stress, decreased tight junction viability, and increased intestinal permeability [26]. Acetate is important for muscle, heart and brain cells, propionate is used in host hepatic neo-glucogenesis and butyrate is important for enterocytes [26]. SCFAs and butyrate have anti-inflammatory effects by inhibiting the pro-inflammatory transcription factor NF-κB, increasing colonic Treg cell numbers and increasing their transcription factor Foxp3 production. Thus, lack of SCFAs in IBD and SpA exposes enterocytes to increased risk of injury and intestinal wall permeability may rise in a pathologic manner [26]. *F. prausnitzii* leads to lower production of pro-inflammatory cytokines such as IL-12 and IFNγ and higher production of anti-inflammatory IL-10 than other bacteria such as *L. acidophilus* [27]. Polysaccharide-A (PSA) of *Bacteroides fragilis* (an organism representing 5% of all *Bacteroidetes* and 2.5% of all bacteria in the enumeration study of the human colonic microbiota) is also inducing an IL-10 response in intestinal T cells, which prevents the expansion of Th17 cells and potential damage to the mucosal barrier [28].

PSA of *B. fragilis* can induce the anti-inflammatory function of Tregs by signaling directly through TLR2 on CD4+ T cell. Modulation of Treg activity by PSA restrains intestinal Th17 cell responses during commensal colonization. PSA from *B. fragilis* impacts the development of systemic T cell responses [28]. PSA-producing *B. fragilis* elicits higher Th1 cell frequencies in the circulation [29]. Together, these findings show that commensal bacteria have a general impact on immunity that reaches well beyond mucosal tissues.

II. Spondyloarthritis and Gut Microbiota

The exact triggering factor in most autoimmune diseases is unknown, yet an infectious cause has long been suggested to have an important role in the development of autoimmunity.

Spondyloarthritis (SpA) is a family of immune-mediated inflammatory disorders. SpA subtypes correspond to different clinical features such as enthesitis, dactylitis and

uveitis. The different SpA subsets share common genetic and pathophysiologic factors [1]. There is considerable clinical overlap between SpA and inflammatory bowel disease (IBD). Inflammatory bowel diseases (IBD) divided histologically and clinically into Crohn's disease (CD) and ulcerative colitis (UC) are strongly associated with SpA. Bowel inflammation is common in SpA, which may be classified as acute or chronic. There is a complex relationship between the gut microbiota and host immune regulation. Rosenbaum and Davey proposed that HLA-B27 alters the intestinal microbiome, due to a similarity between B27 peptide structure and bacterially derived proteins, (with several Gram-negative bacteria) [30], in patients with spondyloarthritis, who also have increased titers of anti-bacterial antibodies. This concept is supported by theories of a disrupted gut environment in spondyloarthropathy, with altered intestinal permeability perhaps leading to a deregulated immune response. This drives microbial dysbiosis and/or microbiota mediated intestinal inflammation leading to epithelial permeability [31].

The early changes to the microbiota may be relevant to the pathogenesis of SpA-related diseases, despite their adult onset [3]. Microscopic gut inflammation occurs in approximately two-thirds (60–70 %) of patients with SpA [32]. Inflammation in the gut can be classified as acute and chronic. Acute gut inflammation was mainly seen in patients with ReA and chronic gut inflammation was predominant in patients with AS [32]. Asymptomatic gut inflammation was present in 46.2% of patients (16.9% acute inflammation and 29.2% chronic inflammation). SpA patients develop clinical IBD within of 5 years (6.5%). Conversely, 30% of patients with IBD develop articular symptoms with a pattern similar to those in SpA including oligoarticular peripheral arthritis, or even AS. IBD patients have been reported to develop uveitis (9%) [3]. Remission of joint inflammation was associated with disappearance of gut inflammation, and chronic arthritis was accompanied by persistence of gut inflammation. As compared to SpA patients with normal gut histology, those with chronic gut inflammation had a higher occurrence of axial involvement [2]. Initial chronic gut inflammation in a SpA patient is associated with a higher risk of evolution to ankylosing spondylitis.

The Ghent group has described a series of patient characteristics and findings that associate with microscopic gut inflammation [33]:

a. Age (younger age has higher risk to develop SpA)
b. Male gender
c. Higher disease activity, measured by the **B**ath **A**nkylosing **S**pondylitis **D**isease **A**ctivity **I**ndex (BASDAI)
d. Restricted spinal mobility, measured by the **B**ath **A**nkylosing **S**pondylitis **M**etrology **I**ndex (BASMI).

Either local inflammation drives damage to the epithelium (e.g. the loss of SCFA-producing bacteria), or a disrupted epithelium promotes a breakdown of mucosal homeostasis with resulting inflammation and dysbiosis. There is an overlap in genetic polymorphisms associated with both SpA and IBD:

- the IL23 receptor, which affects neonatal development of tolerance to microorganisms and is a component of pro-inflammatory pathways in both diseases

- CARD9 (caspase recruitment domain 9) polymorphisms affect immunologic responses to infectious microorganisms and modulate the pro-inflammatory Th17 pathway [34].

Mucin-2 produced by goblet cells is an important component of intestinal barrier function [35]. Altered mucin expression in the intestinal epithelium triggers endoplasmic stress (ER) and unfolded protein response (UPR). ER stress and UPR induce pro-inflammatory cytokines such as TNFα, IFNγ, IL-23 and IL-17, aggravating inflammation [36]. Studies point out the role of autophagy in stimulating IL-23 production in the gut of patients with ankylosing spondylitis who have subclinical intestinal inflammation [37]. Gut bacteria stimulate production of IL-17 and IL-22 cytokines, which in turn recruit neutrophils and stimulate antimicrobial production and mucin to control infection and inflammation [38]. Depleted population of *Clostridiales* and increased populations of *Proteobacteria*

were associated with decreased IL-22-producing cells [39]. The relative abundance of *Enterobacteriaceae* was increased together with reduced abundance of *Clostridia* group XIVa and IV in gut of IBD patients and with arthropathy [40]. Intermediaries of benzoate metabolism influence microbial dysbiosis as a stress response [41] and have the ability to promote *Enterobacteriaceae* growth and virulence [42]. Recent microbiome studies are using a metagenomic approach based on next-generation sequencing (NGS) techniques which can provide unbiased bacterial identification.

1. Ankylosing Spondylitis

High abundance of *Veillonellaceae*, *Prevotellaceae*, *Porphyromonadaceae* and *Bacteroidaceae* was found in terminal ileal biopsies of recent-onset AS patients as compared to healthy controls [43]. Stebbings *et al*. [44] found that sulfate-reducing bacterium (SRB) *Bacteroides* was significantly enriched in AS as compared to controls. Costello *et al.* revealed distinct microbial colonization in the terminal ileum of patients with AS, based on increase in families *Lachnospiraceae*, *Veillonellaceae*, *Prevotellaceae*, *Porphyromonadaceae* and *Bacteroidaceae* [45]. Global dysbiotic changes in this system manifest with a loss of members of the dominant *Bacteroidetes* phylum, as seen in IBD patients, and expansion of less abundant proteobacterial and verrucomicrobial species [46]. *Klebsiella pneumoniae* is a candidate pathogen implicated in initiation and development of AS, which is increased in feces of patients with AS [47]. AS patients who harbored a higher number of *K. pneumoniae* in the gut, had Crohn's-like lesions in the ileocecal regions [48]. Presence of *K. pneumoniae* and a dysbiotic gut microbiota suggests that these bacteria could act as keystone pathogens in a mode similar to that of *P. gingivalis* in periodontitis. Over-representation of members of *Prevotellaceae*; underrepresentation of members of genus *Lactobacillus* in the *Firmicutes* phylum leads to a dysbiotic microbiota by reduced production of interleukin 18 by colonic epithelial cells, which in turn upregulates CCL5 chemokine expression and inflammatory cell recruitment leading to spontaneous inflammation [49]. Anti-*Saccharomyces cerevisiae* antibodies were detected in 20–30% of patients with AS [50]. Other antibodies detected have been: anti-I2 (associated with anti-*Pseudomonas* activity), anti-*Escherichia coli* outer membrane porin C (anti-OmpC), anti-flagellin (anti-CBir1) antibodies [51].

2. Psoriatic arthritis

Microscopic gut inflammation was observed in all patients with PsA; 40 % of patients showed macroscopic inflammation in the absence of bowel symptoms [52]. The reduced bacterial diversity found in gut of PsA was similar to that of IBD patients. A reduction in abundance of *Akkermansia, Ruminococcus* and *Pseudobutyrivibrio* in feces of PsA in comparison with healthy controls has been also described [53]. *Coprococcus* species is decreased in patients with PsA as compared to healthy subjects [53].

3. Juvenile SpA

Juvenile SpA is classified as undifferentiated and differentiated [54]. Patients with juvenile SpA exhibit decreased abundance of *Clostridium leptum* [55]. *F. prausnitzii* was decreased in patients with juvenile SpA compared to healthy controls. There is also some evidence of a cellular immune response to the outer membrane protein of *Salmonella typhimurium* in juvenile SpA patients compared to healthy controls [56].

4. Reactive arthritis

Reactive arthritis (ReA) is an immune-mediated synovitis resulting after an infection of genitourinary or gastrointestinal tract and showing intra-articular persistence of viable noncultivable bacteria and/or immunogenetic bacterial antigens [57]. The primary causative pathogenic enterobacteria in ReA are *Yersinia spp., Salmonella spp., Shigella spp., Campylobacter spp.* [57].

III. The Microbiota in Auto-Immunity

The presence of an auto-immune disease in a patient increases the risk of developing another auto-immune disease for the same patient, and for their relatives. An overlap between different auto-immune diseases clearly exists, e.g. between SpA and IBD [58]. The intestinal microbiota could be a crucial factor explaining this observation, because disturbance of the microbiota (dysbiosis) can affect the immune homeostasis and cause inflamma-

tion and autoimmune disease [58]. A reduced abundance of lactobacilli, bifidobacteria, and *F. prausnitzii,* and/or increased abundance of such pathogens as *E. coli*, *Salmonella*, and *Helicobacter* might contribute to this dysbiosis. The cause of immune mediated inflammatory disease may thus be the gut, acting at local and distant sites.

The immune system can influence the composition of the microbiota, and the microbiota can modulate the immune system [59]. Microbial ATP is responsible for the differentiation of Th17, whereas polysaccharide A from *B. fragilis* was found to induce Treg cells, reducing the response of Th17 [58]. Th17 is a producer of IL-17A, IL-17F, TNF, IL-21 and IL-22 cytokines, and is able to stimulate osteoclast formation and bone resorption [58, 60].

IV. Pathogenesis of Enteric-Infection and Reactive Arthritis

The direct association of reactive arthritis with preceding enteric infection supports an important role of the organisms in pathogenesis. *Yersinia*, *Salmonella*, *Shigella* and *Campylobacter* are all intracellular, Gram-negative bacteria with a lipopolysaccharide-containing outer membrane. The invasiveness is a critical property, this may be influenced in some way by HLA-B27, hence this allele's association with disease. These organisms share certain antigenic peptides that could be presented through HLA-B27. The beta-urease subunit (19 kDa protein), ribosomal protein L23 and the 60 kDa heat shock protein (hsp60) from *Y. enterocolitica* O:3 have been identified as candidate antigens [60-63]. It is possible that the patients with reactive arthritis have an aberrant immune response to bacterial heat shock proteins and that HLA-B27 may contribute to these altered responses [64, 65].

The persistence of the microorganisms which trigger reactive arthritis is consistently reported. *Yersinia* antigens have been detected in the peripheral blood up to 4 years after the initial infection. *Salmonella enteritidis* has been shown to persist in intestinal epithelial cells for up to 14 days after infection, permitting more widespread dissemination [64].The dissemination could result in infection of the synovial fibroblasts. In a patient with *Y. pseudotuberculosis*-associated reactive arthritis, evidence of viable bacteria within the joint was demonstrated over a year [64].

Molecular homology of peptide sequence has been found between *K. pneumoniae* and HLA-B27 [47]. Molecular similarities (hexameric amino acid sequence; glutamine, threonine, aspartic acid, arginine, glutamic acid, and aspartic acid, "QTDRED") have been found between *Klebsiella* nitrogenase reductase enzymes and HLA-B27 self antigen molecules [65, 66]. Other homologous structures have been found:

- in pullulanase pul-D secretion proteins (aspartic acid, arginine, aspartic acid, and glutamic acid, "DRDE" molecules) and HLA-B27 haplotype (aspartic acid, arginine, glutamic acid, and aspartic acid, "DRED" molecules)

- in *Klebsiella* pullulanase Pul-A secretion proteins (amino acid sequences glycine-x-proline -Gly-x-Pro present) and collagen type I, III, and IV fibers. The immune system is important in the elimination of bacteria, and the role of the immune system in the pathogenesis of reactive arthritis has been extensively investigated [60, 65].

In *S. flexneri* strains associated with reactive arthritis, the studies show the presence of a unique 2-Md plasmid, pHS-2. It is possible that any of the pHS-2 open reading frames could encode a pentapeptide that might be produced and trigger reactive arthritis. This pentapeptide shares homology with the hypervariable region of the *a1* domain of HLA-B27 (residues 71-75).

The inferred 22-amino acid polypeptide (Met-Cys-Leu-Met-GIy-Thr-Val-Cys-Ala-Gln-Thr-Asp-Arg-His-Ser-Leu-Ser-Cys-Ile-Ala-Met-Gln) encoded by pHS-2 could form a disulfide bridge through one of its cysteine residues with HLA-B27 cysteine [67]. In patients with *Salmonella*-triggered ReA, the persisting humoral immune response is directed primarily against LPS. Intra-articular LPS are powerful macrophagic stimulators and can trigger the synthesis of proinflammatory cytokines (TNF-α, IL-1, and IL-6). LPS contributes to the degradation of cartilage matrix by collagenases and other neutral proteases secreted by chondrocytes. LPS also suppresses proteoglycan synthesis and induces the production of nitric oxide by chondrocytes [57, 65].

V. Conclusion

Understanding the complexity and dynamic nature of the gut microbiome and its role in inflammatory disorders including SpA is a work in progress. In the process of homeostasis, host microbe interactions in the gut guide the normal development of host immune response, whereas microbial dysbiosis is implicated in disease pathogenesis.

The gut plays a role in the etiopathogenesis of more than half of patients with SpA. Control of the gut inflammation (spontaneously or drug induced), may equally control the articular disease.

The identification of SpA-associated microbiota phenotypes may aid in the diagnosis or prognosis of HLA-B27 dependent disease. Microbiome research has the potential to revolutionize research, diagnosis and treatment of spondyloarthritis [68].

Conflict of interests: No conflict of interest to declare.

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