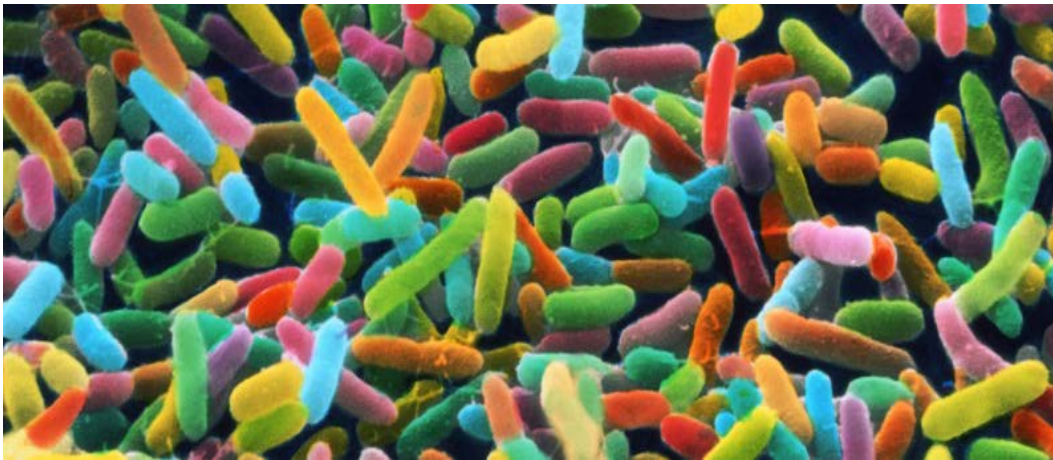


The Microbiome and Spondyloarthritis



Internal Medicine Grand Rounds

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This is to acknowledge that Andreas Reimold, M.D. has disclosed that he has financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Reimold will be discussing off-label uses in his presentation.

Dr. Reimold is an Associate Professor in the Rheumatic Diseases Division at UTSW and Chief of the Rheumatology Section at the Dallas VA Medical Center. He has been actively involved in establishing nationwide VA registries in spondyloarthritis, rheumatoid arthritis, and gout. Research coming out of these efforts has helped to characterize the susceptibilities, disease course, and treatment options in these conditions.

Purpose & Overview – This presentation discusses the role that intestinal inflammation plays in spondyloarthritis. The discussion includes the description of dysbiosis and the alterations in bacterial species found in spondyloarthritis patients. Finally, therapies such as the use of probiotics are considered.

- **Objectives**

1. To describe the range of conditions among the spondyloarthritis.
2. To review the overlap of intestinal inflammation as is seen in inflammatory bowel disease with that seen in spondyloarthritis.
3. To evaluate existing and emerging therapeutic options for spondyloarthritis.

The Spondyloarthritides: a Plethora of Conditions

Spondyloarthritides make up a group of conditions, many of which have in common a genetic association with the HLA-B27 marker. Ankylosing spondylitis, with its preponderance of axial disease, is the archetype for the group and shows HLA-B27 positivity in over 90%. Reactive arthritis (formerly Reiter’s Disease) is strongly associated with antecedent gastrointestinal or genitourinary infection, while end-stage axial involvement can be identical to ankylosing spondylitis. Psoriatic arthritis and enteropathic (IBD-related) arthritis are part of a spectrum of disease that in others does not progress past the skin or gut involvement, respectively. Uveitis can be associated with rheumatic manifestations and can be strongly HLA-B27-associated, leading some to classify it with the spondyloarthritides. Finally the pediatric age group can have enthesitis-related arthritis, a name that recognizes that the axial erosion and fusion of ankylosing spondylitis is infrequently seen in pediatrics, but inflammation at enthesial sites is.

Spondyloarthritides
Ankylosing spondylitis (AS)
Nonradiographic axial spondyloarthritis (nr-AxSpA)
Reactive arthritis
Psoriatic arthritis
Enteropathic arthritis (IBD-related)
Juvenile SpA (Enthesitis-related arthritis)
Undifferentiated SpA
(Acute anterior uveitis)

A new entity within the spondyloarthritides has been brought to attention and further characterized in recent years. It is “nonradiographic axial spondyloarthritis,” abbreviated as nr-axSpA. Patients with this entity have inflammatory back pain, are often getting a workup for possible ankylosing spondylitis, but do not have radiographic changes that meet the Modified New York Criteria for Ankylosing Spondylitis (1). These criteria specify that the sacroiliac radiographic changes should show at least grade III on one side, or at least grade II bilaterally. It is immediately clear that nr-axSpA is a misnomer, since radiographic changes can be as severe as grade II on one side and grade I on the other, not qualify for Modified New York Criteria, and then be labeled “nonradiographic.” Newer ASAS Classification Criteria for Axial Spondyloarthritis (2) include a provision for findings of sacroiliitis on MRI as well as 11 features of SpA ranging across history, clinical, and lab findings.

Modified New York Criteria for Ankylosing Spondylitis
1. Clinical Criteria <ol style="list-style-type: none"> a. Low back pain and stiffness for more than 3 months which improves with exercise, but is not relieved by rest. b. Limitation of motion of the lumbar spine in both the sagittal and frontal planes. c. Limitation of chest expansion relative to normal values correlated for age and sex.
2. Radiological Criterion: <ol style="list-style-type: none"> a. Sacroiliitis grade ≥ 2 bilaterally or grade 3-4 unilaterally
Definite ankylosing spondylitis if the radiological criterion is associated with ≥ 1 clinical criterion.

ASAS Classification Criteria for Axial Spondyloarthritis (SpA)
In patients with ≥ 3 months back pain and age at onset < 45 years---- Sacroiliitis on imaging plus > 1 SpA feature OR HLA-B27 plus ≥ 2 other SpA features
Imaging: <ol style="list-style-type: none"> a. Active (acute) inflammation on MRI highly suggestive of sacroiliitis associated with SpA b. Definite radiographic sacroiliitis according to the modified New York criteria
SpA Features: Inflammatory back pain <ul style="list-style-type: none"> Arthritis Enthesitis (heel) Uveitis Dactylitis Psoriasis Crohn's/colitis Good response to NSAIDs Family history for SpA HLA-B27 Elevated CRP

Nonradiographic axial spondyloarthritis has substantial similarities to ankylosing spondylitis. The findings of patients in both groups are equivalent in terms of symptom severity and functional impairment. Both groups have a similar prevalence of microscopic gut inflammation of 46%. A limited group of medications has efficacy in ankylosing spondylitis (e.g. NSAIDs, anti-TNF α biologics) and these medications work equally well in nr-axSpA. On the other hand, nr-axSpA occurs in males: females at 45%:55%, whereas the ratio in older ankylosing spondylitis literature is up to 90% male: 10% female. Progression of nr-axSpA to ankylosing spondylitis meeting criteria occurs in only 12% of cases, showing that X ray changes do not reflect the full range of symptoms and pathology (3). Finally, the disease classification is better-accepted by

European Medicines Agency (EMA), where the anti-TNF α drugs adalimumab (Humira) and certolizumab pegol (Cimzia) are approved for treating nr-axSpA, while in the US, the FDA has ruled against approval of these drugs for nr-axSpA, after discussing difficulties in positively diagnosing these cases, and fear of overuse of expensive injectables for run-of-the-mill low back pain. Further clinical trials in this condition will likely need to enroll candidates who have an elevated CRP level as an objective sign of inflammation, or MRI findings showing bone marrow edema or subtle erosions that were not seen on plain X rays.

Enteropathic Arthritis

Enteropathic arthritis is the spondyloarthritis that can be found in approximately 30% of patients with inflammatory bowel disease (Crohn's disease or ulcerative colitis). Involvement can be axial (i.e. spine and sacroiliac joints) and/or peripheral arthritis (4).

Prevalence of IBD and enteropathic arthritis in the U.S.

Peripheral arthritis	13%
Asymptomatic sacroiliitis	18%
Symptomatic sacroiliitis	10-18%
Axial arthritis	10-50%
Ankylosing spondylitis	5-10%
US Prevalence: IBD	5.0/1000
US Prevalence: Peripheral arthritis	0.65/1000
US Prevalence: Axial arthritis	0.5-2.5/1000

Sources: Dekker-Saeyns 1978; de Vlam 2000; Steer 2003; Reveille 2012

Two types of peripheral arthritis have been described, type I being a pauciarticular arthritis involving fewer than 5 joints and type II a polyarthritis in 5 or more joints (5).

Characteristics of peripheral arthritis in enteropathic arthritis patients.

	Type I (Pauciarticular)	Type II (Polyarticular)
# of Joints	<5	≥5
Duration	Acute, <10 weeks	Chronic
IBD Association	During IBD flares	Independent of IBD activity
Distribution	Asymmetrical	Symmetrical
Joints	Large joints: knee	Small hand joints

The presence of HLA-B27 allele has prognostic implications for enteropathic arthritis patients with axial involvement. HLA-B27 is present in about 8% of the Caucasian population, in 9-41% of patients with IBD and sacroiliitis (but no ankylosing spondylitis), and in 50-61% of patients with IBD and ankylosing spondylitis. By comparison, ankylosing spondylitis without IBD shows HLA-B27 positivity in up to 95% of patients. Therefore, having IBD with HLA-B27 positivity means a 7 to 10 fold increase in the risk of sacroiliitis and spondylitis compared to IBD without HLA-B27 (6).

Therapy of enteropathic arthritis borrows from existing approaches to IBD and ankylosing spondylitis. For peripheral type I arthritis, treatment of the underlying IBD may be helpful, especially bowel resection in certain patients with ulcerative colitis but not Crohn's. For axial arthritis, oral DMARDs such as methotrexate, sulfasalazine, and azathioprine have not shown a benefit. One is left with use of NSAIDs and anti-TNF α medications. When considering NSAIDs, there is a concern of GI bleeding risks in IBD patients, minimizing their usefulness. Anti-TNF α drugs are the most effective agents for the combination of IBD and axial arthritis, with the exception of etanercept, which is not useful in the treatment of IBD.

FDA-approved uses of anti-TNF α medication in IBD and ankylosing spondylitis

Drug	IBD	Ankylosing spondylitis
Infliximab (Remicade)	Yes (CD and UC)	Yes
Etanercept (Enbrel)	No	Yes
Adalimumab (Humira)	Yes (CD and UC)	Yes
Golimumab (Simponi)	Yes (UC)	Yes
Certolizumab (Cimzia)	Yes (CD)	Yes

The Connection of the Gut with the Joints

Joint involvement occurs in a substantial 30% of IBD patients. Remission of joint inflammation can be seen with resolution of gut inflammation in peripheral pauciarticular disease (but unlikely with polyarticular or axial disease). On the other hand, gut involvement has also been studied in spondyloarthritis patients. Full-blown IBD develops in about 7% of SpA patients within 5 years (7). However, microscopic gut inflammation is very common in SpA patients. In the 1980's, Mielants published that microscopic gut inflammation could be found on colonoscopic biopsies in about 50% of SpA patients without bowel symptoms, and studies of colonoscopies plus fecal markers of inflammation can bring the number up to about 66%. These numbers have been confirmed by more recent studies in the Ghent Inflammatory Arthritis and Spondylitis (GIANT) cohort. While the chronic inflammation appeared the same in SpA and early Crohn's under the light microscope, further characterization of the cell types has shown some differences. SpA patients have rare CD14+ macrophages and expanded M2 macrophages (which make IL-10, an anti-inflammatory cytokine), while Crohn's patients commonly show CD14+ macrophages (8). The findings of gut inflammation have now been described in ankylosing spondylitis, reactive arthritis, nr-axSpA, pediatric SpA, and psoriatic arthritis (though less common). Gut inflammation is more extensive in axial rather than peripheral SpA, but the same in ankylosing spondylitis and nr-axSpA (9).

Several groups have helped to define the associations of gut inflammation with SpA disease characteristics. Initial chronic gut inflammation in a SpA patient is associated with more bone marrow edema on sacroiliac joint MRI and a higher risk of evolution to ankylosing spondylitis. The Ghent group has described odds ratios for a series of patient characteristics and findings that associate with microscopic gut inflammation (10):

1. Age (younger age has OR 0.85)
2. Male sex (OR 8.9)
3. Higher disease activity, measured by BASDAI (OR 2.05)
4. Restricted spinal mobility, measured by BASMI (OR 1.94)

There is overlap in genetic polymorphisms associated both SpA and IBD. Examples include the IL23 receptor, which affects neonatal development of tolerance to microorganisms and is a component of pro-inflammatory pathways in both diseases. In addition, CARD9 (caspase recruitment domain 9) polymorphisms affect immunologic responses to infectious organisms and modulate the pro-inflammatory Th17 pathway (11).

Microbiota and the Microbiome

Definitions:

1. Microbiome: the total of all the microbial genomes found in and on the body.
2. Microbiota: the population of microorganisms that contain the microbiome. These include bacteria, fungi, and archaea. Viruses are variably included in this definition.
3. Dysbiosis: loss of diversity of the microbiota in the gut.

The microbiota outnumber human cells in the body by a factor of 10, and the microbiome in and on our bodies contains 100-fold more genes than the human genome. Microbiota can be found in surface locations, mucus membranes, and additional areas of the body. The list includes the conjunctiva, oral mucosa, saliva, teeth (with different microbiota possible on different teeth), the respiratory tract, the GI tract, the GU mucosa, the mammary glands, the skin, and an airborne microbiome.

Study of the microbiome formerly relied on culture of resident microorganisms, which excluded numerous species that were not amenable to culture. A more complete picture has become possible since high throughput sequencing techniques have been put in place. A common method is 16S ribosomal RNA sequencing, which focuses on this RNA species found in bacteria but not mammalian genomes. Differences in 16S ribosomal sequences allow differentiation among bacterial species. Further techniques of next generation sequencing and massive parallel whole genome shotgun sequencing allow study of all sequences in the sample under study. This already generates massive amounts of data. For the future, the field is gaining

additional complexity with the addition of transcriptomics and metabolomics to study the transcripts and metabolic products present in each sample.

The diversity of the microbiota is a key feature in assessing dysbiosis. In the intestine, there are an estimated 100-160 bacterial species in a given individual, with at least 1150 total species identified in studies of healthy individuals (12). There are 9 major divisions of bacteria with four phyla predominating: anaerobic Firmicutes (50-75%, including Clostridia), Bacteroidetes (10-50%, including Bacteroides, Prevotella, and Porphyromonas), Proteobacteria (<1%, including E. coli), and Actinobacteria (1-10%, including Bifidobacteria). These four may account for up to 98% of 16S rRNA sequences, a reflection of their relative abundance. Although still controversial, 3 enterotypes have been described that characterize healthy controls, with prominence of Bacteroides, Prevotella, or Ruminococcus species. On the skin, the predominant bacteria are Actinobacteria and Proteobacteria species. The teeth show Porphyromonas gingivalis, among others, and individual teeth can have distinctive microbiota. The list of sites and conditions where dysbiosis is implicated in pathogenesis is large and growing, as highlighted in the table.

MEDICAL SPECIALTY	CONDITIONS
GI	IBD, IBS
Dermatology	Psoriasis
Neurology, Pain Management, Psychiatry	Pain, Stress, Depression, Neurodevelopmental disorders
Heme-Onc	Cancer
Allergy	Allergies
Pulmonary	Inflammatory lung diseases
Endocrine	Obesity, metabolic syndrome
Rheumatology	Autoimmune conditions

Development and Functions of the Intestinal Microbiota

The microbiota have vast effects beginning with humans' early development after birth, influencing the growth and complexity of the innate and adaptive immune systems, contributing to the physical barrier between enterocytes and the gut lumen, producing antibacterial substances, and contributing to nutrition of the host in a symbiotic manner.

The microbiota are first influenced by a baby's delivery method, where GU and GI organisms are transferred during vaginal birth. There are changes and shifts in the microbiota during the first years, influenced by genetic predispositions, diet, infections, hygiene, and antibiotic use. A more stable phenotype is found starting at 3 years of age. The microbiota have an "ecological memory," that favors a return to previous microbiota after dietary- or antibiotic-induced

changes. In later life, perturbations in microbiota come from diet, smoking, long-term antibiotics, pathogenic infections, and obesity.

The microbiota have a complex relationship with the intestinal wall. A layer of mucus about 50 microns thick separates the microbiota from the intestinal villi. Dr. Hooper's group at this institution has demonstrated that anti-bacterial properties of the lectin RegIII γ are essential in maintaining the 50 micron separation between intestinal organisms and the enterocyte surface (13). Other such molecules, which have functions in maintaining the mucus barrier and controlling luminal bacteria, are under active study.

Besides occupying a physical space that might otherwise be available to pathogens, commensals in the GI tract can have an inflammatory or an immunoregulatory/anti-inflammatory effect. The development and education of the entire immune system is shaped by intestinal recognition of non-self, with hypoplastic Peyer's patches and submucosal immune tissue described in germ-free animals. The innate immune system is engaged by conserved pathogen-associated molecular patterns (PAMPs) found on all microbes. Genetic effects from differences in Toll-like receptors, NOD-like receptors, and the HLA system restrict and shape the initial response to organisms. The adaptive immune system can also be activated, with dendritic cells and macrophages sampling bacterial products, activation of T cell subsets (e.g. Th1 and Th17 during inflammatory responses, and Treg for immunoregulatory responses including production of IL-10), and B cell activation. IgA is the mucosal immunoglobulin produced in large quantities in the gut and can have specificity for the gut organisms (14).

In addition, the products of microbial metabolism may have additional effects on the intestinal wall. As an example of a symbiotic relationship, certain organisms such as *F. prausnitzii* metabolize short chain fatty acids (SCFA), which become a nutrient for enterocytes. One of the SCFAs, butyrate, has been studied separately and found to have anti-inflammatory effects, inhibiting the pro-inflammatory transcription factor NF- κ B, and increasing colonic Treg cell numbers and increasing their FoxP3 production. Without these protective actions of SCFAs in IBD and SpA, enterocytes are at increased risk of injury and intestinal wall permeability may rise in a pathologic manner (15).

Microbiota in IBD

Multiple studies have described alterations in microbiota in patients with IBD. Dysbiosis is a feature of IBD, with decrease in the diversity of microorganisms found. One prominent example is the decrease in the anaerobe *Faecalibacterium prausnitzii* in IBD. *F. prausnitzii* is a member of the phylum Firmicutes and is the major bacterium of the *Clostridium leptum* group. Products

such as butyrate from *F. prausnitzii* metabolism provide energy to colonocytes and produce an anti-inflammatory milieu. In IBD, the prevalence of *F. prausnitzii* can decrease dramatically from its usual 5% of fecal mass, especially during periods of active inflammation (16). Treatments employed at times for IBD, such as antibiotics in Crohn's disease or probiotics in ulcerative colitis, provide further examples of altering the microbiota for therapeutic benefit. Animal models also support the role of microbiota in IBD. The T-bet and recombination activating gene 2 (RAG-2)-deficient mice develop a spontaneous form of UC driven by $TNF\alpha$ (TRUC model). The colitis improves with broad-spectrum antibiotics, the disease does not occur in offspring of antibiotic-treated mice, and vertical transfer of colitis can occur even to T-bet sufficient mice (17).

Microbiota in Animal Models of Spondyloarthritis

Animal models have made clear that the introduction of as little as one commensal organism species can have a profound effect on the immune response and the course of arthritis. A specific example is the K/BxN murine model of arthritis, which has features of rheumatoid arthritis (18). These animals do not develop arthritis in the germ-free state. However, reintroducing segmented filamentous bacteria, which are Gram-positive anaerobes, caused Th17 cells to develop in the lamina propria of the intestine and arthritis to develop in the joints. The arthritis could be prevented by blocking IL 17 with an antibody, or early use of antibiotics targeting Gram-positives.

Several animal models of spondyloarthritis further reinforce the relationship of microbiota to clinical disease. The HLA-B27 transgenic rat model develops colitis, peripheral arthritis, and spondylitis but not in the germ-free state (19). Reintroduction of *Bacteroides vulgatus* leads to a return of colitis, while feeding *Lactobacillus rhamnosus* GG maintains remission. A similar finding emerged from study of the peripheral arthritis in HLA-B27 transgenic mice, where arthritis was absent in the germ-free state but developed with introduction of anaerobic bacteria (20). A third animal model is the ZAP-70^{w163c}-deficient SKG mouse, which develops ileitis and arthritis when in standard housing conditions. The disease is abrogated in the germ-free state and can develop in germ-free animals injected with microbial β 1,3-glucan (curdian) or by introduction of limited microbiota (21).

Microbiota in Human SpA

A long-established connection between bacteria and SpA exists in the example of reactive arthritis occurring after GI infection (*Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*) or GU infection (*Chlamydia*, others). A genetic predisposition is found here, with presence of HLA-B27 overrepresented, but not to the same extent as in ankylosing spondylitis. Over 10-20 years, 20% of reactive arthritis patients develop ankylosing spondylitis. Bacterial products, including

metabolically-active Chlamydia, have been found in joints as one explanation of joint inflammation. Treatment of reactive arthritis that is PCR-positive (peripheral blood or synovial biopsy tissue) for *C. trachomatis* or *C. pneumoniae* using doxycycline + rifampin or azithromycin + rifampin for 6 months was more effective than placebo in reducing joint inflammation and converting to a PCR-negative status (22).

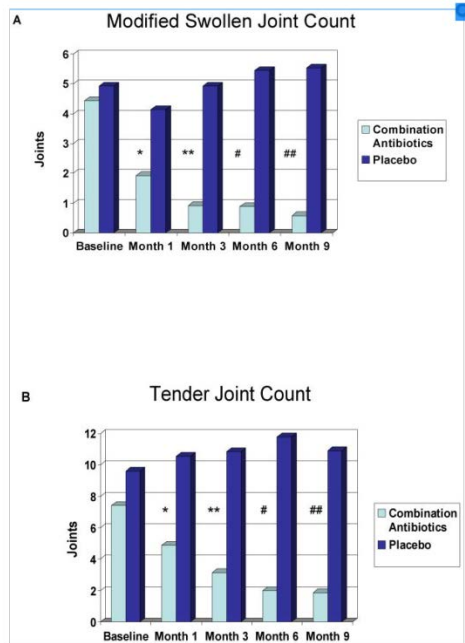


Figure. Combination antibiotics treatment of Chlamydia-related reactive arthritis showed improvement in swollen and tender joint counts (light columns) compared to placebo-treated patients (dark columns). (22)

Stebbing et al compared the fecal microbiota of 15 AS patients with 15 healthy controls (23). Their methodology in 2002 was denaturing gradient gel electrophoresis and fluorescent in situ hybridization using specific DNA probes, which is far less comprehensive than the next-generation sequencing used in later papers. The findings were of overall similarity of organism profiles, with trends for decreases in *Bacteroides-Provotella* and *Clostridium leptum* groups and increases in *Bifidobacterium* in AS.

A study using more modern techniques of PCR amplification followed by 16S rDNA sequencing was performed in pediatric patients with enthesitis-related arthritis (ERA) (24). Here, 25 children with ERA were compared to 13 healthy controls. There were several differences between the groups:

1. ERA patients had significantly less *Faecalibacterium prausnitzii* (3.8% vs 10%, $P=0.008$), a finding previously seen in studies of microbiota in IBD.
2. Two clusters were seen in principal coordinates analysis. One group of 8 ERA patients had elevation of an unspecified *Bacteroides* species. 7 of the other ERA patients had marked elevation in *Akkermansia muciniphilia*, an otherwise very rare species.

An extension of this study to children with other forms of JIA showed that only the ERA group had such dramatically reduced *F. prausnitzii* levels.

Costello et al. published a recent study of 9 AS patients and 9 controls who studied terminal ileal biopsies obtained during colonoscopy (25). The findings were:

1. Increases in Lachnospiraceae, Veillonellaceae, Prophyromonadaceae, Bacteroidaceae.
2. Decreases in Ruminococcaceae and Rikenellaceae.

In a comparison of these 3 trials, the similarities seen in at least two of the studies include decreases in the Clostridiales order (e.g. *F. prausnitzii*) and an increase in Bacteroides, Bifidobacterium species, and Verrucomicrobiaceae family (e.g. *A. muciniphila*).

The decrease of *Faecalibacterium prausnitzii* in both IBD and SpA has led to further interest in its properties. *F. prausnitzii* cultured with human peripheral blood mononuclear cells leads to lower production of pro-inflammatory cytokines such as IL-12 and IFN γ and higher production of anti-inflammatory IL-10 than other bacteria such as *L. acidophilus* (26). In addition, *F. prausnitzii* metabolic activity produces short-chain fatty acids (SCFAs) such as butyrate. SCFAs are nutrients for enterocytes, so that their reduction can lead to cell stress, decreased tight junction viability, and increased intestinal permeability. Butyrate itself has been found to have anti-inflammatory properties such as increasing Treg cell numbers and promoting their FoxP3 expression (15).

Treatment

Antibiotic use has a defined role in Crohn's disease and reactive arthritis but is not effective in axial SpA. Changes in diet can result in rapid alterations in microbiota, with microbial populations often found to rebound to their previous status once the dietary changes are no longer maintained. Some literature describes the ability of exclusive enteral nutrition to induce remission in pediatric IBD at rates similar to corticosteroids. No such studies of diet change or enteral nutrition exist for SpA.

Probiotics, Prebiotics, and Synbiotics

Probiotics are defined by the World Health Organization as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host." With the recognition that dysbiosis involves altered numbers or strains of bacteria in the intestine, the next step becomes trying to achieve a healthier composition for the microbiota. This does not imply that a host's pre-morbid microbiota will be restored, only that a known non-pathogenic microbiota will be the goal.

A prebiotic is defined as “a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host wellbeing and health.” Some dietary fibers are among the few substances that actually meet this definition. Examples are bifidogenic, non-digestible oligosaccharides, such as inulin, its hydrolysis product oligofructose, and trans-galactooligosaccharides. Salutary secondary effects such as cancer prevention, cholesterol reduction, and immunomodulation are presumed to be mediated by the microbiota acting on prebiotics and are not well-proven.

A synbiotic is a synergistic combination of probiotics and prebiotics, representing a category that will likely be filled out in future years as the components are better studied.

Probiotics in Animal and Clinical Studies

Probiotics have multiple potential benefits in the treatment of dysbiosis. First, they may have a barrier effect, competing out other intestinal bacteria from reaching the lamina propria of the gut and stimulating beneficial mucosal immunity. Second, probiotics may enhance mucus production and alter mucus consistency. Third, probiotics may act on the mucosal immune system to cause secretion of IgA, protective defensins and bacteriocins in the gut. They may act on dendritic cells to make them less responsive, leading to an anti-inflammatory rather than a pro-inflammatory state.

One example from a rodent model involves giving *Lactobacillus casei* to rats with collagen-induced arthritis (27). This treatment reduced proinflammatory cytokine levels, increased IL-10 (a generally anti-inflammatory cytokine), improved arthritis score compared to controls, and reduced histopathologic changes such as lymphocytic infiltrates (an effect similar to using methotrexate).

In human rheumatoid arthritis, there is a pilot randomized, double-blind, placebo-controlled trial of probiotics *Lactobacillus rhamnosus* GR-1 and *L. reuteri* RC-14, in 30 clinically-active patients (28). The result showed an improvement in disability scores (the Health Assessment Questionnaire) but not in the ACR-20 response (a 5-component measure of RA disease activity). Follow-up of this pilot trial has not appeared in the last 4 years.

Probiotics in UC

The use of probiotics in UC builds upon the recognition that 1) UC is mainly a mucosal disease, and 2) that the genetic background of the host permits certain gut lumen bacteria to initiate and perpetuate an inflammatory response, even when an acute infection is not present.

Studies of probiotics in UC date back more than a decade. In one, use of nonpathogenic *E.coli* Nissle 1917 (Mutaflor, Ardeypharm) induced remission and maintained remission for at least 1

year in mild to moderate UC (29). This treatment was as effective as mesalamine use. In a second study, the authors used a combination of 8 strains called VSL#3 (Bifidobacterium breve, B. longum, B. infantis, Lactobacillus acidophilus, L. plantarum, L. paracasei, L. bulgaricus, Streptococcus thermophiles)(30). Here, patients with mild to moderate UC again went into remission and maintained remissions at least 6 months. Overall, Lactobacillus species were generally not effective in UC, with some reports not in agreement (e.g. Lactobacillus GG studied by Zocco et al, 2006).

Probiotics in Ankylosing Spondylitis

A double-blind, placebo-controlled study of 63 patients with ankylosing spondylitis by Jenks et al. was done to compare using a combination of 3 probiotics (Streptococcus salivarius, Bifidobacterium lactis, and Lactobacillus acidophilus) versus placebo for 12 weeks (31). The strains were chosen for their previous use in animal models of arthritis and in studies of IBD. There was no difference in the primary outcome measures including Bath Ankylosing Spondylitis Functional Index (BASFI) 10% improvement, nor in secondary measures such as pain, spinal mobility, CRP, fatigue, quality of life, or ASAS20 responses.

Current Limitations in Use of Probiotics

While the presence of dysbiosis leads to research attempts to restore non-pathogenic microbiota, numerous questions remain:

1. Are we searching for dysbiosis in the right locations? Do we need to study the microbiome in different subsections of the mouth and gut and tailor treatments according to dysbiosis in one or multiple compartments?
2. Probiotic strains: What are the most useful strains to use? The strains may differ by disease state and by individual so that individualized therapy may be needed.
3. Probiotic growth in vitro: At this time, the role of multiple intestinal organisms is coming to light due to identification by sequencing technology. It is not currently possible to culture many of these, so advances need to be made before these organisms are grown in culture and to industrial scale as potential probiotics.
4. Probiotic combinations: Do we treat with one probiotic, introduce multiple microorganisms, or replace the entire microbiota?
Is fecal microbial transplant, which is already used for refractory C. difficile infection, a current option while the microbiota get better-characterized? It has not been studied in SpA.
5. Probiotic administration: What route for administration is effective? Oral probiotics need to get past stomach acid, while rectal administration has less patient acceptance.

6. Probiotic dose: What is the proper dose of probiotics? Probably millions to trillions are needed if given orally.
7. Duration of treatment: What is the best duration of treatment? It takes at least 7 to 10 days to get substantial numbers of organisms past stomach acid. Treatment needs to be ongoing as there is reversion to the original microbiota if treatment is stopped quickly.
8. Quality control: What is the quality control of probiotic supplements available over the counter?
9. Should there be more investigation of antibiotics as a “conditioning regimen” to wipe out the current dysbiotic microbiota to create a niche for probiotic species?
10. Should we be banking our stool so that we can reset our microbiota to an earlier, healthier version?

Future Studies

Instead of focusing only on the microbiota, the complex interactions of microorganisms and the host may be studied as a unit. In **pharmacometabolomics**, a drug’s action and metabolization is studied based on genome, microbiome, and environment to give a highly individualized picture of a patient’s clinical condition. Study of the microbiome already runs into the millions of datapoints, and integration with additional factors from the human genome and environment will further increase the complexity of analysis.

The association between disease in the gut and inflammation in the joints remains a clinical observation without a definitive pathogenic pathway. Future studies will be needed to better address if there is translocation of lymphocytes and other inflammatory cells from the gut to the joints, or if there might be translocation of microbial products or fragments between these locations, as is seen at times in reactive arthritis caused by Chlamydia.

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