PROBIOTICS FOR THE MANAGEMENT OF RHEUMATOID ARTHRITIS

By

HARMAN KAUR DHANOA

_____

A Thesis Submitted to The Honors College

In Partial Fulfillment of the Bachelors Degree
With Honors in

Public Health

THE UNIVERSITY OF ARIZONA

M A Y 2 0 1 8

Approved by:

_____

Dr. Ronald Watson
Department of Health Promotion Sciences

Abstract

The human body contains millions of diverse microorganisms that collectively make up the human microbiome. There are variations in the density of bacteria across body sites, and the composition of these microbial species is regulated by several factors including genetics, diet, infectious agents, and other environmental triggers. Pioneering research in this field hypothesized that host-microbe equilibrium is essential for the health of the host and that changes or dysbiosis within the equilibrium are associated with the pathogenesis of several diseases. Recent studies have utilized new sequencing technologies to draw an association between dysbiosis and inflammatory disease. Rheumatoid arthritis (RA) is an inflammatory autoimmune disease in which synovial inflammation causes joint failure. Probiotics have the potential to stabilize the host-microbe equilibrium and decrease the inflammatory process. The available randomized clinical trials (RCTs) show that probiotics can reduce proinflammatory cytokines; however, the clinical effects of probiotics on RA disease activity remain unsettled.

Introduction

There are millions of commensal and symbiotic microorganisms within the human body. There are roughly as many bacteria as human cells in the body.[1] The sites of colonization are the following: the skin, oral cavity, upper respiratory tract, female genital tract, and intestinal tract. The process of colonization begins right at birth when the baby is exposed to bacteria in the vaginal canal of the mother.[2] In-depth analyses of the microbiome have found that the gut is the most prevalent site of colonization and contains the greatest bacterial diversity.[3] Individual gut microbiome composition is influenced by factors like age, drug use, nutrition, stress, and infection.[2]

The human microbiome has a significant role in the development and maintenance of the immune system.[4] Thus, it is essential that the commensal bacteria maintain local homeostasis with the host's intestinal immune system otherwise the immune response may be disrupted. The gut normally provides protection against antigens from many microorganisms. While healthy microbiota can be beneficial to prevent disease, the host immune system utilizes multiple methods to protect itself against the gut microbiome and other pathogens including a mucus layer, tight junctions between epithelial cells, and responses from the innate immune system.[2] The initial, innate response in RA is non-antigen specific site and involves macrophages, dendritic cells, natural killer cells, cytokines, and γ/δ T cells.[5] Macrophages and dendritic cells first stimulate the innate immune system for a rapid effector response. The adaptive immune response that causes RA is antigen-specific, and the response follows after the activation of T cells. The proinflammatory cytokines GM-CSF, IL-1, TNFα, IL-12, IL-15, and IL-18 are known to contribute to the articular destruction in RA patients.[6] One focus of diet therapy for RA is to heal the microbiome and restore a healthy immune system.

Rheumatoid Arthritis

Arthritis affects 54.4 million men and women in the United States.[7] It includes more than 100 disorders of the joints, tissues that surround the joint, and connective tissue. RA is a specific type of arthritis which has a prevalence between 0.5 and 1% among adults worldwide, with greater prevalence among women.[8] It is an inflammatory autoimmune disease in which the immune system attacks healthy cells of the body to cause inflammation, pain, and eventually joint deformities. RA generally inflicts joints of the hands, wrists, and knees. Current treatment options for RA include disease-modifying antirheumatic drugs (DMARDs), non-steroidal anti-inflammatory agents (NSAIDs), corticosteroids, biological response modifiers, and self-

management strategies.[7] Such treatment options simply manage the disease. This is because the exact etiology of the disease is still unknown.

It is understood that genetic factors are involved in the development of RA.[4] However, research has shown that there must be an environmental factor that triggers autoimmunity in genetically predisposed individuals.[3] Several studies conducted on monozygotic and dizygotic twins found a low percentage of concordance for the development of RA.[4] There is no definitive proof that bacteria cause RA. Yet, RA has become one of the most carefully studied autoimmune disorders with respect to microbial dysbiosis. There is knowledge that RA development is at least related to abnormal immune function, excess production of autoantibodies, and pro-inflammatory T lymphocytes.[2] The microbiome begins to explain gaps in the search for a causative agent for RA.

Probiotics

As early as 1907, Russian scientist Elie Metchnikoff published *The Prolongation of Life: Optimistic Studies* to provide rationale for the health benefits of regular consumption of fermented milk.[9] This observation ushered in a wave of research on what are now considered modern probiotics. Probiotics are defined as live microorganisms (bacteria or yeasts) that provide a health benefit to the host when consumed in adequate amounts.[10] Metchnikoff had hypothesized that some microorganisms present within the body are toxic by nature and that the harmful microbes can be modified by beneficial microbes through diet.[9] It is now understood that the bacteria in the human host can be classified as either harmful, beneficial, or a type that exhibits an intermediate property. The harmful bacteria include *Clostridium, Enterobacteriaceae, Veillonella, and Proteus.*[10] The beneficial bacteria of the *Bifidobacterium* and the *Lactobacillus* genera will be discussed in this chapter as potential forms of probiotic diet therapy, both of

which are Gram-positive and nonspore-forming rods.[10] One type of health benefit related to consumption of beneficial bacteria is regulation of the immune system. Probiotics yield an immunomodulatory effect either by stimulation or inhibition of natural immune responses. In relation to RA, probiotics are believed to affect the imbalance of cytokine production that is known to cause inflammation in RA patients. Cytokines are the main group of immune response mediators that provide cell-to-cell communication during innate and acquired immune responses.[10] RA patients suffer from an overproduction of pro-inflammatory cytokines and insufficient production of anti-inflammatory cytokines. Probiotics are known to induce the production of certain cytokines, namely of the Interleukin gene cluster.[10] Thus, probiotics may have a balancing effect on the immune system and could offer a simple intervention to treat RA. The health outcomes for probiotic diet therapy differ among population groups depending on the specific strain of bacteria, the dose, and the frequency of treatment. The general estimate for a probiotic dose is at least 10^9 colony forming unit (CFU) each day; however, optimum dose for each strain remains to be determined.[10] Across species, probiotics generally have a wide safety margin and minimal side-effects like nausea, bloating, and thirst.[8]

RA and Gut Bacteria

The gut mucosa is the primary mucosal site attributed to the onset of RA. When functioning properly, the complex interactions in this region between the human host and 30-400 trillion microorganisms allow for gut homeostasis to be maintained in a symbiotic relationship.[11] The microbiota digest and ferment carbohydrates, synthesize vitamins, and prevent colonization by harmful pathobionts that are associated with chronic inflammatory conditions. In return, the host provides a healthy environment for the microbiota to survive. The health of the host is compromised when dysbiosis upsets normal immune system functions.

Research has found that patients with early RA exhibit an altered gut microbiome when compared to non-RA controls, as evidenced by significantly different fecal samples between the two groups.[12] In addition, it was found that almost 20% of Inflammatory Bowel Disease (IBD) patients have episodes of arthritis.[2] Thus, the hypothesis is that intestinal bacteria influence RA. Other biomarkers of dysbiosis within the gut include increased inflammation, defective gut barrier function, and changes in the differentiation of naive CD4+ T-cells into effector T cells or Tregs.[2] Tregs are regulatory T cells that supply self-tolerance to the body by suppressing immune responses against autoantigens, and the cells are crucial for the prevention of RA.[13] Bacterial dysbiosis may cause Tregs to be defective in their ability to regulate other types of cells that release pro-inflammatory cytokines. Several studies have identified an important relationship between Tregs and pathogenic T-helper 17 (Th17) cells that also contribute to autoimmunity.[14] The decline of Treg cells creates a Treg/Th17 imbalance that results in a chronic, proinflammatory state. A decline in Treg cells also leads to the production of autoantibodies like rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibodies that lead to RA.[14] Finally, dysbiosis of the intestinal mucosa affects the ability of Toll-like receptors (TLRs) to function as innate immune response sensors.[15] There are 10 types of TLRs in humans that induce signal pathways for the production of cytokines in the presence of an infection. TLRs are highly expressed within the joints of RA patients, and studies suggest that abnormal TLR ligands contribute largely to the chronic inflammation that is present in RA.[16] Gut bacteria significantly impact local homeostasis within the host.

RA and Oral Bacteria

The production of RA-related antibodies also occurs at the oral cavity mucosal site. There are over 700 species of microorganisms colonizing this cavity, yet the periodontal pockets are

the most significant sites as they may contain up to 10^8 bacteria.[17] There is a relationship between RA and periodontopathic bacteria, which may allow RA-related antibodies to be identified in periodontal disease patients prior to the onset of RA symptoms.[17] RA patients also have a considerably increased comorbidity with periodontal disease and patients with periodontal disease are more likely to have RA. An epidemiological relationship may exist between the two diseases because of their similar risk factors, mechanisms for disease progression, and the fact that treatment of periodontal disease positively impacts RA severity in patients.[18] Periodontal diseases, such as periodontitis and gingivitis, are dysbiotic and polymicrobial conditions. Inflammation and tissue damage occurs when the host's innate and adaptive immune functions fail. The pathogens *P. gingavalis* and *A. actinomycetemcomitans* are may cause the development of RA-related antibodies, known as anti-citrullinated protein antibodies (ACPAs), through the production of citrullinated proteins.[17] The critical Th17 cells that are present in diseased gut mucosa also appear in the sites of periodontal disease where chronic inflammation exists.[2]

A murine model by Chukkapalli *et al* [18] found that oral bacteria exacerbate RA by infecting mice with three oral pathogens (*P. gingavalis, T. denticola,* and *T. forsythia*). These strains are common in human periodontal disease as well. The infection occurred 24 weeks before inducing collagen-induced arthritis (CIA). The infected mice showed increased inflammation and more severe destruction of cartilage while suffering from active arthritis. The injected bacteria were present within the synovial joints of the mice, thus supporting the hypothesis that the bacteria of the oral cavity have an important role in RA. The study suggests that pathogenesis of RA follows in two steps: harmful subgingival bacteria first attack the host immune system in the form of periodontal disease and the resulting inflammatory conditions cause tissue damage in the form of RA.[18] These findings provide knowledge about the human

microbiome and autoimmune disease that could lead to more effective treatment for both periodontal disease and RA in the future.

Probiotics as RA therapy

RA patients are often required to undergo long term therapy. Though primarily efficacious, the treatment options of pharmaceutical drugs can yield unpleasant side effects. Some 30-60% of RA patients utilize complementary and alternative medicine (CAM) for relief of pain and overall well-being.[19] Probiotics are one of several available nutritional supplements that may provide adjuvant therapy for RA. A limited number of high-quality studies with murine models and human subjects have found a positive relationship between oral administration of certain probiotic strains and decreased RA disease activity. These studies differ by probiotic strain and dose. They highlight the most favorable observations for each strain.

Lactobacillus casei

The supplementation of *Lactobacillus casei (L. Casei)* improved RA symptoms and inflammatory biomarkers among study subjects.[20,21] In the human RCT by Alipour *et al* [20], female patients with inactive to moderate levels of RA were studied. All participants had been under treatment with DMARDs. The study examined several measurements of RA in patients: tender and swollen joint counts, global health score, disease activity score, serum high-sensitivity C-reactive protein (hs-CRP), and serum levels of pro-inflammatory cytokines IL-1b, IL-6, IL-12 and TNF-α and the regulatory cytokine IL-10. Patients in the probiotic group (n=22) received a daily capsule of at least 10^8 colony-forming units of *L. casei 01* and maltodextrin, while the placebo group (n=24) received maltodextrin only. At the end of the study, significant differences were observed between the study group and the placebo group.[20]

Compared to baseline measurements, patients in the *L. casei* group had reduced serum hs-CRP levels, less swollen and tender joints, and reduced disease activity.[20] There was also a significant improvement in the IL-10, IL-12 and TNF-α levels of patients in the *L. casei* group. The study reported no adverse effects for probiotic supplementation. The study also contributed to the current understanding of the dose-dependent effects of probiotics. The researchers found their intervention to be more efficacious than previous studies that had much larger doses of probiotics. There is some evidence that low doses of live microorganisms can be ideal for treatment, since high doses have sometimes shown opposite effects to those beneficial effects seen at low doses.[22]

The Alipour *et al* [20] study did not fully explore the mechanisms by which probiotics improved RA symptoms in their subjects. It was briefly explained that probiotics can regulate the immune system in RA patients by increasing the strength of Treg cells, limiting Treg apoptosis, and preventing the production of harmful Th17 cells. The researchers suggest taking a daily capsule of 10^8 CFU of *L. casei 01* to improve disease activity at a chemical level and alleviate physical symptoms of swollen joints in patients.

So *et al* [21] observed similar, beneficial effects of *L. casei* supplementation in a murine model; however, this study further investigated the effector functions of CD4+ T cells to explain the underlying mechanisms of *L. casei* treatment for RA. In their study, female Lewis rats were given experimental CIA. Rats in the experimental *L. casei* group were fed 5×10^9 CFU/dose and compared to a placebo group. *L. casei* inhibited the production of several proinflammatory molecules: IL-1β, IL-2, IL-6, IL-12, IL-17, IFN-γ, TNF-α and Cox-2 by CD4+ T cells. Probiotic supplementation also increased anti-inflammatory IL-10 levels. The treatment group exhibited reduced swelling in the paws.

To explain the underlying mechanism of *L. casei* in arthritis, the researchers hypothesize that *L. casei* down-regulates the production of Th1-type cytokines while up-regulating the Th2-type cytokines.[21] Data from quantitative real-time PCR analysis shows that the changes in cytokine expression were related to CD4+ T cells. Th1 cells are a type of T cell that produce the pro-inflammatory cytokines known to exacerbate synovial inflammation, while Th2 cells secrete anti-inflammatory cytokines needed to suppress autoimmunity. The researchers found that *L. casei* reduced the type II collagen-reactive effector function of Th1 cells, thereby inhibiting the release of proinflammatory cytokines. These findings suggest that *L. casei* can regulate the immune response in a manner that would benefit patients of RA and similar immune disorders.

Lactobacillus helveticus

Lactobacillus helveticus (L. helveticus) can also reduce RA disease activity by mechanisms similar to those identified by Alipour *et al* [20] and So *et al*.[21] The Kim *et al* [23] study is significant because it utilized an *ex vivo* screening system prior to testing the *in vivo* model in mine. The screening system was used to determine which strain of probiotic would provide the best candidate for treatment in experimental CIA. This was completed by culturing lymphocytes from the lymph nodes with probiotics. *L. helveticus* HY7801 was selected based on its IL-10^high/IL-12p^low expression profile. This balance of anti-inflammatory/inflammatory cytokines was selected for its implications in RA development. The researchers believe this could make an important selection marker when choosing a probiotic. More research on *ex vivo* screening for probiotics is needed.

The study found that supplementation of probiotics three weeks prior to induction of CIA had a preventative effect on the development of CIA.[23] First, the researchers tested three strains as pretreatment options: *Bifidobacterium longum (B. longum), L. helveticus, and*

Lactobacillus johnsonii (L. johnsonii). The mice were given a dose of 5×10^8 CFU/day. All three probiotics reduced the development of CIA; however, *L. helveticus* HY7801 most effectively reduced CII-reactive immunoglobulin antibodies. Thus, this strain was selected to treat ongoing arthritis in the second phase of the experiment. The study later found that *L. helveticus* HY7801 delayed the onset of experimental RA and reduced symptoms of paw swelling.

It is believed that *L. heleveticus* improved disease activity by reducing pro-inflammatory cytokines TNF-α, IFN-γ, and IL-17A and enhancing IL-10 expression by CD4 T cells.[23] These findings are consistent with the So *et al* [21] study. Their findings demonstrate the ability of probiotics to treat experimental RA, which may translate to beneficial effects in humans. The study contributes to the literature by providing a strategy to select probiotic strains *ex vivo* prior to *in vivo* intervention.

Lactobacillus plantarum

A recent study of complete Freund's adjuvant (CFA)-induced arthritis in rats found that the cell wall content of *L. plantarus* can reduce progression of inflammation in arthritis.[24] Gohil *et al* [24] created a model of chronic polyarthritis by testing six groups of female Wistar rats. In the treatment groups, rats received standard dexamethasone or one of three dosages for cell wall content of *L. plantarum*: 10^5 CFU/animal, 10^7 CFU/animal, or 10^9 CFU/animal. The arthritis development was physically measured by body weight, paw volume, lesions, joint inflammation, gait, and mobility. The biochemical measurements were erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF), and serum TNF-alpha. CFA-injected rats receiving treatment with *L. plantarum* displayed improvements in all parameters, suggesting anti-arthritic activity for the probiotic.[24] The beneficial effects of the treatment in improving joint

health, gait, and mobility were only observed with the highest dose of 10^9 CFU/animal *L. plantarum*.

Though this study utilized a murine model, its results are significant for understanding human RA. CFA has features that resemble RA in humans, and CFA includes cell-mediated autoimmunity. The results illustrate that cell wall content of *L. plantarum* can relieve painful symptoms of arthritis while decreasing the presence of inflammatory molecules in the joints. This study did not identify the mechanism by which *L. plantarum* causes changes in disease activity. Future studies should include quantitative real-time PCR as a measurement tool.

Lactobacillus plantarum and Lactobacillus brevis

In addition to probiotic supplementation, the symptoms of RA patients can also be treated with functional foods. Nenonen *et al* [24a] demonstrated the therapeutic effects of an uncooked, lactobacilli-rich, vegan diet in RA patients. The diet provided large of amounts of probiotics, chlorophyll, and fiber. Compared to the control group that ate an omnivorous diet, the 'living food' diet decreased subjective symptoms of rheumatic pain, joint swelling, and morning stiffness in RA patients. Furthermore, a return to an omnivorous diet caused RA patients to experience aggravated symptoms.[24a] There were no observed statistically significant changes in the objective measures of the disease which included the number of swollen and tender joints, DAS 28, HAQ, CRP, and ESR.

The decrease in RA disease activity is linked to fermented wheat drink, wheat grass drink, fiber, and iron. Of these, fermented wheat drink is the rich source of lactobacilli. Patients in the experimental group received fermented wheat drink containing 2.4-4.5x10^{10}/day of *L. plantarum* and *Lactobacillus brevis (L. brevis).* Analysis of the faecal microbiota of this group showed an increase in faecal lactobacilli. Thus, Nenonen *et al* [24a] associate the decreased RA activity in the experimental group with diet-induced changes in patients' intestinal microflora. Unfortunately,

half of the patients in the diet group experienced adverse effects that caused them to stop the treatment prematurely. The nausea and diarrhea associated with this diet suggest that extreme diet therapy may not be advisable for all patients. It remains possible that a vegan diet, rich in lactobacilli, may have therapeutic effects on objective measures of RA.

Lactobacillus rhamnosus

Probiotic therapy with *Lactobacillus rhamnosus* GG (LGG) did not yield clinically significant benefits related to RA activity; however, more patients in the LGG group (71%) reported better subjective well-being.[25] No seriously adverse effects of LGG were reported. The research subjects were separated into the LGG that group received >5 x 10^9 CFU/capsule twice and a placebo group that did not receive any treatment. At a length of 12 months, this study is considered long-term and is unlike most probiotic interventions that have been tested for RA. All patients were examined by the same physician at 0, 1, 4, 8 and 12 months from the start. The researchers were primarily interested in evaluating the number of swollen and tender joints on the body and changes in the Health Assessment Questionnaire (HAQ). The LGG was associated with a reduced number of swollen and tender joints, yet the sample size was too small to detect statistically significant differences between the treatment and placebo groups. The study observed negligible changes in HAQ index after the study.

In an effort to better understand the mechanisms of LGG by which LGG affects RA activity, researchers also analyzed secondary outcome variables: cytokines, faecal urease activity, and changes in medication. Again, there were no major changes in these variables. There was a small increase in the IL-1B cytokine, but conclusions could not be made because baseline levels had been low. There were no significant changes in other proinflammatory cytokines (IL-6 and TNF-a), anti-inflammatory cytokines (IL-10 or IL-12), or serum levesl of myeloperoxidase (MPO). The lack of results from a biochemical perspective may partially be

explained by the fact that patients in the study were in a stable phase of the disease, meaning their disease parameters were not likely to reduce to a great extent.[25] The improvement in self-reports of subjective well-being by patients is beneficial to their personal wellness but does not suggest clinical implications for LGG therapy in RA.

Bacillus coagulans

In separate studies of humans and rats, oral intake of *Bacillus coagulans (B. coagulans)* improved the disease activity of RA.[26,27] Mandel *et al* [26] created an intervention for humans by means of a randomized, double-blind, placebo-controlled, parallel-design study. They provided adjunctive treatment to adult RA patients with oral administration of *B. coagulans* GBI-30, 6086 at a daily dose of $2x10^{10}$ CFU, and the placebo group received microcrystalline cellulose. Both groups continued use of their standard arthritis medications. The researchers measured arthritis activity by clinical criteria, the Stanford Health Assessment Questionnaire Disability Index (HAQ-DI), and biomarkers for ESR and CRP. No seriously adverse effects of *B. coagulans* were reported. There were statistically significant improvements in the treatment group.[26]

At the end of the study, the clinical criteria established by the American College of Rheumatology was used to evaluate physical improvements in research subjects. Patients in the probiotic group showed statistically significant improvement in Pain Scale and borderline statistically significant improvement in their own Pain Assessment. Some patients also reported better self-assessed disability, consistent with their ability to walk two miles and carry out daily activities at the end of the study. Biochemical analysis showed a total reduction in CRP levels thus indicating a reduction in RA disease activity.

The suggested mechanism of action for this probiotic is related to anti-inflammatory properties of the bacteria. *B. coagulans* produces proteins known as bacteriocins and lactic acid

that lowers local pH levels. This process may eliminate some of the microbes that contribute to an inflammatory response in the host. *B. coagulans* also produces a butyric acid, a short-chain fatty acid, that aids in the healing process of cells in the small and large intestines.[26] These immunomodulating and anti-inflammatory properties may explain the ability of *B. coagulans* to alleviate RA. The sample size of the present study was low (n=45); however, the favorable results suggest that supplementation of *B. coagulans* is a safe and effective addition to RA treatment.

B. coagulans has also shown improvement in the RA activity of rats. Abhari *et al* [27] created an *in vivo* model of CFA in male Wistar rats. They investigated the effects of prebiotic, probiotic, and synbiotic diets on several inflammatory markers of arthritis. These results were compared to a treatment control group that received indomethacin as a reference nonsteroidal anti-inflammatory drug (NSAID). In the probiotic treatment group, rats received *B. coagulans* at a dose of 10^9 spores/day. The arthritis was measured by paw thickness, fibrinogen (Fn), serum amyloid A (SAA), and TNF-α and alpha-1-acid glycoprotein (α1AGp). Fn is a critical autoantigen in RA. SAA is known to activate proinflammatory Th1 cells and regulates the behavior of leukocytes, angiogenesis, and matrix degradation. Fn, SAA, and TNF-α have important roles in RA joint damage. There were statistically significant improvements in the treatment group.[27]

The *B. coagulans* group had a significant decrease in production of SAA, Fn, and TNF-α proinflammatory cytokine. This group also demonstrated a significant reduction in paw thickness. The proposed mechanism of action for *B. coagulans* in relation to paw thickness is explained by the properties of the Indomethacin reference drug.[27] Indomethacin is thought to reduce paw thickness and other RA-related inflammation by inhibiting enzymes that produce

prostaglandins. Prostaglandins are downregulated by anti-inflammatory cytokines. Abhari *et al* believe that *B. coagulans* may act in a similar way by lowering prostaglandins production to activate anti-inflammatory cytokines. These results support the supplementation of *B. coagulans* in RA treatment.

Lactobacillus acidophilus, Lactobacillus casei, and Bifidobacterium bifidum

A combined probiotic treatment with *Lactobacillus acidophilus (L. acidophilus), L. casei,* and *Bifidobacterium bifidum* demonstrated beneficial effects for RA patients. Zamani *et al* [28] designed a short-term RCT to test the effects of a multispecies probiotic at a total dose of 6×10^9 CFU/g. Each individual bacterial strain was measured at 2×10^9 CFU/g. Patients in the treatment and placebo groups were continued use of their standard arthritis medications. The arthritis of patients was measured primarily by Disease Activity Score of 28 joints (DAS28) and inflammatory factors. The secondary outcome measurements were of insulin resistance, lipid concentrations, biomarkers and oxidative stress.

The results show that the mixed probiotic improved DAS-28.[28] It reduced serum insulin levels, homeostatic model assessment-B cell function (HOMA-B), and (hs-CRP) concentrations. There was also a borderline statistically significant improvement in the cholesterol levels of the probiotic group. They found no relationship between the probiotic treatment and glucose homeostasis parameters, lipid profiles or biomarkers of oxidative stress. Zamani *et al* explained the mechanism of action for the mixed probiotic in a manner similar to Mandel *et al* [26] by referencing the ability of probiotics to produce bacteriocins and butyric acid that down-regulates inflammation in arthritis.

Lactobacillus rhamnosus and *Lactobacillus reuteri*

Oral administration of *Lactobacillus rhamnosus GR-1* and *Lactobacillus reuteri RC-14* did not significantly clinical criteria for RA; however, the experimental group displayed functional improvements that the control group did not.[29] Pineda *et al* [29] conducted a pilot study on RA patients with chronic synovitis. Patients in the probiotic group received a dose of $2x10^{10}$ CFU twice daily. The primary outcome was the ability of probiotic patients to achieve an ACR20 response versus the placebo group. Arthritis activity was also physically measured by swollen and tender joints, Physician Global Assessment, HAQ, Patient Assessment of Pain, and morning stiffness. The biochemical parameters included ESR, CRP, and 15 inflammatory cytokines (IL-1α, IL-1β, IL-6, IL-8, TNF-α, IL-12p70, IL-15, IL-10, GM-CSF, G-CSF, IL-17, sCD40 ligand, MIP-1α, MIP-1β, MCP-1). Based on the primary outcome variable, there was no statistically significant difference between the treatment and placebo groups.

Interestingly, patients within the probiotic group showed statistically significant improvement in their HAQs score from baseline to end of study.[29] This bears resemblance to the Hatakka *et al* [25] in which patients reported better subjective well-being despite insignificant clinical improvements. There is some reason to believe that HAQ accurately assesses the ability of RA patients to function with their illness better than other clinical evaluations. This remains unclear, so it is difficult to conclude the efficacy of *Lactobacillus rhamnosus* and *Lactobacillus reuteri* as an adjunctive treatment for RA. Still, the sample size of the present study was small (n=29) with just 15 patients in the probiotic treatment group. This may partially explain why the number of patients who received ACR20 response was too low to demonstrate statistical significance. Further research is needed to determine if probiotics can improve functionality in the long-term.

Limitations of current research

The experiments described in this paper have individually demonstrated a beneficial link between probiotic supplementation and RA. Mohammed *et al* [8] conducted a systematic review and meta-analysis to determine overall efficacy of probiotics in various RCTs. After conducting a comprehensive search of the existing literature, they compared nine studies involving 361 patients. The studies include those by Hatakka *et al* [25], Mandel *et al* [26], Alipour *et al* [20], Pineda *et al* [29], Nenonen [et al] and Zamani *et al* [28]. The meta-analysis investigated the following outcome variables: DAS 28, CRP, ESR, HAQ, swollen and tender joints, and cytokines (IL1β ,IL6, IL10, IL12, and TNF-α).

It was determined that probiotics significantly lowered pro-inflammatory cytokine IL-6.[8] There was no difference of DAS 28 between the probiotic and placebo groups. The remaining outcome variables did not show a statistically significant difference. The change in IL-6 is of relevance to joint destruction in RA patients; however, the lack of additional clinical improvements shows only a small therapeutic effect for probiotics in RA treatment.

Mohammed *et al* [8] attribute the absence of significance in the outcome variables with the large variability between RCTs. First, separate studies used different strains of probiotics at varying dosages. It is suggested that future RCTs investigating RA follow careful guidelines. RA patients should be grouped by severity of disease. There were also major differences in sample size among the studies. The sample size should be calculated with 5% type I error rate and 20% type II error rate. The therapy should continue for at least 12 weeks to accurately assess changes in outcome variables, which should include those meta-analyzed variables in Mohammed *et al.* Furthers RCTs of suitable statistical power are needed to determine the effects of probiotics on RA.

Conclusion

RA is an inflammatory autoimmune disease without a known cause. New findings have shown that the host-microbe equilibrium has a critical role in the pathogenesis of the disease. Probiotics provide an immunomodulatory benefit to the host with minimal reported complications. There is some evidence that inflammation in RA can be reduced by adjunctive probiotic therapy. The likely mechanism of action is related to the release of proinflammatory cytokines, such as IL-6; however, the current state of evidence remains too low to make definitive conclusions. The data suggests that future RCTs are needed to prove the efficacy of probiotics in treating RA symptoms and biochemical activity.

Reference List

1. Sender R, Fuchs S, Milo R. Revised estimates for the number of human and bacteria cells in the body. PLos Biol. 2016;14:e36103.
2. Jethwa H, Abraham S. The evidence for microbiome manipulation in inflammatory arthritis. Rheumatology (Oxford, U.K.). 2017;56:1452-1460.
3. Sandhya P, Danda D, Sharma D, Scaria V. Does the buck stop with the bugs?: An overview of microbial dysbiosis in rheumatoid arthritis. Int J Rheum Dis. 2016;19:8-20.
4. Bedaiwi MK, Inman RD. Microbiome and probiotics: Link to arthritis. Curr Opin Rheumatol. 2014;26:410-415.
5. Arend WP. The innate immune system in rheumatoid arthritis. Arthritis Rheumatol. 2001;44:2224-2234.
6. Brennan FM, McInnes IB. Evidence that cytokines play a role in rheumatoid arthritis. J Clin Invest. 2008;118:3537-3545.
7. Centers for Disease Control and Prevention. Rheumatoid Arthritis (RA) | Arthritis Basics | Arthritis Types | Arthritis | CDC. https://www.cdc.gov/arthritis/basics/rheumatoid-arthritis.html. Updated July, 07 2017. Accessed January 17, 2018.
8. Mohammed AT, Khattab M, Ahmed AM, Turk T, Sakr N, Khalil A, Abelhalim M, Sawaf B, Hirayama K, Huy N. The therapeutic effect of probiotics on rheumatoid arthritis: a systematic review and meta-analysis of randomized control trials. Clin Rheumatol. 2017;36:2697-2707.
9. Anukam KC, Reid G. Probiotics: 100 years (1907-2007) after Elie Metchnikoff's Observations. In: Mendez-Vilas A ed. Communicating Current Research and Educational Topics and Trends in Applied Microbiology. Bajadoz, Spain: Formatex, 2007:466–474.
10. Gill H, Prasad J. Probiotics, immunomodulation, and health benefits. Adv Exp Med Biol. 2008;606:423-54.
11. de Oliveira GLV, Leite AZ, Higuchi BS, Gonzaga MI, Mariano VS. Intestinal dysbiosis and probiotic applications in autoimmune diseases. Immunology. 2017;152:1-12.
12. Eerola E, Möttönen T, Hannonen P, Luukkainen R, Kantola I, Vuori K, Tuominen J, Toivanen P. Intestinal flora in early rheumatoid arthritis. Br J Rheumatol. 1994;33:1030-1038.
13. Cooles FAH, Isaacs JD, Anderson AE. Treg Cells in Rheumatoid Arthritis: An Update. Curr Rheumatol Rep. 2013;15:352.
14. Alunno A, Manetti M, Caterbi S, Ibba-Manneschi L, Bistoni O, Bartoloni E, Valentini V, Terenzi R, Gerli R. Altered immunoregulation in rheumatoid arthritis: the role of regulatory T cells and proinflammatory Th17 cells and therapeutic implications. Mediators Inflammation. 2015;2015:751793.
15. Frosali S, Pagliari D, Gambassi G, Landolfi R, Pandolfi F, Cianci R. How the Intricate Interaction among Toll-Like Receptors, Microbiota, and Intestinal Immunity Can Influence Gastrointestinal Pathology. J Immunol Res. 2015;2015:489821.

16. Huang Q, Pope R. The role of toll-like receptors in rheumatoid arthritis. Curr Rheumatol Rep. 2009;11:357-364.
17. Cheng Z, Meade J, Mankia K, Emery P, Devine DA. Periodontal disease and periodontal bacteria as triggers for rheumatoid arthritis. Best Pract Res, Clin Rheumatol. 2017;31:19-30.
18. Chukkapalli S, Rivera-Kweh M, Gehlot P, Velsko I, Bhattacharyya I, Calise S, Satoh M, Chan E, Holoshitz J, Kesavalu L. Periodontal bacterial colonization in synovial tissues exacerbates collagen- induced arthritis in B10.RIII mice. Arthritis Res Ther. 2016;18:161.
19. Fernández-Llanio Comella N, Fernández Matilla M, Castellano Cuesta JA. Have complementary therapies demonstrated effectiveness in rheumatoid arthritis? Reumatología Clínica. 2016;12:151-157.
20. Alipour B, Homayouni-Rad A, Vaghef-Mehrabany E, Sharif S, Vaghef-Mehrabany L, Asghari-Jafarabadi M, Nakhjavani M, Mohtadi-Nia J. Effects of Lactobacillus casei supplementation on disease activity and inflammatory cytokines in rheumatoid arthritis patients: a randomized double-blind clinical trial. Int J Rheum Dis. 2014;17:519–527.
21. So J-S, Kwon H-K, Lee C-G, Yi H, Park J, Lim S, Hwang K, Jeon Y, Im S. Lactobacillus casei suppresses experimental arthritis by down-regulating T helper 1 effector functions. Mol Immunol. 2008;45:2690-2699.
22. Borchers A, Selmi C, Meyers F, Keen C, Gershwin M. Probiotics and immunity. J Gastroenterol. 2009;44:26–46.
23. Kim J, Chae C, Kim G, Hwang W, Hwang J, Hwang S, Kim Y, Ahn Y, Park S, Jun C, Rudra D, Im S. Lactobacillus helveticus suppresses experimental rheumatoid arthritis by reducing inflammatory T cell responses. J Funct Foods. 2015;13:350-362.
24. Gohil P, Vimal Patel B, Shrikalp Deshpande B, Mehul Chorawala B, Gaurang Shah B. Anti-arthritic activity of cell wall content of Lactobacillus plantarum in freund's adjuvant-induced arthritic rats: involvement of cellular inflammatory mediators and other biomarkers. Inflammopharmacology. 2017;26:171-181.
24a. Nenoen M, Elorinne, AL, Helve TA, Hänninen, Osmo. Uncooked, lactobacilli-rich, vegan food and rheumatoid arthritis. Br J Rheumatol. 1998;37:274-281.
25. Hatakka, K, Martio, J, Herranen M, Poussa, T, Laasanen, T, Saxelin, M, Vapaatalo, H, Moilanen, E, Korpela R. Effects of probiotic therapy on the activity and activation of mild rheumatoid arthritis – a pilot study. Scandinavian Journal of Rheumatology. 2003:211-215.
26. Mandel DR, Eichas K, Holmes J. Bacillus coagulans: a viable adjunct therapy for relieving symptoms of rheumatoid arthritis according to a randomized, controlled trial. BMC Complementary Altern Med. 2010;10:1.
27. Abhari K, Shekarforoush SS, Hosseinzadeh S, Nazifi S, Sajedianfard J, Eskandari MH. The effects of orally administered Bacillus coagulans and inulin on prevention and progression of rheumatoid arthritis in rats. Food Nutr Res. 2016;60:30876.

28. Zamani B, Golkar HR, Farshbaf S, Emadi-Baygi M, Tajabadi-Ebrahimi M, Jafari P, Akhavan R, Taghizadeh M, Memarzadeh M, Asemi Z. Clinical and metabolic response to probiotic supplementation in patients with rheumatoid arthritis: a randomized, double-blind, placebo-controlled trial. Int J Rheum Dis. 2016;19:869-879.
29. Pineda M, Thompson SF, Summers K, de Leon F, Reid G. A randomized, double-blinded, placebo-controlled pilot study of probiotics in active rheumatoid arthritis. Med Sci Monit. 2011;16:347-354.