Analysis on Intestinal Microbiota in Rheumatoid Arthritis

Ruining Peng
School of Engineering and Applied Sciences, University of Pennsylvania, Philadelphia, PA 19104, U.S.A.

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Abstract: Rheumatoid Arthritis (RA) is a prevalent symmetrical facet joint involved systemic autoimmune disease, which is mainly characterized by swelling and deformity of the joints, causing mobility problems and affecting living quality. Factors such as heredity, environment, and microbial infection often lead to the onset of RA, which is also related to immune dysfunctions, but the specific pathogenesis is still unclear. Tens of billions of microbes in the gastrointestinal tract, the biggest immune organ, participate in immunological regulation of the human body, changes in the composition and function of which are closely related to rheumatoid diseases such as RA. Hence, this review focuses on the relationship between intestinal microbes and RA. Based on the analysis and summary of existing materials, it is found that the composition of microbes in the gastrointestinal tract in RA patients is different from that in healthy group. Reductions in Bacteroides and increases in Prevotella are strongly correlated with disease in new-onset untreated rheumatoid arthritis (NORA) subjects. In addition, intestinal microbes and their metabolites affect the production and differentiation of regulatory T cells (Treg cells). Besides, these substances also disrupt the balance between Treg and helper T cells, induce the release of pro-inflammatory factors, interfere with the host immune system, and get involved in the occurrence of autoimmune diseases. The study of the intestinal-articular axis provides a new perspective of RA pathogenesis.

1 INTRODUCTION

RA is a chronic systemic immune disease with synovitis and pannus as the main pathological manifestations. The global incidence of RA is about 1%. Previous studies believe that RA susceptibility genes are the most common cause of the disease. However, recent epidemiological studies have reported that the proband-wise concordance rate of RA in monozygotic twins is lower than that in dizygotic twins (Svendsen, Anders et al 2002), indicating that the environment should be the environment instead of genetic factor play a role in such autoimmune diseases. Environmental changes, such as humid climate, imbalance the immune homeostasis through epigenetic modification, leading to RA and other rheumatoid disorders (Calabresi, Emanuele et al. 2018).

With in-depth research on microorganisms and new understandings of their functions in the human body, the relationship between microbes and RA has gradually been revealed. The early hypothesis was that Europeans were exposed to a certain version of the pathogen in America that led to the RA on-set; recent studies have found that RA's pathogenesis may involve the colonization of specific microorganisms in the human body. It has been reported that RA might be caused by intestinal flora participating in cell, especially immunological cells, interactions, changing immune homeostasis, triggering inflammatory reactions.

Changes in the composition of intestinal microbes in RA patients, pathogenesis involved microorganisms and their role in immune regulation, and the application prospects of probiotics as new treatments are current research hotspots. This review collects, analyzes the existing research results, discusses the genetic and environmental susceptibility factors, changes in the microbial composition in RA patients, the participation of intestinal flora in human immunity, and the possible role of RA pathogenesis. As the specific pathogenic cause of RA is still unclear, the relationship between gut microbes and RA will provide new research perspectives and new ideas for adjuvant therapeutic methods.

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2 RA PATHOGENESIS

RA is a systemic immunological disease, which pathogenesis includes innate immune dysfunction and acquired immune responses, involving antigen-presenting cells, self-reactive T cells, and antibodies. Thus immunological dysfunction should be the leading cause of joint damages and RA.

Cytokines and inflammatory mediators, for example, tumor necrosis factor α (TNF-α), interleukin-17 (IL-17), and granulocyte-macrophage colony-stimulating factor (GM-CSF) could induce a strong inflammatory response, play a key role in RA pathogenesis, which is one of the critical floating activity indicators for RA diagnosis and prognosis. Besides, cytokines and their signaling pathways work as effective therapeutic targets applied in RA clinical treatment.

The high level of serum TNF-α, one of the inflammatory factors involved in the pathogenesis of RA, indicates the active stage. TNF is mainly produced by activated mononuclear macrophages in the synovial membrane, and its various effector functions, such as inflammatory effects, are related to RA pathogenesis. TNF triggers the activation of several immune cells (leukocytes, endothelial cells, etc.) as well as the production of a series of cytokines and chemokines (IL-1, IL-6, IL-8, GM-CSF, etc.) (McInnes, Iain B, and Georg Schett. 2007) TNF also drives the differentiation of osteoclasts. Meanwhile, it inhibits the formation and function of osteoblasts, which disrupts the balance between bone formation and destruction, causing damages to tissues in the patient's joints. Study shows that THF antagonist can clinically reduce the production of several cytokines, slow down inflammatory responses, and relieve joint damages.

The production of self-reactive antibodies is another decisive factor in the progression of RA disease. B cells, induced by the antigen-antibody recognition process, stimulate T cell activation, initiate immune responses, and trigger the production of autoantibodies (for example, rheumatoid factors), which will then precipitate with IgG leading to the onset and progression of RA. Regulatory B cells (Breg cells) direct Th cells to develop into the memory T cell and reduce inflammatory T cells' proliferation, inhibiting RA progression. Since it is well-known that the imbalance between pro- and anti-inflammatory T cells is closely correlated with the RA disease. Th 17 cell, a new subtype of CD4+ T cell, could induce autoantibody through the secretion of IL-17, which is one of the pro-inflammatory factors that amplify inflammatory responses. It has been reported that the large amount of IL-17 positively correlated with RA severity. Sun J, et al. found that metallothionein-1 (MT-1) can correct the relationship between pro- and anti-inflammatory T cells, alleviate the pathological symptoms (for example, synovitis) of RA by inhibiting Th17 cell while inducing the proliferation of Treg cells (Sun, Li, Li, Ding, Liu, Chen, Zhang, Qi, Du, Huang 2018).

3 RA SUSCEPTIBILITY FACTORS

RA is closely related to the genetic background while also involves several other susceptibility factors. Among them, the body's immunity and microbial infection are at the central position; endocrine and environmental factors also increase RA's susceptibility (Figure 1) (Deane, Kevin D et al. 2017). Certain microorganisms, such as Epstein-Barr virus and parvovirus B19, infect the human body and then mediate autoimmune responses via polypeptide fragments; or work as an initiating factor, first cause local inflammatory responses (for example, pharyngitis and sinusitis), causing immunity to start the regulation process to fight against inflammation but might trigger systemic immune disease including RA as well (Mathew, Ashish Jacob, and Vinod Ravindran. 2015). In addition, microbial infection may change the micro-ecological environment in the oral cavity and gastrointestinal tracts, which disrupts the normal composition of microorganisms, interferes with immune regulation, leading to the enhanced susceptibility of RA. Except for microbial infections, the incidence of RA in women is significantly higher than that in men at the same age, indicating endocrine and gender might participate in RA progression. Finally, cold and humid climates, smoking, obesity, etc. can also aggravate disease conditions.

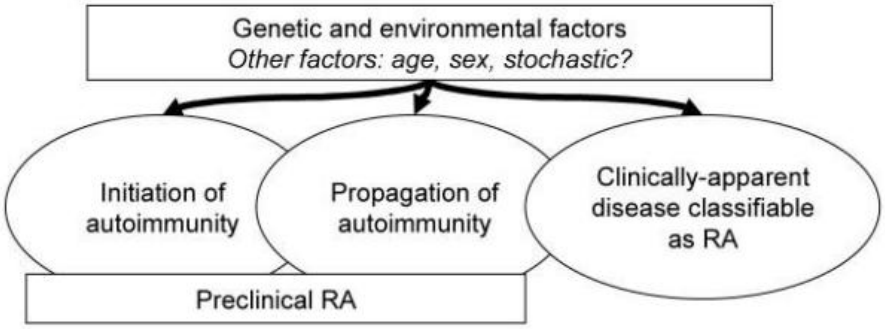

Figure 1: A general model of RA susceptibility factors.

4 RA AND GASTROINTESTINAL MICROBES

Joint deformities and dysfunction are common clinical manifestations of RA. While patients in the earlier stage only have elevated serum autoantibodies level with no obvious clinical synovitis. And research suggests that synovial self-reactive immunity in oral mucosa, lungs, intestines, etc., may cause systemic autoimmune diseases, including RA (Mankia, Kulveer, and Paul Emery. 2015). Wu et al. found that mice under sterile conditions had alleviated RA symptoms with the reduced number of Th17 cells and serum self-reactive antibody titers. When using intestinal segmented filamentous bacteria (SFB) infected these mice, Th17 cells in lamina propria and self-reactive antibody recovered. It indicated the critical functions of intestinal microbes in regulating pro- and anti-inflammatory T cells and the progression of autoimmune diseases. Still, it should not be ignored that the difference between human and mouse intestinal microbiota. Analysis with RA patient's saliva and stool samples using sub-genome shotgun sequencing and whole-genome association studies (MGWAS) demonstrates the imbalance of microbiota in both oral cavity and gastrointestinal tract. Specifically, Haemophilus spp. decrease and Lactobacillus salivarius increase significantly compared to the control, indicating the probable role of such microorganisms in autoantibody production and disease activity.

When the environment changes, the mutual relationship in microbial flora will be disrupted; conditioned pathogens gain an ability to cause disease. Prevotella is a commensal bacterium locates ubiquitously in mucous membranes in healthy subjects and rarely causes inflammation. Epidemiological studies have found Prevotella can lead to periodontitis which associates with an increased risk of systemic immune diseases. Research by Maeda Y, et al. showed that patients in RA early-stage carry Prevotella dominated intestinal flora (Maeda, Kurakawa, Umemoto, Motooka, et al. 2016), which correlated with the decreased population of Bacteroides and other beneficial bacteria. Thus, intestinal microbe plays an essential role in RA pathogenesis. In addition, infection of Prevotella goes along with the increased number of Th17 cells in mice, causing severe arthritis symptoms (Maeda, Kurakawa, Umemoto, Motooka, et al. 2016). And Prevotella can induce secretion of IL-6 and IL-23, stimulate the proliferation of bone marrow-derived dendritic cells, and promote five times higher IL-17. It can be seen that changes in intestinal flora correspond to RA disease activity.

5 THE ROLE OF GASTROINTESTINAL MICROBES IN RA PATHOGENESIS

The onset and progression of RA are related to dysfunctional immunity, while the specific mechanism is still unclear. It is believed that the imbalance of Treg cells and Th cells, which can cause immune system disorders, might get involved in RA development and disease activity. The typical healthy human digestive tract is planted with a larger population of Treg cells as well as 1014 microorganisms, which is ten times the number of human cells. These large, various microbes have significantly enriched the diversity of the host genome, encoding 3.5 million genes, which is about 150 times more complex than the host self-genes (Figure 2) (Cresci, Gail A, and Emmy Bawden. 2015). It has been reported that intestinal microbes

and these genes can encode proteases that host cannot possess, which play an important role in regulating host metabolic functions (Kho, Zhi, and Sunil K Lal. 2018). Intestinal microbes and their metabolites affect the production and proliferation of Treg cells, that is, by interfering with immune cells differentiation to disrupt the normal capacity of immune system and trigger the development of systemic immune disease. It is also agreed that intestinal microbes participate in cell communications, induce inflammatory responses via mucosal barrier devastation caused by mutual recognition of immunological molecules. In summary, intestine-colonized microbes can affect the production of Treg cells, induce releases of pro-inflammatory mediators using molecular simulation mechanisms, interfere with the body's immune regulation functions, then initiate systemic autoimmune diseases, including RA.

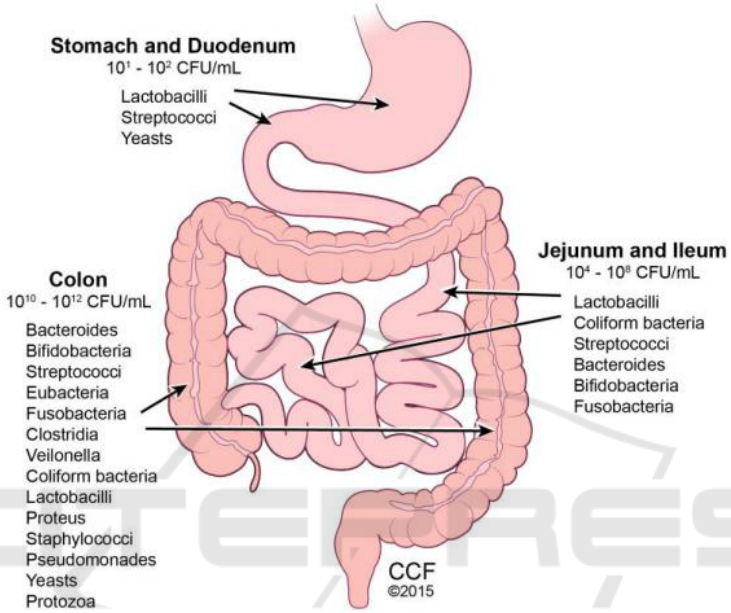

Figure 2: Typical microorganisms colonized human gastrointestinal tracts.

The host immune system is functioned by various immune mediators, cells, and complicated biological processes to defend against pathogen invasions and diseases. When biological, pharmaceutical, environmental factors simulate the host, inflammatory responses will immediately be initiated to fight against infected tissues, and repair processes will then start. Breg cells differentiate in response to early inflammation and restrain excessive inflammatory responses through the secretion of IL-10. Studies showed that in the inflammatory stage of RA, intestinal microbes can regulate the differentiation of Breg cells colonized in immune tissues and organs, such as spleen and mesenteric lymph nodes, by releasing IL-1β and IL-6.

Antibiotic-treated mice (ABX mice) and specific pathogen-free mice (SPF mice) developed a lower number of IL-1β and IL-6, compared to the conventionally housed mice (CNV mice), in response to the induction of arthritis. Besides, compared to the control, ABX mice showed severer arthritis symptoms with a lower-functional small number of Breg cells due to changes in the composition of intestine-colonized microbiota. It indicated the participation of microorganisms in regulating immune responses, inducing Breg cells differentiation and retraining excessive inflammation could alleviate RA progression (Rosser, Elizabeth C et al. 2014). Another study showed that germ-free mice (GF mice) developed milder arthritis (WuHJ, IvanovII, DarceJ, etal. 2010). In addition, GF mice had lower number of splenic autoantibody-secreting cells, Th17 cells and Treg cells, as well as lower serum self-reactive antibody titers. After the infection of intestinal-specifically colonized single commensal SFB, Th17 cell populations in lamina propria and serum autoantibody titers have recovered. Colonization of SFB can induce the proliferation of CD4+ T helper cells (Th17 cells), which produce IL-17 and IL-22 in lamina propria. Additionally, together with the up-regulation of acute subtype serum amyloid, activation of dendritic cells and production of other immune mediators lead to mouse joints destruction (Ivanov II, Atarashi K, Manel N, et

al. 2009). Animals fed with Lactobacillus casei developed milder level of pro-inflammatory factors (IL-1β, IL-2, IL-6, IL-12, etc.), TNF-α, and IFN-γ; together with increased population of IL-10 and TGF-β ameliorate arthritis (Vaghef-Mehrabany, Elnaz et al. 2013). It suggested the beneficial effects of intestinal-specifically colonization of certain microbes on the amelioration of RA symptoms via regulating immune functions. In conclusion, intestine-planted microbes have significant impacts on the maintenance of normal immune function; certain or conditioned pathogens can disrupt host immune structure and affect immune tolerance, leading to the occurrence and development of diseases.

Intestinal microorganisms can affect host immunity via their metabolites. Short-chain fatty acids (SCFAs), mainly produced by the decomposition of carbohydrates, are among the most important microbial metabolites linked with immune regulation. As a key energic source for intestinal epithelial cells and intestinal microbes, SCFAs are also involved in cell differentiation, anti-inflammatory responses, and many other critical metabolic processes and are therefore of great significance in regulating host immunity. Studies showed that SCFAs recruit granulocytes, thus aggravate local inflammation. The host can detect and respond to the appearance of SCFAs using the surface located G-protein-coupled receptor 41 and 43, which then promotes Treg cells differentiation and clustering, enhances the IL-10 production, and thereby inhibits inflammatory responses (Lopez, Christopher A et al. 2014). SCFAs can also stimulate mucin 2 expression in intestinal epithelial cells. It may play an important role in mucoprotective function (Willemsen, L E M et al. 2003).

Above, microbes may affect the host immune metabolism process mainly in two ways: microorganisms and microbial metabolites. Both can enhance the susceptibility of RA through, for example, inducing immune cell differentiation, pro-inflammatory mediator releases. Microbes and their metabolic products may become new therapeutic targets in RA clinical treatment.

6 MICROBIOME IN RA TREATMENT

Probiotics, living microorganisms that are believed to be beneficial, change the microbial composition of the human body, influence disease progression. According to clinical researches, eight weeks of oral administration of Lactobacillus casei leads to significantly reduced disease activity index of RA patients. Also, patients developed lower serum inflammatory factors (TNF-α, IL-6, IL-12) with increased serum regulatory cytokine IL-10 (Vaghef-Mehrabany, Elnaz et al. 2013). Many other studies, however, demonstrated that probiotics could not significantly change inflammatory parameters (such as erythrocyte sedimentation rate, TNF-α, IL-6, IL-10) and oxidative stress indicators (total antioxidant capacity and malondialdehyde); while it did improve the RA disease activity index (Aqaeinezhad Rudbane, Seyed Mohammad et al. 2018). The current research evidence is not sufficient to prove the effectiveness of probiotic interventions in RA treatment, which still needs extended animal and clinical trials for further evaluation. Besides, screening for the best bacteria species and optimizing the dose intake is also necessary.

Probiotics can change intestinal flora. While, due to the larger quantities of microorganisms that colonize gastrointestinal tracts, oral probiotics can sometimes be difficult to achieve the desired effects. Therefore, fecal flora transplantation has become an alternative way to adjust patient's intestinal microbial composition. It helps reorganize the micro-environment of intestinal tract via transplanting healthy people's intestinal flora into the patients in need, thereby improving disease conditions caused by disorders of intestinal microbes.

In recent years, traditional DMARDs, methotrexate represented, treatments have played a certain role in controlling RA development. However, such therapeutic methods cannot cure the disease, the long-term use of which will also increase the risk of infections and cancers. By contrast, oral probiotics and fecal transplantation are much safer, with more advantages in inhibiting inflammation and improving arthritis symptoms.

7 CONCLUSIONS

Human intestinal microbiota is closely related to the host immune system. It participates in the occurrence and development of autoimmune diseases such as RA by interfering with immune cell differentiation, inflammatory mediator, and self-reactive antibody production, while the specific mechanism of which remains to be explored. And due to the relationship between intestinal flora disorders and incidence of RA, it's plausible to predict the disease occurrence and evaluate disease stages via detecting the human

intestinal microenvironment, which is helpful to disease control. In addition, advanced techniques, such as high-throughput sequencing, make precision medicine achieved. Patients can obtain targeted treatments, targeted use of certain probiotics, specific changes in daily diet, etc. Future studies could combine multiple analyses (microbiota, genomics, and proteomics) to explore mechanisms involved intestinal microorganisms with host immune system. It would deepen the current understanding of the intestinal microenvironment, leading to discovering new biomarkers for RA diagnosis and prognosis, providing an innovative reference for staged treatment of RA.

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