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Utility of gut flora in pathogenesis of diseases with special reference to rheumatoid arthritis

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Abstract
Gut flora, or gut microbiota, or gastrointestinal microbiota, is the complex community of microorganisms that live in the digestive tracts of humans and other animals, including gut immune cells. These gut immune cells are important gatekeepers. They must detect and destroy foreign invaders that ride on the food we eat. However, the gut's immune system has to reach a delicate balance between fighting invaders and allowing nutrients to enter the body. If the immune system is too lax, we get infected. If it is too aggressive, we get allergic reactions and possibly autoimmune diseases. Rheumatoid arthritis is a chronic disease that causes painful swelling in the joints. The disease usually starts in the hands and feet and works its way toward central joints over time. Without effective treatment, rheumatoid arthritis can cause severe deformity and disability. Currently, the most effective treatments for rheumatoid arthritis are drugs that suppress the immune system. Patients who first develop rheumatoid arthritis have a substantially lower number of helpful bacteria in their gastrointestinal systems. People with rheumatoid arthritis have significantly less Bifidobacteria, Bacteroides-Porphyromonas-Prevotella species, *Bacteroides fragilis* species and the Eubacterium rectale-Clostridium coccoides species. Despite intense research, scientists still do not know exactly what causes rheumatoid arthritis. However, evidence now strongly suggests that the number and types of bacteria present in the gastrointestinal tract influences the development of rheumatoid arthritis. *Prevotella copri* may be a bacterial trigger of rheumatoid arthritis. Likewise, patients with early rheumatoid arthritis seem to have abnormally low amounts of many helpful bacterial species. Thus the gut microbiota plays a vital role in pathogenesis as well as recovery of gut microbiota.

Keywords: Gut microbiota, gut microbiota, auto immunity

Introduction
Rheumatoid arthritis (RA)
Rheumatoid arthritis is an autoimmune disease that involves multiple molecules and pathways. Autoantibodies and cytokines present classes of immune cell secreted proteins postulatd to have a variety of roles in rheumatoid arthritis, from regulating the initiation and perpetuation of chronic inflammatory responses to joint destruction. However, the precise mechanisms leading to the expression of auto-antibodies and cytokines in early rheumatoid arthritis are not completely understood.

Although only scant evidence exists that auto antibodies are directly pathogenic in rheumatoid arthritis, they represent important markers for diagnosis and classification of rheumatoid arthritis. By contrast, auto antibodies have been observed infrequently in other types of arthritis. Proinflammatory cytokines such as tumour necrosis factor (TNF) alpha and interleukin (IL) probably play important parts in regulating immune activation, driving the inflammatory process and promoting joint distraction in a variety of inflammatory joint diseases. Chemokines are chemotactic cytokines produced by fibroblast-like synoviocytes, cells of the innate immune system and other immunoregulatory cells, and there is solid evidence that, among their many roles, they are important potentiators of autoimmune arthritis. As expression of cytokines and chemokines in synovial tissue occurs early in the course of rheumatoid arthritis, they are under evaluation as biomarkers in early rheumatoid arthritis. The advent of proteomics technologies has enabled large-scale analysis of proteins to identify biomarkers that delineate disease subtypes of rheumatoid arthritis, and to gain insights into the mechanisms underlying these subtypes [1].

Rheumatoid arthritis (RA) progresses in three stages. The first stage is the swelling of the synovial lining, causing pain, warmth, stiffness, redness and swelling around the joint. Second is the rapid division and growth of cells, or pannus, which causes the synovium to thicken. In the third stage, the inflamed cells rel ease enzymes that may digest bone and cartilage, often are causing the involved joint to lose its shape and alignment, more pain, and loss of movement.

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About 1% of the world's population is afflicted by rheumatoid arthritis, women three times more often than men. Onset is most frequent between the ages of 40 and 50, but no age is immune. The incidence of RA is in the region of 3 cases per 10,000 population per annum. RA is prevalent throughout the world and involves all ethnic groups. The figures of prevalence vary substantially ranging from 0.3% to 1% of the population Indian data suggests the prevalence to be around 0.65% to 0.75% of the population. 1-3%of women may develop RA in their life time Onset is uncommon under the age of 15 and from then on the incidence rises with age until the age of 80. The prevalence rate is 1%, with women affected three to five times as often as men. It is 4 times more common in smokers than non-smokers. It can be a disabling and painful condition, which can lead to substantial loss of functioning and mobility [2].

Causes

The cause of RA is unknown. It is considered an autoimmune disease. The body's immune system normally fights off foreign substances, like viruses. But in an autoimmune disease, the immune system confuses healthy tissue for foreign substances. As a result, the body attacks itself.

RA can occur at any age. Women are affected more often than men.

RA usually affects joints on both sides of the body equally. Wrists, fingers, knees, feet, and ankles are the most commonly affected. The course and the severity of the illness can vary considerably. Infection, genes, and hormones may contribute to the disease [3].

Symptoms

The disease usually begins gradually with:
- Fatigue
- Loss of appetite
- Morning stiffness (lasting more than 1 hour)
- Widespread muscle aches
- Weakness

Eventually, joint pain appears. When the joint is not used for a while, it can become warm, tender, and stiff. When the lining of the joint becomes inflamed, it gives off more fluid and the joint becomes swollen. Joint pain is often felt on both sides of the body, and may affect the fingers, wrists, elbows, shoulders, hips, knees, ankles, toes, and neck. Additional symptoms include:
- Anemia due to failure of the bone marrow to produce enough new red blood cells
- Eye burning, itching, and discharge
- Hand and feet deformities
- Limited range of motion
- Low-grade fever
- Lung inflammation (pleurisy)
- Nodules under the skin (usually a sign of more severe disease)
- Numbness or tingling
- Paleness
- Skin redness or inflammation
- Swollen glands

Joint destruction may occur within 1-2 years after the appearance of the disease.

Diagnostic criteria [4]

The American College of Rheumatology has defined (1987) the following criteria for the classification of rheumatoid arthritis:
- Morning stiffness of >1 hour most mornings for at least 6 weeks.
- Arthritis and soft-tissue swelling of >3 of 14 joints/joint groups, present for at least 6 weeks
- Arthritis of hand joints, present for at least 6 weeks
- Symmetric arthritis, present for at least 6 weeks
- Subcutaneous nodules in specific places
- Rheumatoid factor at a level above the 95th percentile
- Radiological changes suggestive of joint erosion

At least four criteria have to be met for classification as RA. These criteria are not intended for the diagnosis for routine clinical care; they were primarily intended to categorize research.

Exams and Tests

Table 1: Laboratory and Radiological Findings of Rheumatoid Arthritis

Laboratory Test	Associated Findings
Erythrocyte sedimentation rate	Often increased to >30mm per hour; may be used to monitor disease course.
C-reactive protein	Typically increased to >0.7 picograms per mL; may be used to monitor disease course.
Hemoglobin/Hematocrit	Slightly decreased hemoglobin averages around 10gm perdL(100 g per L); norm chromic anemia, also may be normocytic or microcytic.
White blood count	May be increased
Platelets	Usually increased
Liver function	Normal or slightly elevated alkaline phosphate
Urinalysis	Microscopic hematuria or proteinuria may be present in many connective tissue diseases.
Rheumatoid factor	Negative in 30 percent of patients early illness; if initially negative can repeat 6-12 months after the disease onset can be positive in numerous other processes (e.g, lupus; scleroderma; sjogren's syndrome; neoplastic disease; scleroidosis various viral, parasitic, or bacterial infections) ; not an accurate measure of disease progression.
Antinuclear antibody	Limited value as a screening study for rheumatoid arthritis
Radiographic findings of involved joints	May be normal or show osteopenia or erosions near joint spaces in early disease; wrist and ankle films are useful as baselines for comparison with future studies.
Anticyclic citrullinated	Tends to correlate well with disease progression; increases peptice antibody sensitivity when used in combination with rheumatoid factor more specific than rheumatoid factor (90 versus 80 percent); not readily available in many laboratories.
Complement levels	Normal or elevated
Immunoglobulin	Elevated alpha-1 and alpha-2 globulins possible.
Joint fluid evaluation	Consider if an affected joint can be tapped and diagnosis is uncertain, straw-colored fluid with fibrin flakes often

	seen; fluid may clot at room temperature; 5,000 to 25,000 white blood cells per mm3 (5 to 25 x 109 per L) with 85 percent poly morpho nuclear leukocytes a common finding; in rheumatoid arthritis, cultures are negative, there are no crystals, and fluid glucose level typically is low.
Renal function test	Estimated during drug therapy

Non Invasive Techniques
X-RAY
X-rays are to be taken of infected joints in rheumatoid arthritis to grade the degree of degeneration.

Joint Ultrasound
Joint ultrasound is a much less expensive way to look for joint inflammation before X-rays show damage. Although not currently used often, this procedure may gain wider use over the next few years as doctors increase their efforts to document early evidence of the disease.

Magnetic Resonance Imaging (MRI)
A MRI can detect early inflammation before it is visible on an X-ray, and are particularly good at pinpointing synovitis (inflammation of the lining of the joint)

Bone Densitometry (DEXA)
Bone densitometry is an important imaging study for measuring bone density, used primarily to detect osteoporosis. Osteoporosis may be especially severe in people with RA due to joint immobilization, the inflammatory response itself and the use of certain therapies (such as glucocorticoids) that may hasten bone loss. Some doctors suggest that a bone density test should be part of the evaluation and monitoring of all people with RA, particularly for women after menopause.

Differential diagnosis
Several other medical conditions can resemble RA, and usually need to be distinguished from it at the time of diagnosis:

Crystal induced arthritis (gout, and pseudogout) - usually involves particular joints and can be distinguished with aspiration of joint fluid if in doubt

Osteoarthritis - distinguished with X-rays of the affected joints and blood tests

Systemic lupus erythematosus (SLE) - distinguished by specific clinical symptoms and blood tests (antibodies against double-stranded DNA)

One of the several types of psoriatic arthritis resembles RA - nail changes and skin symptoms distinguish between them

Lyme disease causes erosive arthritis and may closely resemble RA - it may be distinguished by blood test in endemic areas

Reactive arthritis (previously Reiter's disease) - asymmetrically involves heel, sacroiliac joints, and large joints of the leg. It is usually associated with urethritis, conjunctivitis, iritis, painless buccal ulcers, and keratoderma blennorrhagica.

Ankylosing spondylitis - this involves the spine and is usually diagnosed in males, although a RA-like symmetrical small-joint polyarthritis may occur in the context of this condition.

Table 2: Complications [5-7]

Complications	Inferences/Comments
Cardiac Complications	Pericarditis-one third of patients may have asymptomatic pericardial effusion at diagnosis; atriaventricular block rare; myocarditis-diffuse inflammation can occur, may or may not be symptomatic.
Cervical spine disease	Tenosynovitis of transverse ligament can lead to instability of atlas on axis. Caution must be used during endo-tracheal intubation; may see loss of lordosis of the neck and decreased range of motion; C4-C5 and C5 – C6 subluxations are possible; may see joint space narrowing on lateral cervical spine films; avoid flexion films until odontoid fracture ruled out if injury is suspected; myelopath can occur, with gradual onset of upper extremity weakness and paresthesias.
Ophthalmic Complications	Episcleritis rarely occurs.
Upper extremity deformities	Ulnar deviation at metacarpo-phalangeal joints; boutonniere deformity- flexed PIP hyper-extended DIP; swan neck deformity the reverse of boutonniere, with flexed DIP and hyper extended PIP, thumb hyperextension; increased risk of tendon rupture.
Respiratory Complications	Lung nodules can coexist with cancers and form cavitary lesions; cricoarytenoid joint inflammation can arise, with hoarseness and laryngeal pain; pleuritis present in 20 present at onset of disease, not usually associated with pleuritic pain; interstitial fibrosis rales may be noted on lung examination.
Rheumatoid nodules	Often have necrotic tissue in their centers; found in 20 to 35 percent of patients with rheumatoid arthritis; usually found on extensor surfaces of the limbs or other pressure points; may form nearly anywhere, including on the sclera, vocal cords, sacrum, or verterbral bodies.
Vasculitis	Forms include distal arteritis, pericarditis, peripheral neuropathy, cutaneous lesions, arteritis of viscera, and coronary arteritis; increased risk of developing if male sx, high rheumatoid factor titers, treatment with steroids, number of disease modifying antirheumatic drugs prescribed; associated with increased risk of myocardial infarction.

Prognosis
- Predictors of poor outcomes in the early stages of rheumatoid arthritis include a relatively low functional score early in the disease progression, lower socioeconomic status, lower education level, strong family history of the disease, and early involvement of many joints.
- Prognosis is worse in patients who have a high ESR or CRP level at disease onset, positive rheumatoid factor, or early radiologic changes.
- Thirty percent of patients with rheumatoid arthritis, usually those with the most severe forms of the disease, will not demonstrate an ACR 20 response to any treatment.
- Patients with milder disease tend to benefit from early treatment.
- A study of patients with rheumatoid arthritis onset in the 1980s showed no increase in mortality with rheumatoid arthritis in the first eight to thirteen years following diagnosis.
- The standardized all-cause mortality ratio for patients with rheumatoid arthritis compared with the general population is 1.6, but this may decrease with long-term use of new DMARDs.

Gut Flora [9-12]

Normal gut flora /Gut microbiota (formerly called gut flora) is the name given today to the microbe population living in our intestine. Flora contains tens of trillions of microorganisms, including at least 1000 different species of known bacteria with more than 3 million genes (150 times more than human genes). Microbiota can, in total, weigh up to 2 kg. One third of our gut microbiota is common to most people, while two thirds are specific to each one of us.

The newborn's digestive tract is quickly colonised by microorganisms from the mother (vaginal, faecal, skin, breast, etc.), the environment in which the delivery takes place, the air, etc. From the third day, the composition of the intestinal flora is directly dependent on how the infant is fed: breastfed babies' gut microbiota, for example, is mainly dominated by Bifidobacteria, compared to babies nourished with infant formulas. Scientists consider that by the age of 3, microbiota becomes stable and similar to that of adults, continuing its evolution at a stadier rate throughout life. Gut microbiota's balance can be affected during the ageing process and, consequently, the elderly have substantially different microbiota to younger adults. While the general composition of the intestinal microbiota is similar in most healthy people, the species composition is highly personalised and largely determined by our environment and our diet. The composition of gut microbiota may become accustomed to dietary components, either temporarily or permanently. Japanese people, for example, can digest seaweeds (part of their daily diet) thanks to specific enzymes that their microbiota has acquired from marine bacteria. Although it can adapt to change, a loss of balance in gut microbiota may arise in some specific situations. This is called dysbiosis. Dysbiosis may be linked to health problems such as functional bowel disorders, inflammatory bowel disease, allergies, obesity and diabetes. Many studies have demonstrated the beneficial effects of prebiotics and probiotics on our gut microbiota. Serving as "food" for beneficial bacteria, prebiotics help improve the functioning of microbiota while allowing the growth and activity of some "good" bacteria. Present in some fermented products such as yoghourt, probiotics help gut microbiota keep its balance, integrity and diversity. The picture of the bacteria living in the gastrointestinal tract is becoming clearer. Researchers now use a range of techniques, including the tools derived from molecular biology, to further clarify the mysteries of microbiota. While there are still some things that are yet to be discovered, more and more findings are being presented every day.

Role of Gut Micro Biome in Pathogenesis in Rheumatoid Arthritis

Studies with probiotics in patients with RA have not been consistent. Many studies carried out in patients have used various species of *Lactobacillus* or *Bifidobacterium*. However, while some studies have shown some useful effect of these probiotics, it has not been very significant. Supplementation with multistrain synbiotic bacteria in RA patients has shown some benefit. However, use of gut-residing commensals is still in its infancy.

Recently, a human gut–derived commensal, *P. histicola,* has shown remarkable protection from arthritis in humanized mice [9]. This commensal was isolated from the upper gut and is therefore more likely to interact with immune cells. The advantage of using a single strain is that it is easier to monitor and control adverse effects. Moreover, since microbes can proliferate at different rates, variability of expansion of

multistrains in various individuals is difficult to predict and control.

Most of the bacteria in the gut are anaerobic and unculturable, making it hard to define the mechanism. However, one area that has gained some interest is the inflammation caused by pathogenic or opportunistic microbes. Expansion or abundance of certain microbes can cause local changes in the immune system, leading to differentiation of T-follicular helper cells that can, along with B cells, lead to generation of germinal centers and antibody production in the gut. There is some evidence from experiments done in mouse models that supports this hypothesis. Inflammation caused by the microbes can augment gut permeability, leading to exposure of luminal contents to systemic immunity. Also, extravasation of inflammatory gut-residing immune cells can lead to systemic inflammation involving distant organs. Genetic predisposing factors and hormones may also determine what kind of bacteria can colonize the gut and contribute to disease via the gut microbiome.

It's clear that gut microbiota play a significant role in immune regulation and that alterations in composition can cause an abnormal immune response. Onset of RA occurs long before the actual clinical symptoms are apparent. It remains unknown if alterations in gut microbiota cause the changes in immune response that accumulate over several years before clinical symptoms of RA become obvious. Also, RA is a heterogeneous disease: some patients have progressive, severe arthritis with involvement of other organs like the heart and lungs, while other patients have mild disease. A better understanding of gut microbiota and metabolites produced by them, as biomarkers that can predict the course and heterogeneity of disease, will help bridge the knowledge gap.

Gut microbiota are involved in drug metabolism. Knowledge of specific gut microbes required for activation or inactivation of the drugs used for RA patients will help determine what drug is the best option for a patient based on his or her intestinal microbiota. Finally, determining which beneficial microbes are missing in a patient, and supplementing those microbes, will enable an individualized treatment approach for each patient. Monoclonal microbial treatment of RA is an approach that needs to be explored. Microbes derived from the human gut are endogenous, and it is expected that they will have minimal to no adverse effects. Based on the results of humanized mouse studies with human gut-derived *P. histicola*, such therapies will be possible in the near future.

Conclusion

The role of the gut microbiota in human RA and in mouse models of arthritis. Several studies have suggested that increased abundance of *P. copri* was observed in early RA patients. In contrast, another report showed other *Prevotella* species suppressed the induction of arthritis. Further studies are required to determine the precise mechanistic links between dysbiosis and the development of human RA. The modulation of the gut microbiota may offer a novel therapeutic or preventive approach to RA patients.

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