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Rheumatoid Arthritis Complementary Approaches and the Role of Microbial Infection

Rheumatoid Arthritis and Complementary Health Approaches

Rheumatoid arthritis (RA) is a health condition that causes pain, swelling, stiffness, and loss of function in the joints. Conventional medical treatments are highly effective for RA; however, researchers are also studying complementary health approaches as possible additions to RA treatments. Some complementary health approaches for RA are intended to reduce joint inflammation, and some are intended to reduce symptoms such as pain. This fact sheet provides basic information on RA; summarizes scientific research on the effectiveness and safety of selected mind and body practices, dietary supplements, and other approaches that have been studied for RA; and suggests sources for additional information.

Key Points

- In general, there is not enough scientific evidence to prove that any complementary health approaches are beneficial for RA, and there are safety concerns about some of them. Some mind and body practices and dietary supplements may help people with RA manage their symptoms and therefore may be beneficial additions to conventional RA treatments, but there is not enough evidence to draw conclusions.

- Some complementary health approaches—particularly dietary supplements—may have side effects or may interact with conventional medical treatments or each other. Although many dietary supplements (and some prescription drugs) come from natural sources, "natural" does not always mean "safe." In particular, the herb thunder god vine *(Tripterygium wilfordii)* can have serious side effects.

- Conventional treatments are highly effective in slowing or stopping permanent joint damage in RA. Do not replace conventional medical therapy for RA with an unproven health product or practice.

- Tell all your health care providers about any complementary health approaches you use. Give them a full picture of what you do to manage your health. This will help ensure coordinated and safe care.

About Rheumatoid Arthritis

Rheumatoid arthritis is an inflammatory autoimmune disease—a type of condition in which the immune system, which normally protects the body by fighting infections and diseases, instead targets the body. RA is different from other types of arthritis such as osteoarthritis, a wear-and-tear condition that most commonly occurs as people age. In RA, the immune system attacks the tissues that line the joints, causing pain, swelling, and stiffness in the joints and affecting their ability to work properly. Over time, RA may damage bone and cartilage within the joints and weaken muscles, ligaments, and tendons that support the joints.

RA often begins in middle age and occurs more frequently in women than in men. Although RA primarily affects the joints, particularly the wrists and fingers, some people with RA may have other health problems, such as anemia, dry eyes or mouth, and heart or lung problems. People with RA may have fatigue, occasional fevers, or a general sense of not feeling well. They may also experience other symptoms such as depression, anxiety, a feeling of helplessness, and low self-esteem.

Early treatment to avoid permanent joint damage is key for preventing disability and progression of RA. Treatment for RA combines a variety of approaches and is aimed at relieving pain, reducing joint swelling, slowing or preventing joint damage, and improving physical function and well-being. Conventional medicines used for RA include:

- Disease-modifying antirheumatic drugs (DMARDs) to slow the progress of the disease
- Biologic response modifiers to reduce inflammation and structural damage to the joints
- Nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids to reduce inflammation.

Other treatments include surgery, physical therapy, modified exercise programs, and devices that ease physical stress on the joints (such as splints). People with RA are also encouraged to make lifestyle changes such as balancing activity with rest, eating a healthy diet, and reducing emotional stress.

To find out more about RA, contact the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) (see For More Information).

About Scientific Evidence on Complementary Health Approaches

Scientific evidence on complementary health approaches includes results from laboratory research as well as clinical trials (studies in people). It provides information on whether an approach is helpful and safe. Scientific journals publish study results, as well as review articles that evaluate the evidence as it accumulates; fact sheets from the National Center for Complementary and Alternative Medicine (NCCAM)—like this one—base information about research findings primarily on the most rigorous review articles, known as systematic reviews and meta-analyses.

What the Science Says

In general, there is not enough scientific evidence to prove that any complementary health approaches are beneficial for RA, and there are safety concerns about some of them. Some mind and body practices and dietary supplements may be beneficial additions to conventional RA treatments, but there is not enough evidence to draw conclusions. This section describes the scientific evidence on several complementary health approaches studied for RA.

Mind and Body Practices

Results from clinical trials suggest that some mind and body practices—such as relaxation, mindfulness meditation, tai chi, and yoga—help people with RA manage their symptoms and therefore may be beneficial additions to conventional treatments.

- **Acupuncture** has been studied for a variety of pain conditions, but very little acupuncture research has focused on RA. Reviews of the research on acupuncture have found conflicting evidence regarding its usefulness for RA.

- A 2010 systematic review looked at the benefits of mind and body techniques such as **mindfulness meditation** (which involves nonjudgmental attention to experiences in the present moment), **biofeedback**, and **relaxation training** on the physical and psychological symptoms associated with RA. There was some evidence that these techniques may be helpful, but overall, the research results have been mixed.

- A 2008 study compared cognitive-behavioral therapy that emphasizes pain management with **mindfulness meditation** for RA. The researchers found that mindfulness meditation, which helps regulate emotions, improved participants' ability to cope with pain. The researchers noted that participants with a history of depression responded better than others to mindfulness meditation.

- A few small studies have been conducted on **tai chi** for RA. In general, tai chi has not been shown to be effective for joint pain, swelling, and tenderness, although improvements in mood, quality of life, and overall physical function have been reported.

  - A 2010 study examined the effect of practicing tai chi on 15 patients with RA. The researchers found that tai chi improved muscle strength and endurance, but there was no evidence that it reduced disease activity or pain.
  - A 2007 systematic review of the research concluded that the value of tai chi for treating RA is still unproven. Many factors—including differences in tai chi styles, number of movements, length of the practice, and qualifications of instructors—add to the challenge of designing quality tai chi studies. Some people have reported soreness, but most studies have found that tai chi is relatively safe for people with RA.

- **Yoga** incorporates several elements of exercise that may be beneficial for arthritis, including activities that may help improve strength and flexibility. However, only a few studies have examined yoga for RA. Preliminary studies have found that yoga may improve physical function and decrease the number of tender and swollen joints. Yoga exercises should be performed with caution by people with RA who have limited mobility or spinal problems. People with RA may need assistance in modifying some yoga postures to minimize joint stress and may need to use props to help with balance.

Dietary Supplements

No dietary supplement has shown clear benefits for RA, but there is preliminary evidence for a few, particularly fish oil, gamma-linolenic acid, and the herb thunder god vine. Dosage and safety issues and potential interactions with conventional medicines need to be more thoroughly evaluated.

Fish oil contains high levels of omega-3 fatty acids—substances the body needs to perform a number of important functions. Types of fish high in omega-3s include herring, mackerel, salmon, and tuna. Fish oil supplements are available as capsules or oils.

- Clinical trials on RA have found that fish oil supplements may help to relieve tender joints and morning stiffness. Studies have also found that fish oil may reduce the need for NSAIDs and other conventional RA medicines. For example, the results of a randomized, controlled clinical trial published in 2008 found that people who received a blend of cod liver oil and fish oil over a 9-month period reduced their NSAID intake by more than one-third, compared with those who took a placebo.

- Because the omega-3 fatty acids in fish oil may make blood clot more slowly, people who take medications that affect clotting, such as anticoagulants, should discuss the use of fish oil supplements with a health care provider. Products made from fish liver oils (for example, cod liver oil) may contain vitamins A and D as well as omega-3 fatty acids; these vitamins can be toxic in large doses.

- For more information on omega-3 fatty acids, see the NCCAM Web site at www.nccam.nih.gov/health/omega3.

Gamma-linolenic acid (GLA) is an omega-6 fatty acid found in the oils of some plant seeds, including evening primrose (*Oenothera biennis*), borage (*Borago officinalis*), and black currant (*Ribes nigrum*). In the body, GLA may be converted into substances that reduce inflammation.

- There is some preliminary evidence that GLA may be beneficial for RA; however, the quality of the studies on GLA has been inconsistent. The more rigorous studies suggest that GLA may relieve symptoms such as joint pain, stiffness, and tenderness; in some cases, GLA led to a decreased need for NSAID medication.

- Side effects of GLA may include headache, soft stools, constipation, gas, and belching. Some borage oil preparations contain chemicals called pyrrolizidine alkaloids that may harm the liver.

Thunder god vine *(Tripterygium wilfordii)* has been used for centuries in traditional Chinese medicine. Extracts are prepared from the skinned root of the herb, as other parts of the plant are highly poisonous. <u>Thunder god vine can cause severe side effects.</u>

- Findings from laboratory and animal studies suggest that thunder god vine may fight inflammation and suppress the immune system. A 2011 systematic review looked at three human studies of oral (taken by mouth) thunder god vine and one study of topical (applied to the skin) thunder god vine for RA. The data showed that both oral and topical thunder god vine may improve some RA symptoms, but the study methods were not consistent among the trials.

- <u>A systematic review of the research on thunder god vine for RA concluded that serious side effects occurred frequently enough that the risk of using it outweighs its benefits.</u> Depending on the dose and type of extract, thunder god vine may cause serious side effects. Thunder god vine can affect the reproductive system, possibly causing menstrual changes in women and infertility in men. Long-term use may decrease bone mineral density in women, potentially increasing the risk of osteoporosis. Other side effects can include diarrhea, upset stomach, hair loss, headache, and skin rash.

Research on **other supplements** for RA symptoms is still in the early stages. For example:

- Varieties of **boswellia** *(Boswellia serrata, Boswellia carterii,* also known as frankincense) produce a resin that has shown anti-inflammatory and immune system effects in laboratory and animal studies, but no rigorous clinical trials in people with RA have been conducted.

- Laboratory studies have identified anti-inflammatory compounds in **ginger** *(Zingiber officinale)*. Most of this research has focused on the anti-inflammatory properties of gingerol compounds—the components of ginger that give it flavor. A 2009 study funded in part by NCCAM examined whether nongingerol compounds had an antiarthritic effect in rats. The researchers found that ginger extract with both gingerol and nongingerol components prevented joint inflammation and destruction better than ginger extract containing only gingerols. They concluded that the nongingerol compounds may play a role in the antiarthritic properties of ginger. Although these laboratory and animal studies show some promise, studies regarding ginger extracts for RA symptoms in people are lacking.

- A 2010 NCCAM-funded review has found evidence that substances found in **green tea** might be useful for RA and osteoarthritis, but the effects of these substances in either type of arthritis have not been fully tested in people.

- In animal studies, extracts of **turmeric** *(Curcuma longa)* containing the chemical curcumin were found to protect joints from inflammation. Building on previous laboratory research that examined turmeric's anti-arthritic properties, a 2010 study, funded in part by NCCAM, looked at whether turmeric essential oils (TEO) protected joints in rats. The researchers found that an oral dose of TEO had an anti-inflammatory effect specific to the joints. There may be a potential role for turmeric or its components in preventing or slowing RA disease, but this has not yet been demonstrated in people.

Other Types of Complementary Health Approaches Studied for Rheumatoid Arthritis

Other complementary health approaches have been studied for RA:

- An NCCAM-funded preliminary study of **Ayurvedic medicine**, a system of healing that originated in India and involves using individually prescribed combinations of herbs, found that classic, individualized Ayurvedic approaches, methotrexate (a conventional medication frequently used to treat RA), or a combination of both were equally effective in reducing symptoms of RA. Because this was a small, preliminary study, its results, although promising, are insufficient to show definitively that Ayurvedic medicine is helpful for RA.

- **Balneotherapy** is the technique of bathing in tap or mineral water for health purposes. Preliminary research on balneotherapy for RA has been conducted in areas where it is most popular, such as Europe and Israel's Dead Sea region. Although some benefits have been reported, there is not enough reliable evidence to draw conclusions.

- Some people with RA may try following **special diets**—such as vegetarian and vegan diets, the Mediterranean diet, and periods of fasting—to control symptoms. Research on these diets has been inconclusive. Although a few studies suggest that decreasing or eliminating meat, dairy, or foods likely to cause allergies may help in some cases, others do not. One drawback is that special diets may be difficult for people to follow over time. In addition, some diets could put people at risk for nutritional deficiencies.

- **Traditional Chinese medicine (TCM)** encompasses multiple practices, including acupuncture, Chinese herbal medicine, and others. Several practices that are part of TCM, including acupuncture, tai chi, and the herb thunder god vine, have been studied individually for RA, as described above. Some research has also been done on TCM as a whole for RA symptoms and for relief of side effects from conventional RA treatments, but no conclusions can be reached because of the poor quality of some of the research, variations in study design, and insufficient data on safety.

If You Are Considering Complementary Health Approaches for Rheumatoid Arthritis

- Do not replace proven conventional treatments for RA with unproven health products and practices. Do not change your use of prescribed RA medications without consulting your health care provider. Going without effective treatment for RA could lead to permanent joint damage.

- Be aware that some complementary health approaches—particularly dietary supplements—may interact with conventional medical treatments. Also consider the possibility that what's on the label of a dietary supplement may not be what's in the bottle; for example, some tests of dietary supplements have found that the contents did not match the dose on the label, and some herbal supplements have been found to be contaminated. To learn more, see the NCCAM fact sheet *Using Dietary Supplements Wisely at* nccam.nih.gov/health/supplements/wiseuse.htm.

- Women who are pregnant or nursing or people who are thinking of using a complementary health approach to treat a child should consult their (or their child's) health care provider before using any complementary health approach.

- Tell all your health care providers about any complementary health approaches you use. Give them a full picture of what you do to manage your health. This will help ensure coordinated and safe care. For tips about talking with your health care providers about complementary health approaches, see NCCAM's Time to Talk campaign at nccam.nih.gov/timetotalk.

NCCAM-Funded Research

Recent NCCAM-supported research includes projects studying:

- Approaches such as yoga, fish and borage seed oils, tai chi, and relaxation for RA symptoms, physical function, and quality of life
- How celastrus, a Chinese herb, works on a cellular level and if it has the potential to treat certain autoimmune diseases such as RA.

Microbial Infection and Rheumatoid Arthritis

Abstract

Rheumatoid arthritis (RA) is a complex autoimmune disease affecting 1–2% of general worldwide population. The etiopathogenesis of RA involves the interplay of multiple genetic risk factors and environmental triggers. Microbial infections are believed to play an important role in the initiation and perpetuation of RA. Recent clinical studies have shown the association of microbial infections with RA. Accumulated studies using animal models have also found that microbial infections can induce and/or exaggerate the symptoms of experimental arthritis. In this review, we have identified the most common microbial infections associated with RA in the literature and summarized the current evidence supporting their pathogenic role in RA. We also discussed the potential mechanisms whereby infection may promote the development of RA, such as generation of neo-autoantigens, induction of loss of tolerance by molecular mimicry, and bystander activation of the immune system.

Introduction

Rheumatoid arthritis (RA) is one of the most common inflammatory autoimmune diseases. It is characterized by persistent synovitis, systemic inflammation and production of autoantibodies [1]. The molecular mechanisms of RA pathogenesis are not fully understood. It is believed that approximately half of the risk factors for RA are attributed to genetic

factors such as the human leukocyte antigen (HLA) alleles while the other half of the risks are environmental factors including infection and smoking [2]. Clinical and animal model studies have suggested that infections by many microorganisms, such as *Porphyromonas gingivalis* (*P. gingivalis*), *Proteus mirabilis* (*P. mirabilis*), Epstein–Barr virus (EBV), and mycoplasma contribute to the etiopathogenesis of RA (Table 1).

For this review, we first identified the most common microbial infections associated to RA in the literature [3–5] and then performed a key word search using "arthritis" and "name of the microorganism" for original publications in English in the databases including Pubmed/ Medline, Embase, EBSCO, SCOPUS, and Cochrane Library till November, 2013. The candidate microorganisms included in our search were *P. gingivalis, P. mirabilis*, EBV, cytomegalovirus (CMV), human immunodeficiency virus (HIV), parvovirus, hepatitis virus, herpes virus, human T-lymphotropic virus 1 (HTLV-1), mycoplasma, *Streptococcus pyogenes* (*S. pyogenes*), Salmonella, mycobacterium, and enterobacterium. Thus, this review will discuss studies regarding to those microorganisms with RA and emphasize on *P. gingivalis* which shows the strongest association with RA. Our discussion is organized in three sections, namely, clinical association of infection with RA, induction of arthritis by infection in animal models, and the pathogenic mechanisms of infection in RA.

Clinical Association of Infection with RA

Clinical co-existence of infection and RA

Periodontal disease (PD) is the most commonly associated RA disease. The association between the two has been considered since the early 1820s. PD is caused by chronic infection of approximately twenty different bacterial species, of which *P. gingivalis, Prevotella intermedia, Tannerella forsythia,* and *Aggregatibacter actinomycetemcomitans* are the most common ones. PD can progress from gingivitis to periodontitis and cause bone degeneration in the jaw. Clinical association studies consistently show that the prevalence of periodontitis is increased about two-fold in RA patients than non-RA patients. In a large study involving 4461 participants aged 60 or older in the US population, subjects with RA were more likely to have periodontitis (odds ratio (OR)=1.82) or complete tooth loss (edentulism, OR=2.27), compared to non-RA subjects after adjusting for age, gender, race/ ethnicity, and smoking [6]. Another study reported that moderate to severe periodontitis was more prevalent in RA patients (51%) than age and gender matched osteoarthritis patients (26%) [7]. A recent study in the Dutch population confirmed the higher prevalence of severe periodontitis in RA patients [8]. They also reported that RA patients with severe periodontitis had higher DAS28 scores than RA patients with no or moderate periodontitis, suggesting that the severity of periodontitis is related to the severity of RA [8].

However, it is less clear that whether subjects with PD have increased incidence of RA. In a large prospective study involving 81,132 American women in the Nurses' Health Study cohort, there is no increased risk of later-onset RA in subjects with a history of periodontal surgery and/ or tooth loss compared to subjects with healthy periodontal conditions [9]. In another large prospective study using the National Health and Nutrition Examination Survey cohort, subjects with PD experienced higher odds of prevalent/incident RA, but most odd ratios were not statistically significant [10]. It is also important to keep in mind that both

studies are not specifically designed to examine the relationship between PD and RA. It is quite possible that there are missing data about PD and RA status in these cohorts and differential RA and PD ascertainment bias may also complicate the interpretation of data. Taken together, clinical studies have clearly shown the association of periodontal infection with RA. However, more longitudinal studies using well-defined populations are necessary to support the conclusion that periodontal infection is a risk factor for the development of RA.

Another common infection associated with RA is *Proteus*-caused urine tract infection. Patients with RA had significantly increased incidence of urinary tract infection and subclinical/asymptomatic bacteriuria compared to non-RA subjects [11]. *P. mirabilis* bacteria were isolated at a higher rate from urine samples of both female (63%) and male (50%) patients with RA than from healthy female (32–35%) and male (7–11%) subjects and patients with other autoimmune diseases including osteoarthritis, fibromyalgia, and psoriasis [12]. These studies implicate a plausible role of *Proteus* microorganisms in the development of RA.

Furthermore, it has been well documented that infections by a range of bacteria and viruses frequently manifest rheumatic diseases, including reactive arthritis. Gastrointestinal or genitourinary infections with *Salmonella, Shigella, Campylobacter, Yersinia*, and *Chlamydia trachomatis* may cause inflammatory oligoarticular or polyarticular sterile arthritis, usually starting within four weeks of infection [13]. Viruses including HIV, parvovirus, hepatitis viruses B and C, alpha-viruses like Chikungunya can cause acute or chronic forms of arthritis, and in some cases, mimic RA [13]. The precedence of infection over clinical arthritis suggests a causal relationship of the two events.

In summary, the clinical association studies suggest that infection is a risk factor for the development of RA. In addition, antibiotics such as sulphasalazine, minocycline, and rifampicin have been reported to be beneficial for the treatment of RA [14,15]. Reversely, periodontal treatments (oral hygiene and supragingival scaling) decreased the DAS28-CRP scores in RA patients [16], further implicating the pathogenic role of microbial infection in RA.

Presence of microbial contents in RA tissues

Besides the disease association, the presence of microbial contents in RA tissues provides additional evidence for the correlation between infection and RA. Molecular techniques such as PCR and DNA/RNA-*in situ* hybridization have been widely used to detect bacterial or viral infections. *P. gingivalis* [17], mycoplasma [18,19], parvovirus [20], EBV [21,22], and cytomegalovirus (CMV) [22,23] have been identified in the synovial fluid, synovial membranes or serum samples from RA patients. Herpes viruses were detected in salivary cells and circulating lymphocytes from RA patients [24,25]. Besides nucleic acids, other microbial components can be used in the detection of infections in RA patients. Bacterial fatty acids, peptidoglycan, and muramic acid quantified by techniques such as gas-liquid chromatography (GLC), enzyme-linked immunosorbent assays (ELISA) and mass spectrometry have been used to detect the presence of microbes in RA samples [26–29]. For example, in a cohort of patients with early RA before any specific treatment, analyses of

bacterial cellular fatty acids by GLC revealed the abundance of anaerobic bacteria in the intestinal flora of RA patients, implicating a possible role of intestinal anaerobic bacteria in the development of RA [26–29].

Immune response to microbes in RA patients

Another strategy to detect previous and ongoing infections is to measure the immune responses to microbial components in patients. Indeed, antibodies against infectious microbes were detected in the sera of early RA patients and the levels of these antibodies correlated with the disease activity of RA. For example, elevated levels of IgM and IgA antibodies to *P. mirabilis* were found in rheumatoid factor (RF)-positive early RA patients [30]. The levels of anti-*P. mirabilis* antibodies in RA patients went down after one year of treatment and this decrease was significantly correlated with the decrease in a modified Stoke disease activity index in RA patients [31]. The specific antigens from P. mirabilis were later identified as haemolysin and urease [32,33].

Another prominent example is that increased antibody responses to *P. gingivalis*, one of the common bacteria causing PD, were detected in RA patient sera and synovial fluid. Furthermore, the anti-*P. gingivalis* antibody levels were correlated with the titers of anti-cyclic citrullinated peptide (CCP) antibodies (the recently added RA diagnosis criteria) in RA patients [34,35]. Interestingly, a recent study showed that anti-*P. gingivalis* antibodies were significantly associated with the presence of RA-related autoantibodies (anti-CCP and rheumatoid factor) in individuals at high risk of RA [36]. This result supports the hypothesis that infection by *P. gingivalis* may play a central role in the early loss of self-tolerance that occurs in the pathogenesis of RA. Increased antibody responses to other infectious agents, such as EBV [37], B19 parvovirus [38], and mycoplasma [39,40], have also been reported in RA patients. Furthermore, T cell responses to EBV [41–43] and CMV [44] were detected in the inflamed joints from RA patients.

In summary, clinical studies using human materials revealed a possible causative link between microbial infection and RA. However, more definitive studies in well characterized cohorts are necessary before we can conclude that microbial infection plays a crucial role in the initiation and perpetuation of RA. Moreover, the "chicken or egg" relationship between infection and RA may be hard to address in human studies. First, the RA-susceptible genetic and environmental factors, such as predisposed genes and living habits, may cause increased risks to infection even before or in the early stage of RA. Second, the RA-associated abnormal immune response and immunosuppressive medicine may contribute to decreased host defense to infection [45]. In this case, studies using animal models are very useful as experimental animals normally have homogeneous genetic and environmental backgrounds.

Induction of Arthritis by Infections in Animal Models

Animal studies can directly address the causal relationship between infection and RA in accordant to Koch's postulates, although there is no perfect animal model for human RA yet. Infections by *P. gingivalis* or mycoplasma induced or aggravated experimental arthritis in mice or rats [46–50]. Interestingly, a very recent study showed that *P. gingivalis* facilitated the development and progression of destructive arthritis in CIA mice through its

unique bacterial peptidylarginine deiminase (PPAD) [51]. PPAD can lead to the generation of RA related citrullinated autoantigens by converting protein arginine residues to citrulline. This result suggests that *P. gingivalis* infection may play an important role in the loss of tolerance to citrullinated proteins in RA, implicating the causative link between infection and RA. In another study, experimental arthritis was strongly attenuated in the K/BxN mouse model under germ-free (GF) conditions, featured with reduced Th_{17} cells. Furthermore, introduction of segmented filamentous bacteria into GF mice reinstated the production of autoantibodies and arthritis symptoms [52]. This study shows that a single commensal microbe can drive the autoimmune arthritis possibly via its ability to promote Th_{17} cells.

Components derived from infectious pathogens can also induce or potentiate arthritis in animal models. For example, bacterial cell wall extracts can induce chronic arthritis in certain susceptible rat strains; and bacterial lipopolysaccharide (LPS) potentiates type II collagen-induced arthritis in mice [53,54]. Interestingly, EBV does not infect mice; and in a humanized mice model, EBV induced erosive arthritis with many features resembling those of RA [55,56]. Human T-lymphotropic virus 1 (HTLV-1) transgenic mice also developed inflammatory arthropathy, resembling RA [57].

In summary, the animal studies provide direct evidence for the causal relationship between infection and RA in experimental arthritis. Combined with the clinical association between infection and RA in human patients, it is convincing that microbial infection contributes to the etiopathogenesis of RA. However, the translation of findings from animals to humans is still arbitrary to some extent. First, adjuvant is used in the immunization protocol in these arthritis animal models. Adjuvant is known to strongly boost immune responses, and in the mean time, may also invoke immune responses to self-antigens. Second, the methods of delivering microbes in animal studies may differ from the routes of microbial evasion in humans. ird, arthritis symptoms in animals only resemble the joint inflammation in human RA, which may not represent the natural course of RA development in human patients. Nevertheless, these problems are common for animal of human diseases, and arthritis animal studies certainly provide valuable information for our understanding of RA pathogenesis.

Pathogenic Mechanisms of Infection in RA

Generation of neo-autoantigens

Autoantibodies play a crucial role in the development of RA. In fact, serum RF and anti-citrullinated protein antibodies (ACPAs) are included in the *2010 ACR/EULAR* diagnostic criteria of RA [58,59]. ACPAs are highly specific for RA and appear years earlier than the clinical diagnosis of RA [60]. Protein citrullination is a post-translational modification catalyzed by the enzyme peptidylarginine deiminase (PAD). *P. gingivalis* is the only prokaryotic organism that contains PAD. Recent studies showed that *P. gingivalis*-mediated citrullination of bacterial and host proteins provided an important mechanism for generating neo-antigens that drive the ACPA responses in RA [61]. Endogenous citrullinated proteins such as citrullinated α-enolase were abundant in *P. gingivalis*; and *P. gingivalis* PAD can citrullinate human proteins including common RA antigens fibrinogen and α-enolase [62]. Interestingly, a recent animal study showed that *P. gingivalis* facilitated the development

and progression of destructive arthritis through its bacterial PAD enzymatic activities [51]. Microbial infections may also facilitate the citrullination process by activating host monocytes and neutrophils which express high levels of PAD [63–65].

Neutrophil extracellular trap (NET) is a structure released by activated neutrophils. It is composed of decondensed chromatins and granular molecules which can enhance the killing of extracellular microbes. It has been shown that bacterial LPS and some inflammatory cytokines can strongly induce NET. NET provides a source of autoantigens for several autoimmune diseases, such as vasculitis, systemic lupus erythematosus, and RA [66,67]. NET contains citrullinated RA autoantigens including α-enolase and vimentin. Furthermore, netting neutrophils have been found in synovial tissues, rheumatoid nodules, and skin from RA patients [67], implicating a role of NET in the pathogenesis of RA.

Collectively, microbial infections directly or indirectly induce the generation of citrullinated neo-autoantigens which may trigger the aberrant immune responses in RA [68].

Loss of tolerance by molecular mimicry

Molecular mimicry plays an important role in the loss of tolerance in autoimmunity. Microbes may have elements that are similar in amino acid sequences or structure to self-proteins thus trigger autoantibody production through epitope spreading. For example, the *P. gingivalis* enolase and human α-enolase share 82% homology at the 17-amino acid immunodominant regions. Therefore, antibodies against bacteria enolase can recognize the homologous human α-enolase and promote the production of anti-human α-enolase autoantibodies. Indeed, the levels of anti-citrullinated human α-enolase antibodies were tightly correlated with the levels of antibodies to bacterial α-enolase in RA patients [69]. In addition, the affinity-purified antibodies to the human α-enolase peptide displayed cross-reactivity with the *P. gingivalis* enolase peptide [69]. Other examples include antibodies against EBV peptide p107 cross-react with the denatured human collagen and keratin [70]. Molecular mimicry also promotes autoreactive T cell activation and proliferation. The mimicry peptides for T cell activation are generally shorter and more linear compared to those of B cells. *E. coli* heat shock protein DnaJ contains a QKRAA motif that is also present in the HLA-DRB1 shared epitopes. DnaJ strongly activated RA synovial T cells which had passed the positive selection in the thymus through weak binding with the corresponding HLA epitopes [71,72]. Mycobacterial 65 kD heat shock protein (HSP65) shares homology with human HSPs. Clonal expansion of mycobacterial HSP65-reactive T lymphocytes was found in the synovial fluids and blood samples of RA patients. In addition, mycobacterial HSP65 can induce the proliferative response of mononuclear cells derived from RA synovial fluids [73,74]. These studies support the hypothesis that microbial molecular mimicry plays an important role in priming autoimmunity in patients with RA.

Bystander activation of the immune system

Bystander activation is a process by which microbial products non-specifically activate lymphocytes and immune effecter cells. It has been shown that bystander activation also plays a role in driving the autoimmunity and tissue injury in RA. The pathogen-associated molecular patterns (PAMPs) can bind to the pattern recognition receptors (PRRs) and lead to

both innate and adaptive immune cell activation [75]. *P. gingivalis* and *E. coli* LPS induced monocyte activation and the production of RA-associated cytokines interleukin (IL)-1 and IL-33 through the TLR pathways [76,77]. Peptidoglycan, a bacterial cell wall component, is a potent arthritogen. It can activate lymphocytes and induce production of cytokines and polyclonal autoantibodies including RF *in vivo* using animal models and *n vitro* using cell culture systems [78,79].

Microbial superantigens

Superantigens have long been suggested to play a role in pathogenesis of autoimmune diseases. The frequency of Vβ14$^+$ T cells in the synovial fluid of affected joints are significantly higher than that in the peripheral blood of RA patients, implicating that the etiology of RA may involve initial activation of Vβ14$^+$ T cells by a Vβ14$^+$-specific superantigen [80]. The skewed accumulation of Vβ14$^+$ T cells in RA synovial joints was confirmed by another study [81]. EBV infection of human lymphocytes can cause *in vitro* expansion of non-specific B cells and CD8$^+$ T cells, leading to polyclonal antibody production and cytotoxic T cell activation [43,82,83]. In animal models, several superantigens, such as mycoplasma arthritidis mitogen and toxic shock syndrome toxin, were able to exacerbate arthritis [50,84].

Direct effects on joint tissues

Microbial infection can have direct activating or damaging effects on the joint tissues. For example, *Streptococcus pyogenes* infection resulted in the increased expression of receptor activator of NF-κB ligand (RANKL) in mouse osteoblasts in cell culture [85,86]. In another study, Salmonella infection led to RANKL upregulation in synovial fibroblasts derived from mice [87]. Furthermore, co-cultures of Salmonella-infected synovial fibroblasts with osteoclast precursors resulted in the differentiation of multinucleated bone-resorbing, osteoclast-like cells and the formation of bone-resorbing pits [87]. This study provided evidence that Salmonella infection can mediate osteoclast differentiation and activation, which may contribute to bone destruction in infected joints. Recently, it was reported that *P. gingivalis* directly promotes early and later stages of apoptosis of human chondrocytes, which may contribute to the cartilage loss in RA patients [88].

Conclusion

RA is a complex autoimmune inflammatory disease. The etiopathogenesis of RA involves the interplay of multiple genetic risk factors and environmental triggers. Numerous studies have shown the clinical association of microbial infection with RA. Infection is often detected in early RA and can precede the occurrence of clinical arthritis. These observations suggest that infection contributes to the initiation and exaggeration of RA, arguing against the theory that the RA-associated infection is simply a sequela of immunosuppressive treatments. The pathogenic role of infection in RA is also suggested by studies using arthritis animal models. Among the RA associated microbes, *P. gingivalis* shows the greatest promise as a significant contributor to RA etiology. *P. gingivalis* is the only known prokaryotic organism that contains enzyme peptidylarginine deiminase (PAD) which is essential for the generation of citrullinated autoantigens. Human studies have shown the

association of *P. gingivalis* infection with RA patients and individuals at high risk for RA. Animal studies also demonstrated that *P. gingivalis* infection facilitated the development and progression of destructive arthritis. And more interestingly, this effect is dependent on *P. gingivalis* PAD. Future prospective studies examining *P. gingivalis* infection in patients before and at the early-onset of RA using serial collections of patient sera are necessary to confirm the etiopathogenetic role of *P. gingivalis* in RA. Multivariate analyses stratified by RA related factors such as susceptible gene alleles and smoking are also required to pinpoint the role of *P. gingivalis* infection in RA. In addition, studies that elucidate the arthritogenic pathways of *P. gingivalis* infection hold great promise to provide therapeutic targets for the prevention and treatment of RA, a disease affecting 1–2% of the general world wide population.

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Table 1

Common RA-associated microbes.

Microbes	Clinical association	Animal study	Arthritogenic mechanism
Porphyromonas	Clinical association between RA and periodontitis [6–10]. Presence of *P. gingivalis* DNA in RA patients [17]. Immune responses to *P. gingivalis* in RA patients [34,35]. Increased anti-*P. gingivalis* antibodies in subjects with high risk of RA [36].	Immunization with *P. gingivalis* or *P. gingivalis* enolase induced or exacerbated arthritis [47–49]. *P. gingivalis* facilitated destructive arthritis in CIA mice dependent on its peptidylarginine deiminase [51].	Neo-antigen generation [62]. Molecular mimicry [69]. Bystander activation [47,49]. Direct joint damage [88].
Proteus	Clinical association between RA and urinary tract infection [11]. Immune responses to *P. mirabilis* in RA patients [30–33].		Molecular mimicry [33].
EBV	Clinical association between RA and EBV infection [24]. Presence of EBV DNA and protein in RA patients [21,22]. Immune responses to EBV in RA patients [37,41–43].	EBV induced arthritis in humanized mice [55,56].	Molecular mimicry [70,89]. Superantigen [43,82,83].
Mycoplasma	Presence of DNA [18,19] and glycoglycerophospholipids (GGPL) [29] in RA patients. Immune responses to mycoplasma in RA patients [39,40].	Immunization with mycoplasma arthritidis induced or exacerbated arthritis [46,50,84].	Superantigen [40,50]. Bystander activation [29].