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Review article

Pseudomonas aeruginosa Lipopolysaccharide Involves in Inflammatory and Autoimmune Response in Patients with Rheumatoid Arthritis

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ABSTRACT

Rheumatoid arthritis (RA) is one of the complex diseases caused by unknown reasons and mechanisms of action of the disease. Studies that address the role of bacterial antigens in stimulating the inflammation, autoimmune response, and activity of RA are scanty in the literature. Therefore, the current review attempts to highlight autoimmune diseases' pathogenicity, especially RA. The review also focused on the role of bacterial infections and lipopolysaccharides in stimulating the autoimmune response as well as the activity of RA. The study showed that *Pseudomonas aeruginosa* LPS increased the inflammation response and activity of RA. Moreover, this review showed the effect of this LPS on increasing the autoimmune response in patients with RA. The study concluded the role of bacterial infection and bacterial LPS in the autoimmune response phenomenon in the laboratory as well as RA disease activity, and inflammation immune response.

Keywords: Bacterial infections, Lipopolysaccharide, *Pseudomonas aeruginosa*, DAS 28, Rheumatoid arthritis, Urinary tract infection.

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1. INTRODUCTION

Autoimmune disease is a situation triggered by the immune system attacking self-molecules because of the deterioration of immunologic tolerance to auto-reactive immune cells. Autoimmune disorders affect approximately more than 3% of the North American and European populations, with 75% of those affected being women. In most cases, the attacks against the body's self-molecules in Rheumatoid Arthritis (RA) disease are unknown, but many studies propose that they are strongly related to factors including genetics, infections, and/or environment [1]. The discrimination between self-molecules and foreign substances ensues through intricate mechanisms depending on certain recognition molecules located on the surface of immune-competent cells, specifically, T and B lymph-B lymphocytes that complement the T and B lymphocytes to provide the first line of defense against possible pathogens. Such cells can be leukocytes [macrophages, natural killer cells, and Polymorphonuclear leukocytes (PMNs)]. Soluble mediators such as cytokines that have a role in the body's defense structure can also be present [2].

The management of RA has progressed during the past 2 decades. Although some patients with RA suffer from mild illness with minimal joint destruction, the disease can progress to significant impairment of the affected joints. RA is systemic and often affects joints in a balanced manner. The main symptoms of RA include joint pain or stiffness, weakness, and muscle aches. Joint deformity takes place in the final stage of

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disease progression. Extra-articular manifestations can also be present [3]. The inflammatory process includes the stimulation of T lymphocytes and B cells and the formation of autoantibodies by plasma cells. Autoantibodies such as rheumatoid factor and anti–citrullinated protein antibodies (ACPAs) may be present earlier than clinical disease. Rheumatoid factor (RF) is generally a polyclonal immunoglobulin IgM antibody and is found in 85%–90% of patients. Though not specific to RA, higher levels of rheumatoid factor are usually related to more severe RA [4]. The research has been conducted to outline the role of immune mediators in the pathogenesis of RA, especially interleukin-l (IL-1). This included the presence of IL-l bioactivity in rheumatoid synovial fluid (SF) on its pro-inflammatory actions on human rheumatoid synovial cells, its stimulation of the release of collagenase and prostaglandin E2, and its induction of both cartilage degradation and bone resorption. The possible advantage of IL-l in RA has been supported by the ability of intra-articular administration of IL-1 in rabbits to reproduce many of the antigen-induced arthritis and by the presence of IL-l receptors on porcine chondrocytes and synovial fibroblasts [5].

Pseudomonas aeruginosa is an opportunistic pathogen that infects virtually all tissues, can also infect immune-compromised individuals, and is involved in hospital-acquired infections [6]. Burn patients, mechanically ventilated patients, and cystic fibrosis (CF) patients are more vulnerable to *P. aeruginosa* infections. It is responsible for morbidity and mortality in patients with cystic fibrosis [7]. This bacterial species contains a variety of virulence factors that contribute to its pathogenicity. It also has other virulence factors such as exotoxin a, exoenzyme S, and lipopolysaccharide (LPS) [8]. Lipopolysaccharide (LPS) is a constituent of the Gram-negative bacterial cell wall which activates B cells, leading to the production of polyclonal antibodies. LPS is also a powerful substance that secretes various kinds of mediators, including interleukin-12 (IL-12) and interferon-c (IFN-c), involved in cellular immunity [9]. Therefore, many studies have demonstrated that LPS plays a role in some diseases in which autoantibodies or self-antigen-specific T cells are involved. For example, LPS enhances MRL/lpr nephritis, experimental autoimmune uveitis, experimental autoimmune myocarditis, and experimental autoimmune enterocolitis [10]. Nevertheless, few studies have shown a role for LPS in the induction of autoimmune arthritis. Experimental models include collagen-induced arthritis in mice, made by immunization with type II collagen (CII) emulsified with complete Freund's adjuvant containing mineral oil (incomplete Freund's adjuvant) and heat-killed mycobacteria, and followed by a booster injection [10].

2. AUTOIMMUNE DISEASES

The immune system is a complicated system of cellular and molecular elements that guard the body against attack. Under normal circumstances, the immune system shows tolerance (an inability to react) to self-molecules and thus does not respond to elements (carbohydrate, nucleic acid, or protein) expressed in endogenous tissues. When self-tolerance is lost, the immune system is expanded against one or more of the body's own molecules. The civil battle against autologous tissue after loss of self-tolerance is the onset of autoimmune diseases [11].

The assumption of autoimmune disease is the danger model in which the trigger that activates quiescent antigen-presenting cells (APCs) is the genesis of one or more alarm signals by injured cells. The injury can result from many sources (neoplasia, pathogens, toxicants, trauma, etc.), that can release elements that cause a damage-associated molecular pattern (DAMP) [12]. Human autoimmune diseases happen extensively (afflicting more than 5% of the population worldwide), and cause a significant burden of morbidity and mortality on the human population [13]. Dozens of autoimmune diseases have been proven, and autoimmune mechanisms are speculated to contribute to the pathogenesis of many chronic inflammatory conditions. Some affect a single organ, while others damage multiple sites of a single organ system (e.g., immune-mediated vasculitis), and yet others disrupt multiple organs (e.g., systemic lupus erythematosus [SLE]) [14]. The actual prevalence is presumed to be higher because the extent of less prevalent autoimmune diseases is underestimated due to the limited epidemiological data. The number of autoimmune disease victims in the United States is slightly greater than the number of people suffering from cardiovascular disease and nearly three times higher than the number with cancer [13]. Nearly 75% of autoimmune disease patients are women and the chance of developing an autoimmune disease is about tenfold greater for post-pubescent women than it is for age-matched young males; however, certain autoimmune diseases are more prevalent in males, especially the several autoimmune renal diseases [15].

The autoimmune diseases are increasing in developed nations, for unknown reasons. Environmental impacts such as exposure to chemical pollutants and overuse of medication as well as stress have been considered as possible causes [16]. Another view is the decreased exposure to microbial and parasitic antigens because of enhanced hygiene and following better pathogen-reducing regimes (e.g. therapies and vaccines), which makes immune elements switch to self-antigens. The countries with emerging economies do not have these traits of prosperity and thus have a much lower rate of autoimmune diseases [16].

3. Mechanisms of autoimmunity

A small number of auto-reactive B and T cells make up the normal part of the immune cell pool since the production of autoantibodies is usually observed in normal healthy individuals. Tolerance is normally preserved by the regulatory interactions of a variety of cell types and soluble mediators. But, under certain conditions, tolerance can be cut off which may lead to an autoimmune pathology. Development of autoimmune disease is highly reliable on a genetic background but other catalytic factors such as viral, bacterial, or chemical attack lead to altered self-reactivity [17]. The discrimination between self and non-self by the mature immune system relies on a complicated meshwork of persistent and well-regulated interactions between the afferent arm and the efferent branch to guarantee that self-elements are tolerated. In general, this is regulated by components of the acquired immune system, in particular the complement of B- and T-lymphocytes along with their membrane bound, antigen-specific recognition molecules. In an autoimmune disease, auto-reactive T- and B-lymphocytes interact in a mutual positive feedback to keep the disease over time [18]. The three essential cell-oriented mechanisms for preventing autoimmunity are deletion (removal), anergy (relaxation), and suppression (restraint). Deletion involves irreversible trimming of self-reactive T-cells. This process of central tolerance takes place mainly in the thymus, the primary lymphoid organ responsible for lymphocyte production. In the thymic cortex, native lymphocytes (Th0 class) that ignore self-elements as potential targets are positively selected for survival, while T-cell precursors that attract self-antigens are eliminated through apoptosis. The survivors move to the thymic medulla to be exposed to self-antigens in the presence of MHC type I receptors but not MHC type II receptors or co-stimulatory molec-

ules. Any T-cells that attach self-antigens with high affinity in the absence of MHC type II and a co-stimulatory signal are negatively selected and converted into an additional round of T cell receptor-mediated apoptosis. Simultaneously, these two rounds of apoptosis provide central tolerance. If required, additional apoptosis is launched in secondary lymphoid organs [19]. Other mechanisms used to reduce auto-reactive immune cell lineages occur in secondary lymphoid organs and/or sites of inflammation. These mechanisms are called peripheral tolerance as they happen from the core central immune organs. The second alternative for preventing autoimmunity by inducing condition of unresponsiveness that suppresses the function of the autoreactive clones while leaving them alive [20, 21]. The pathogenesis of autoimmune disease is also motivated by numerous mechanisms at the molecular level. These pathways promote dysregulated intercellular interaction in one form or another. Both innate immune cells and elements within the acquired immune system can be involved at the molecular level [11].

4. RHEUMATOID ARTHRITIS

RA is a common autoimmune disease accompanied by progressive disability, systemic complications, early death, and socioeconomic costs. The causative agent of RA is unknown, and the diagnosis is careful. However, progress in understanding the pathogenesis of the disease has promoted the discovery of new therapeutics, with improved outcomes [22]. RA involves a complex interaction among genotype, environmental factors, and chance [22]. It is marked by synovial inflammation and hyperplasia, autoantibody production (rheumatoid factor and anti–citrullinated protein antibody [ACPA]), cartilage and bone deformity, and systemic signs, including cardiovascular, pulmonary, psychological, and skeletal disorders [22]. Genome wide analyses proved that immune factors manage the disease (Welcome Trust Case Control Consortium, 2007). Infectious agents such as (Epstein–Barr virus, cytomegalovirus, *Proteus* species, and *Escherichia coli*) and their products (e.g., heat-shock proteins) have long been correlated with RA, and although mechanisms remain mysterious, a state of molecular mimicry is assumed. Immune complexes produced during infection may catalyze the production of rheumatoid factor, a high-affinity autoantibody against the Fc portion of immunoglobulin, which is a diagnostic feature of RA [22]. It has been long known that women are more prone to RA than men. The initiation of RA is also associated with adverse life events. Molecular information for such phenomena is rising from animal models of inflammation, which show a connection between the hypothalamic–pituitary–adrenal axis and cytokine production [23]. The central nervous system is normally involved in immune regulation and homeostasis, and neuro-immunologic interactions manage disease development in rodent models of arthritis. Leukocyte migration is allowed by endothelial activation in synovial microvessels, which leads to extensive expression of adhesion molecules (integrins, selectins, and members of the immunoglobulin superfamily) and chemokines. Correspondingly, neoangiogenesis and insufficient lymphangiogenesis are characteristic signs of early and established synovitis [24]. These micro-environmental changes, linked with profound synovial architectural rearrangement and local fibroblast activation, allow the buildup of synovial inflammatory tissue in RA.

4.1. Pathogenesis of RA

Synovial joints are specialized anatomical structures that permit low-friction movement between contiguous bone/cartilage surfaces. Normal synovium is a thin inner layer of tissue composed of synovial fibroblasts which form the components of lubricating synovial fluid, synovial macrophages, and a capillary vascular network [25]. In RA, the synovial area becomes the site of a regulated inflammatory response, including an influx of neutrophils, monocytes, dendritic cells, T cells (mainly CD4+, memory phenotype), and B lymphocytes. The synovial cover becomes hypertrophic, with an increase of the synovial fibroblast and macrophage populations and angiogenesis. Lymphocytes can infiltrate the synovial membrane diffusely or organize into perivascular or lymphoid follicles. Neutrophils move through the synovium, accumulate in vast numbers in the joint space, and eventually die. Possibly, this has a great force on the phagocytic capacity of synovial cells. There are serious changes in joint physiology, with high synovial fluid volume and pressure, acidosis, and hypoxia [26, 27]. Through direct invasion and the coupled effects of metalloproteinase and prostaglandin production, accompanied by local osteoclast stimulation, inflamed synovial tissue converts to local invasive pannus tissue, damaging cartilage and eroding bone. Many studies have shown excessive local production of prostaglandins, cytokines, chemokines, adhesion molecules and growth factors in RA synovial tissue, and the existence of macrophage and fibroblast-derived regulators including tumor necrosis factor (TNF), Interleukin-1(IL-1), IL-6, IL-12, IL-15, IL-18, granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage *migration inhibitory factor* (MIF) and vascular endothelial growth factor (VEGF) [28, 29].

4.2. Inflammation Mediators and Joint Damage

RA is a complex disease that mainly affects joints but also has multiple systemic manifestations. Hypergammaglobulinemia and rising levels of acute-phase proteins are regular characteristics of this disease [30]. The synovial membrane is hyperplastic and filled with mononuclear cells. T lymphocytes are then activated and B lymphocytes produce rheumatoid factor. Synoviocytes may have a central role in this process. They are found at the site of tissue destruction and release proteases that degrade cartilage, and other mediators of inflammation and tissue damage. Interleukin (IL) -1 and tumor necrosis factor-a (TNF-α) are thought to mediate inflammatory joint destruction by their capacity to stimulate proteases and prostaglandins by synoviocyte production and bone resorption by osteoclasts. IL-1 and TNF-α are essential inducers of IL-6 synthesis [31].

5. *Pseudomonas aeruginosa*

Currently known as *P. aeruginosa*, is an opportunistic bacterial pathogen that has been named many times throughout its history depending on the characteristic blue-green coloration produced during culture. *P. aeruginosa* is a ubiquitous agent found in many diverse environmental sites, and it can be isolated from many living sources, including plants, animals, and humans. *P. aeruginosa* persists in both community and hospital environments because of its ability to survive on minimal nutritional requirements and to tolerate a variety of physical conditions. In the hospital, it can be isolated from a variety of sources, including respiratory therapy equipment, soap, antiseptics, sinks, mops, medicines, and physiotherapy and hydrotherapy pools. Community reservoirs of this organism include swimming pools, hot tubs, contact lens solutions, home humidifiers, whirlpools, soil and rhizosphere, and vegetables. *P. aeruginosa* is occasionally a member of the normal microbial flora in humans [32,33].

6. BACTERIAL LPS

Endotoxin is a term still used today for the lipopolysaccharides that were later discovered. However, endotoxins are not all toxic, just like bacteria are not all pathogenic. They were then used to designate the major group of Gram-negative bacteria those having a second, outer membrane [34]. The invention of techniques for extraction and preparation of pure endotoxins for structural studies was slow. The enterobacterial LPSs (especially those of *E. coli* and *Salmonella enterica* serovar typhimurium) were mainly studied. Strains of these species produced colonies with either a rough or a smooth appearance. *E. coli* produced LPS containing glucosamine, galactose, glucose, L-glycero-D-manno-heptose, and 3-deoxyoct-2-ulosonic acid (Kdo), as well as phosphate and the C12 and C14 lauric, myristic, and hydroxymyristic acids. Strains giving rise to colonies with a smooth texture also produced LPSs with the same components along with many other sugar membranes [34, 35].

It is not astonishing that most LPSs picked for analysis were those of medical or veterinary importance. In many cases, bacteria that were not pathogenic to humans were found in infected, immune-compromised patients. While in other cases, bacteria that were pathogenic for other mammals became pathogens for humans and vice versa. Nowadays, considerable focus is given to the LPSs of Gram-negative, nitrogen-fixing bacteria. Although these are not pathogens, one refers to the symbiotic interaction between plants and bacteria that may lead to an infection [36]. Only small amounts of LPS are liberated into the medium as in the case of cell division and not secreted by the cells. Larger amounts are secreted by bacteria after destruction by antibiotics, the complement complex, phagocytosis or treated with divalent cation chelators. In an infected host, small amounts of LPS can be protective since it induces the immune system to shrink tumors. Nevertheless, large amounts cause high fever, increase heart rate and lead to septic shock and death by lung and kidney failure, and systemic inflammatory response [37, 38].

Most of the biological functions have been associated with the lipid region of the molecule, though the exact role of the polysaccharide moiety is not negligible. This has been explained by the stronger biological activities activated by Re-type LPS consisting of lipid A having only two Kdo residues in contrast to those of isolated or synthetic lipid A. A highly purified lipid A was incapable of inducing the release of interleukin-1 by human monocytes. The polysaccharide moiety is also characterized by its antigenic properties, its impact on the solubility of the LPS molecule, and its charge. Other activities that necessitate oligosaccharides include the stimulation of B cell division (mitogenicity) and the stimulation of human macrophage cell lines [39, 40]. For this reason, exposure to host cells, whether they are responsible for passive immunity or systemic immunity, will lead to the stimulation of the inflammatory response, and this, if it continues for a long time, will lead to damage to the body.

6.1. LPS and rheumatoid arthritis disease

LPS activates B cells, leading to significant production of polyclonal antibodies. LPS is also an effective substance that releases various kinds of mediators, including IL-12 and IFN-c, implicated in cellular immunity. Hence, many studies have shown that LPS plays a role in some diseases in which autoantibodies or self-antigen-specific T cells are included. For example, LPS stimulates nephritis, autoimmune myocarditis, autoimmune uveitis, and experimental enterocolitis [41]. Nevertheless, few studies have shown a role for LPS in the induction of autoimmune arthritis. Experimental models include collagen-induced arthritis in mice, as a result of immunization with type II collagen (CII) emulsified with complete Freund's adjuvant containing mineral oil (incomplete Freund's adjuvant) and heat-killed mycobacteria, and later given a booster injection. In this model of arthritis, which is similar to rheumatoid arthritis in humans in certain clinical and histological features, autoimmunity to CII is critically included because the disease can be transferred to new recipients with anti-CII immunoglobulin G (IgG) and IgG2a antibodies. However, it has not been shown if LPS has the ability as an adjuvant to stimulate this type of autoimmune disease [42]. The CII administration in combination with LPS, but not of CII or LPS alone, was followed by stimulation of arthritis which was related to significant production of anti-CII IgG, and IgG2a antibodies, and release of cytokines including IL-12, IFN-c, IL-1b and TNF-α, indicating that LPS plays an essential role as an adjuvant in the initiation of autoimmune arthritis [43].

Another study demonstrated that LPS produced by *E. coli*, a common member of the intestinal bacterial flora, decreases the onset levels of autoantibody required to passively transfer arthritis to new mice by > 10 to 20fold. LPS was synergistically involved in both triggering and intensifying arthritis in DBA/1 mice. Hence, it was wondered that high absorption of undigested antigens and intestinal bacterial toxins such as LPS together as a result of increases in mucosal permeability might cause a decrease of immune-homeostasis in the gut-associated lymphoreticular tissues (GALT), overwhelming the immune tolerance mechanism and initiating autoimmune arthritis. To demonstrate that dietary collagens could activate the synthesis of serum anti-type II collagen antibody that cross-react with autologous cartilage and causes arthritis, Terato and his colleagues studied the immunogenicity and arthritogenicity of type II orally administered collagen to DBA/1 mice and successfully stimulated autoimmune-mediated chronic arthritis in DBA/1 mice [44, 45].

The presence and role of LPS may be supported by the other review that provided proof for the role of microorganisms in inflammatory arthritis and rheumatic diseases [46, 47]. LPS is also known to increase all of the cytokines involved in RA. Antibodies could be synthesized to LPS that – like the anti-Proteus antibodies might also serve as autoantibodies in RA and usually during the flares [48]. The generalized LPS also applies its effects by activation of cytokines such as IL-6, and TNF-α in response to LPS, IL-8, IL-12, and IL-15, thereby stimulating the innate immune response [49]. LPS may stimulate the production of inflammatory and cytotoxic b-amyloids. Bacteria should be found in relevant tissues of RA patients, whether by culture or by molecular methods (e.g. macromolecular sequencing or antibodies). Significant products such as LPS and other antigens should be found in patients vs. controls. Their numbers (bacteria and/or inflammatory products) should rise with disease severity and during flares. Their numbers and activity (hence disease prevalence/severity) should relate to free iron levels. Treatments that decrease the activity of bacteria and/or their products should be beneficial. These may include iron restraining, antibacterial, anti-LPS, and anti-amyloid treatments. A number of therapeutic strategies based on these and other ideas (including the roles of vitamin D metabolites) seemed successful [50, 51]. In a study conducted by Wang and colleagues, 2015; they treated the macrophage cell line THP-1 with LPS and made qRT-PCR analysis of Semaphorins

(Sema4A). Sema4A expression was also strongly increased in a dose-dependent manner during LPS treatment. They proved that as in RA synovial fluids, LPS treatment also induced Sema4A secretion in THP-1 cells. They also experimented with whether Sema4A affected TNF-α and IL-1β secretion. Sema4A treatment for 48 h induced TNF-α and IL-1β greater than cells treated with phosphate buffer saline (PBS) alone. Then they measured TNF-α and IL-1β production by THP-1 cells treated with Sema4A for 48 h and then activated with LPS (1 μg/ml) for 24 h. Contrasted with the control and consistent with the observations made in RA synovial fluids, they observed that Sema4A significantly released these cytokines in response to LPS, compared with the control without LPS stimulation [52].

7. CONCLUSION

The current study showed that there are several reasons that lead to the development and increase in the activity of RA such as the genetic and environmental factors. The current study focused on the main and important role of bacterial infections in the development of the disease and increasing its activity. The study highlights that stimulating the body with LPS plays an important role in stimulating the inflammatory response and also contributes to generate the autoimmune response in some people suffering from RA and also in the experimental animal.

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Conflict of interest

The authors declare that they have no conflict of interests.

Ethical Approval

This review was approved by the Scientific Committee of the Ministry of Higher Education, Baghdad, Iraq (No 1630, 2021).

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