Asian Journal of Medicine and Health Sciences Vol 7 Issue 2, November 2024

REVIEW ARTICLE

Pathogenic Advances in Rheumatoid Arthritis: A Review.

Nurul Syuhada Nordin[1], Yong Yoke Keong[2], Siti Farah Md Tohid[1], Zetty Nadia Md Zain[3], Muhammad Nazrul Hakim[1,4]*.

[1] *Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[2] *Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[3] *Faculty of Medicine and Health Sciences, Universiti Sains Islam Malaysia, Malaysia*
[4] *Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

Corresponding Author
Muhammad Nazrul Hakim
Department of Biomedical Sciences, Faculty of Medicine and Health Sciences
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
Email: nazrulh@upm.edu.my

Submitted: 25/06/2024. Revised edition: 09/08/2024. Accepted: 16/09/2024. Published online: 01/11/2024.

Abstract

Rheumatoid arthritis (RA) affects approximately one percent of people worldwide. It falls within the category of an inflammatory immune-mediated illness where the primary tissue involved is the joint. Environmental and genetic factors combine to cause RA. However, the exact onset of the disease is unknown, as does the appropriate time to diagnose it as RA. In RA, the synovial membrane and surrounding tissues are attacked by the immune system. The pathophysiology of RA still raises three unanswered problems. First, how the environment or heredity drives the immune system. Secondly, how it persisted in causing inflammation in the surrounding joint, and thirdly, how inflammation results in damage to bones. There are various numbers of cells associated with the progression of RA disease. Proinflammatory cytokines are mostly produced by macrophages, which can also serve as antigen-presenting cells. In joints, the fibroblast-like synoviocytes (FLS) interact with cells of the innate host immune system and activate B and T cells causing an increase in chemokines and cytokines production such as TNF-α, IL-1, and IL-6 thus allowing a feedback loop to occur. B cells, T cells, and macrophages will all play a part in these extra encounters. Matrix metalloproteinases (MMPs), prostaglandins (PGs), and inflammatory cytokines are also produced in varying quantities by the activated fibroblast-like synoviocytes within the synovial membrane. The accumulation of chemokines and nitric oxide (NO) also contributes to inflammation and tissue catabolism. MMPs enter the synovial fibroblast (SF) directly as a result of the positive feedback loop, which can lead to the degeneration of bone and cartilage. We herein summarized the key pathogenic advances addressing these issues, along with the basics of rheumatoid arthritis, its mediators, and the cell signalling pathways involved.
Keywords: *Inflammation, mediators, rheumatoid arthritis.*

Introduction

The term Rheumatoid Arthritis (RA) is based on the Greek words, which means *watery* and *inflamed* joints [1]. RA disease is categorized as an immune-mediated inflammatory disease in which the joint is the main site of tissue. The inflammation within the joint causes redness, warmth, swelling, and pain. It has been identified as one of the primary causes of disability [2], a reduction in the expectation and quality of a person's life, the possibility that a patient may become clinically impaired within 20 years [3], the increased risk of cardiovascular disease, early mortality, infection, and cancer, and the potential to cause death [4]. Rheumatoid arthritis (RA) affects approximately range from 0.24 to 1%, but varies considerably around the world [5].

As it is described as the most typical disease of systemic autoimmune connective tissue, common features include symmetric joint inflammation, and continuous degradation of articular cartilage and periarticular tissues [6]. It manifests as inflammation of the synovial membrane around diarthrodial joints, synovial vaginae, and gliding synovial bursae, which causes localised destruction [7]. This disease is a chronic type involving certain organs and occurs by systemic effect throughout the body [1].

RA is a combination of genetic and environmental factors [5]. RA is commonly identified by its symmetrical pattern. One of the most distinguishing features from other inflammatory arthritis is relying on its ability to expand the disease to new joints [8]. The central new criteria are clinical synovitis, involving the number of affected joints, abnormality in serology, increased acute phase, and duration of symptoms [9].

History and Epidemiology

Around 1500 BC, Ebers Papyrus uncovered an equal case matching rheumatoid arthritis. Subsequently, multiple research findings indicate that mothers from diverse generations exhibit physical abnormalities that are recognised as pathognomonic for arthritis [10].

RA involves 1% of the world's population, and in the United States only, more than 30 billion dollars has been put into managing the prevalence of this disease [11]. The prevalence studies are unrecorded from evolving areas of countries. It has been reported in annual published reports recently that in Northern European and North American nations, RA may differ between 20 and 50 cases per 100,000 and probably lessened in Southern European areas [9].

The Arthritis Foundation of Malaysia estimates that 5 out of every 1000 individuals in the nation have RA, with women accounting for 75% of the disease's victims [12]. As it affects 1% of people around the world, with the highest frequency is in women between 40 and 60 years of age [7]. Based on gender, women are 2:1 to 3:1 strongly affected by this disease compared to men, with the yearly occurrence of RA stated to be around 40/100,000 globally [10].

The highest range age for RA onset is between 45 and 65 years [4] which is commonly the time of hormonal transition in women. Some ethnic groups have a higher possibility of being infected based on the distribution differences and interactions of genetics and environment [13]. RA is available in all regions of the world where it has been investigated. It is assigned as the universal type of disease, where there is no information on any areas or ethnic groups which does not involved with this disease. The challenges of the descriptive epidemiology of RA are based on chronic and complex diseases, and it is unclear how early we should be calling it RA [9,10].

Life expectations of RA are declining about 3 to 10 years compared to the general population, fatality rates are also greater among RA sufferers, and this has not been transformed over the last 2 to 3 decades. The major factor of death in RA patients is based on complications of pulmonary, various secondary infections, hematological diseases, and cardiovascular and gastrointestinal problems [9,13].

Aetiology of RA

Hormonal, genetic, immunological, reactive oxygen species, infectious agents, physiological, and environmental factors interact to cause the complex disease known as RA. Dietary factors components, pollutants, and urbanization also may be the other factors involved [6].

Like many other diseases, RA is a result of a combination of environmental and hereditary factors. 50% to 60% of the development factors for RA are inherited. Genes critical to immune responses are encoded by the major histocompatibility complex (MHC); the MHC allele linked with these genes was first identified as HLA-DR4. Previous research demonstrated the relationship between RA [14] and HLA-DR4, HLA-DRB, and a number of additional alleles classified as common epitopes. PTPN22 is the non-MHC gene in RA. It carries impulses through the T cell receptor and influences lymphocyte development. Genome-wide association studies have discovered over 100 additional loci that impact susceptibility to RA. A few are mentioned in Table 2.1. These genes related to their cellular function that control immune reactions which supports the concept of autoimmune disease in RA.

The investigation into the involvement of epigenetics in RA is still in its early stages. Their methods are crucial for understanding how environmental cues affect gene expression. The complexity of RA analysis is made possible by the apparent existence of epigenetic modifications in certain cells, such as lymphocytes or synovial fibroblasts [15].

One of the extrinsic risk parts of RA development and severity is smoking. Smoking triggers the citrullination process by modifying the HLA-DR-restricted immune activity into autoantigens, this is the procedure that turns arginine amino acid into citrulline. The development of autoimmune diseases is triggered by autoantibodies directed against citrullinated proteins [16]. Previous research has also suggested that smoking may cause the lungs' enzyme that is in charge of citrullination to express itself, hence triggering the autoantibody's antigen targets [17]. Infection by several bacterial and viral pathogens has been considered a cause of RA, although direct infection of RA joints has not been reported [18]. The study of microbiome is a recent part of RA study and another autoimmune disease. This complex task involved viewing the various microbiomes that exist on the skin, gastrointestinal, and respiratory tracts [19]. Other variables that may also raise the risk of RA include birthweight, breastfeeding, birthplace, and socioeconomic status [10].

Pathogenesis of RA

Rheumatoid arthritis will induce immune system to target synovial membrane and tissues near joints. Three unanswered problems remain regarding the pathophysiology of RA. First, how the systemic immune response is triggered by the environment or genetics. The second is how the inflammation persisted in the surrounding joint, and the third is how the inflammation causes damage to the bone [20].

The first stage involves the hallmark of autoimmune priming in the appearance of Rheumatoid factors (RFs) and/or the anti-citrullinated protein antibody (ACPA) which is categorized as the RA-associated autoantibodies. At this stage, the increased serum biomarkers of inflammation and pro-inflammatory cytokines can also be discovered. RFs are autoantibodies targeting the antigen through recognizing a domain of the IgG Fc portion, further, they form immune complexes that contribute to the disease process. The appearance of RF positively correlates with the severity of disease, while the ACPA identifies proteins which undergone the conversion of arginine to citrulline and predicts the severity of disease such as the degree of joint destruction, thus making ACPA much more specific for RA than RF [21].

The next phase of RA is the emergence of clinical arthritis based on the joint inflammation level that is adequate to produce clinical signs and symptoms. The triggers including trauma and infection start to localize in the systemic

autoimmune action to the joint [15]. Activated cells of the innate system recognize the unknown triggers or microbial pathogens, which is done mainly by antigen-presenting cells (APC) like macrophages and dendritic cells via intracellular pattern recognition receptors (PRRs). The molecules of the innate system including APC and effector cells are recruited locally and move to nearby lymphoid tissues when they are unable to defeat the pathogen alone [22]. From there, the APC cells manifest arthritis-associated antigens including the activated B cells to T cells [23]. This start inflammation in the joint and aligns the disease with the local mesenchymal cells, which are synovial fibroblasts (FLS) [24]. The proliferation of synovial fibroblasts and extensive inflammatory cell infiltration such as $CD4^+$ T-cells and cells of the innate immune system cause the hyperplasia of the synovial membrane [20]. The complex interactions in RA synovium between various cellular constituents resulting the production of various cytokines and other inflammatory mediators. The cytokines released will induce blood vessels in membrane of synovia to divide in a process called hypervascularization. The increased blood flow allows the excess synovium tissue growth and leads to the thickening of the synovium [15].

The third phase of RA is inflammation involving the destruction of cartilages, bones, tendons and ligaments. To support synovial expansion and inflammation, angiogenesis occurs to provide spaces for inflammatory cells and their sustained nutrients. The thick synovial membrane, called pannus occupies the small space between the joint's bones and it covers the bone's surface and the articular cartilage. The cytokines initiate the differentiation of monocyte precursors into osteoclasts leading to bone erosion [15]. However, there are still unclear distinctions regarding the underlying mechanisms between these three stages. This is based on the damage to the joint that can be detected in the very early clinical diagnosis of RA by sensitive imaging techniques [25].

There are various numbers of cells involved in the development of RA. The macrophages are the basic element of proinflammatory cytokines and may also perform as antigen-presenting cells [26]. The CD4+T-cells, specifically IL-17-producing helper T (Th17) cells contribute to the enrolment of RA systemic immune response. The Th17 cells' migration into the inflamed site is assisted by mesenchymal cells to produce homeostatic proliferation and further stimulate IL-17 production. In joints, the fibroblast-like synoviocytes (FLS) interact with cells like macrophages, dendritic cells, mast cells, and NK cells, as well as cells of the adaptive immune system, B and T lymphocytes [9]. The activation of B and T cells caused an increase in chemokines and cytokines production such as TNF-α, IL-1, and IL-6 thus allowing a feedback loop to occur. This will involve the additional interactions of B cells, T cells, and macrophages. The activated fibroblast-like synoviocytes also produced various amounts of matrix metalloproteinases (MMPs), prostaglandins (PGs), and inflammatory cytokines within the synovial membrane [23]. The accumulation of chemokines and nitric oxide (NO) also contributes to inflammation and tissue catabolism [27]. The positive feedback loop causes the direct invasion of MMPs into the synovial fibroblast (SF) and can cause cartilage and bone destruction [23]. The hallmark cytokine of the recently identified "Th17" T helper cell population, IL-17 (also called IL-17A), has been linked to the development of a number of autoimmune disorders, including rheumatoid arthritis. A novel category of cytokines with strong pro-inflammatory characteristics was founded by IL-17. Research on mice, mammalian cell culture methods, and clinical settings all point to IL-17's potential role in aggravating rheumatoid arthritis [23-25].

a) Synovial fibroblast

The two visible layers of normal synovial tissue are the subintima/sublining layer, which is mostly made up of two cell types, and the surface layer, also known as the intima/lining layer or synovial

lining. The cells include type A macrophage-like synoviocytes (MLS) and type B fibroblast-like synovial cells (FLS). The FLS is the most commonly present in the rheumatoid joint [28]. It generates the extracellular matrix (ECM) components of the synovial fluid that is essential for lubrication of the joint cartilage integrity. It detains the mononuclear cells in synovium and allows neutrophils to accumulate in the joint space, further regulating the leukocyte aggregation of two areas to eliminate debris from the synovial cavity. The SF also excrete various connective tissue types, including fibronectin and collagen [28].

There are review evidences that relates FLS as major cause of rheumatoid arthritis. The synovial hyperplasia marks the RA which the subintima layer amplify into 15 or more cells thus increases the FLS number. Three different mechanisms involved in this process; that are the hyperproliferation of FLS, reduced apoptosis and senescence.

The FLS secretes inflammatory mediators including IL- 1, 4, 6, 8, 10, 12, 13, 17, 18, 21, TNF-a, TGF-h, IFN-g, VIP, iNOS, and via upregulation of cyclooxygenase-2, prostaglandin E2 during inflammatory response. The resting T cells have been presented to activate the FLS. The direct contact between RA FLS and T cells through the connection of T Cell CD47 receptors on the surface of synovial fibroblast leads to T cell activation, T cell cytokines production and T cell proliferation. RA-SF will induce growth of blood vessels by producing proangiogenic factors, including fibroblast growth factors (FGF), vascular endothelial growth factors (VEGF) and IL-18 in order to sustain pannus formation in arthritis process [28]. FLS shows the number of signal transduction pathways activation specifically of inflammatory responses. Nuclear factor-nB (NF-KB) is known as a key transcription factor in RA. The activation of NF-KB regulates the matrix metalloproteinases (MMPs) expression showing it may regulate the joint destruction based on in vitro. While *in vivo* study, NF-KB expression is elevated at joint destruction sites which specifically at the cartilage–pannus junction [23].

b) Macrophages

In RA, synovial macrophages and monocytes involvement in driving the pathways are still an issue [30]. The matured monocytes enter the bloodstream undergo trans-endothelial migration into synovia. Both cells play their roles in the inflammatory initiation and perpetuation, the migration and adhesion of leukocytes, matrix degradation, and angiogenesis [31]. During joint inflammation, they become activated and control the pro-inflammatory cytokines and enzyme production [32] together with infiltrating macrophages/monocytes thus leading to cartilage and bone destruction [33].

Various cell-surface receptors are expressed by macrophages in chronic RA such as chemokine receptors and cell adhesion molecules (CAMs). Chemokine receptors on macrophages engaged in the aggregation of monocytes/macrophages to sites of inflammation. CAMs control macrophages interaction with other cells. Integrins such as VLA-4 and VLA-5 also facilitate the attachment of monocytes to endothelium during the transmigration process into the synovium. The integrin-dependent activation of macrophages leads to the initiation of transcription factor pathways and inflammatory mediator production including matrix metalloproteinases (MMPs) and cytokines [31].

Interleukin-1 and TNF-α are the pro-inflammatory cytokines released by RA synovial-tissue macrophages and exert overlapping effects in RA. TNF-α activates interleukin-6 production, which is formed by synovial-tissue macrophages and synovial-fluid monocytes. Synovitis primarily is cytokine driven. The initial key observation was when the rheumatoid synovial cell cocultures decreased IL-1 production when infused with neutralizing anti-TNF antibodies resulting with. Another study also shows that the

other pro-inflammatory cytokines including IL-6, IL-8, and GM-CSF were also induced by TNF in cocultures of RA synovial cells. The observations lead to the understanding of the macrophage-produced TNF-α concept as the key mediator of disease and driving the other proinflammatory cytokines among the other cytokines [30].

c) T cells and B cells

Most RA patients carry the cluster of HLA-DRB1 epitope. On the HLA-DR β-chain, these genotypes have a similar amino acid sequence that, through interaction with certain peptides, influences how antigen is presented to T-cell receptors (TCRs). The arthritis-related peptides may exist in disease-associated HLA-DR alleles. It caused the stimulation and augmentation of autoantigen-specific T cells in the joints and lymph nodes [23].

T cells in RA joint commonly have a memory $CD4^+$ phenotype, express numerous activation markers, and often exist close to antigen-presenting cells (APCs) such as activated B cells, macrophages, and dendritic cells [26]. It stimulates macrophages and synovial fibroblast production and induces rheumatoid synovitis [34]. B cells generate Ig and autoantibodies such as rheumatoid factor and anti-collagen antibodies which build immune complexes that can induce local inflammation [26]. B cells are also presented as antigen-presenting cells (APCs) that can activate the pathogenic T cells [35].

In RA, the tissue-localized B cells reveal their pathogenic properties by definite mechanisms including the production of autoantibodies, T-cell activation, and cytokine synthesis [35]. In immunologic action, T helper cells (Th cells) aid the B cell's maturation into memory B cells and plasma cells and also assist the activation of cytotoxic T cells and macrophages. It divides immediately and secrete cytokines which regulate the active immune response. These cells can differentiate into one of several subtypes including Th1 and Th2 [36]. In normal environments, Th1 and Th2 cells are involved in autoimmunity and respectively mediate immune reactions against intracellular and extracellular pathogens [37]. In the immune response towards self-antigens, the naïve T cells including Th1 and Th2 were transformed into $CD4^+$ and $CD8^+$ effector cells subsets [38]. Both $CD4^+$ and $CD8^+$ T cells are the main producers of TNF-α [38]. TNF-α induces IL-1 and IL-6 secretion thus indirectly influencing the Th17 population [38], which is the main key player in autoimmunity. Th17 usually reacted in immune responses against extracellular fungi and bacteria [37]. Pathogenic Th17 cells are the main players in disease development by mediating the growth of pannus, osteoclast genesis, and synovial neoangiogenesis [37]. IL-6 also can trigger *de novo* differentiation of naïve T cells to Th17. The Th17 cells and Th17-cell-derived cytokines further encourage the B-cells proliferation, differentiation, class-switch recombination, and the production of antibodies. This process proved the presence of a positive feedback loop in the inflammatory reaction between B and T cells [35].

d) Neutrophils

An essential component of the innate immune system are neutrophils. They rectify the infection through the phagocytosis process and by the release of neutrophil extracellular traps (NETs) [39]. Around 60% of neutrophils are present from all types of leucocytes in blood circulation and are one of the earliest cells to reach the synovium during RA [40].

In RA patients, these infiltrated neutrophils are being degranulated which is different from healthy neutrophils. They are also available in synovial fluid and synovial membranes for a few days instead of hours. This apoptosis delay is caused by the existence of anti-apoptotic cytokines such as TNF, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-8, and hypoxia, further increasing the neutrophil survival for up to several days in the affected joints and leads to progressive tissue damage due to their extended life span [41]. The defects in apoptotic neutrophil clearance also can produce

autoantibody production by expressing their autoantigens on their surface [41].

The synovial fibroblast-neutrophils can trigger the proliferation of T-cell stimulation [26] and shift via chemotactic gradients approaching the pathogens before finally binding through the pattern recognition receptors or opsonic receptors [41]. Phagocytosis occurs when the phagocytic vesicle of neutrophils encloses the pathogen and discharges the contents of cytoplasmic granules into the vesicle [42]. During phagocytosis, the plasma-membrane-bound NADPH oxidase is triggered and releases oxygen free radicals and reactive oxygen species (ROS) [43].

The inflammatory neutrophils in RA interact with other cells and release cytokines and chemokines such as the secretion of MMP-8, MMP-9, the cytokines repertoire (IL-1β, IL-6, IL-12, IL-17, IL-18, IL-23, and TNF-α) and also chemokines (CCL-2, CCL-4, CCL-5, and CXCL-8) [44]. The cytokines derived by neutrophils further regulate the responses with immune complexes, and the soluble immune complexes trigger higher ROS secretion and also granule enzyme [41]. The tissue damage occurs if large numbers of neutrophils infiltrate the tissues and produce large amounts of cytotoxic products that could decrease the antioxidant and anti-protease protective systems in tissues [42].

Effector Molecules and Pathogenic Mediators of RA

The evidence from previous study specify that the vessel dilate as the blood flow to the injured harmed region may increase up to ten-fold. This is mainly caused by the mediators including nitric oxide, prostaglandins (PGE2) and inflammatory cytokines. The mechanisms by which bone erosion occurs in RA are not clear [25].

a) Nitric Oxide

The short-lived signalling molecule, Nitric oxide (NO) is a chemical modulator of inflammation. It has a main role in various physiologic activities such as blood vessel tone, functions of mitochondrial, inflammation, and apoptosis [45]. The synoviocytes, chondrocytes, and endothelial cells are the major producers of NO in inflammatory joints. It is synthesized through the oxidation of L-arginine by nitric oxide synthases (NOS) [46]. The isoforms of NO are expressed as calcium-dependent (cNOS and eNOS) and calcium-independent inducible enzymes (iNOS). cNOS produces the NO and controls the physiological function, while iNOS produces excessive amounts of NO and allows inflammatory disease pathogenesis [47].

NO is associated with RA pathophysiology through inflammation and the destruction of autoimmune-mediated tissue, thus influencing the catabolism of cartilage. Studies and evidence reported that increased endogenous NO synthesis is responsible for T-cell dysfunction in RA. Under physiological conditions, NO regulates T cell functions through the potential of mitochondrial membrane of T cells and able to induce or inhibit apoptosis. During activation, the antigen-specific T cells interact with antigen-presenting cells and lead to the proliferation and differentiation of CD4 T cells. This formation of immunological synapse produces two main subsets of primary effector cells which are T helper cells 1 (Th 1) and Th 2. They are further specifically characterized by their specific patterns of cytokine expression. The Th1/Th 2 cell balance is essential in chronic inflammatory diseases [45].

The main source of NO in RA is the inflamed joint, which synoviocytic fibroblasts are the main source in rheumatoid synovium. Farrell et al.,[48] reported elevated levels of NO which were measured indirectly as nitrite from synovial fluids and serum in RA patients. Macrophages, neutrophils, endothelial cells, osteoblasts, osteoclasts, and fibroblasts can produce NO in the inflamed synovium [49].

NO may perform as a pro-inflammatory mediator together with other mediators including cytokines such as TNF and IL-1β to generate the production of synovial Matrix metalloproteinases (MMPs)[50]. A close network between iNOS expression and MMP has been suggested by

Yoshida et al.,[51] through the involvement study of NO to the production of MMP-1 in the uterine cervical fibroblast cells. In the cellular reactions to hypoxic and inflammatory conditions, the iNOS and MMP3 expressions controlled by transcription factor Hypoxia-inducible Factor 1-alpha (HIF-1α) and thus are involved in the pathogenesis of RA [52]. These suggested the roles of NO as the activator of MMPs, but the mechanisms are still in partially understood.

b) Matrix metalloproteinase (MMP)

In chronic joint inflammation, elevated levels of different inflammatory cytokines trigger the pannus and generate numerous proteolytic enzymes causing joint tissue degradation [53]. There are four classes of proteolytic enzymes in matrix proteins involved in cartilage degradation that are; serine/threonine proteases, cysteine proteases, aspartic proteases, and metalloproteases. Matrix metalloproteinases (MMPs) are one of the key mediators of the cartilage, bone, synovial fluid, and adjacent soft tissue resorption. In normal joints, the synthesis and degradation of matrix proteins are in equilibrium, but in RA patients the serum levels of MMPs are increased. This shows that the matrix MMPs can mediate the occurrence and development of RA [53].

MMPs are generated by synovial lining cells, sub-lining fibroblasts, infiltrating leukocytes and macrophages [54]. The MMPs regulations cultivate at the gene transcription level and by the activation of proenzymes [47] and their gene expression is regulated by TNF-alpha and IL-1β via the signal transduction pathways, such as by mitogen-activated protein kinases (MAPKs). The MAPKs target the AP-1 and Ets sites in the MMP promoters and demanding for expression. Under the influence of c-Jun/c- Jun homodimers or c-Fos/c-Jun heterodimers, the protein complex AP-1 is phosphorylated by c-Jun N-terminal kinase (JNK) to activate a DNA-binding AP-1 complex. Therefore, the AP-1 also activates the c-fos and c-jun mRNAs synthesis in reaction to inflammation stimulates [55].

MMP-1 (collagenase-1) is generated by the synovial cells that line the joints and primarily is the first collagenase that forms when RA disease occurs in the synovial fibroblasts. It is localized on the superficial surface of cartilage and its expression is generally 10-fold superior to MMP-13 expression [55]. MMP3 (stromelysin 1) has been studied as the major enzyme generated by fibroblasts and macrophage-like cells in the synovium among the other MMPs, and the amount is higher in synovial fluids of RA patients [52]. In a variety of cell types basically, MMP-3 expression is induced by IL-1 [56] and further can initiate the activation of other MMPs and degenerate multiple proteins, including fibronectin, cartilage link protein, and collagen types IV, VII, IX, and XI [55].

The cytokine-induced transcription factor, Nuclear factor-kappa B (NF-kB), is a major signaling pathway in controlling MMP gene expression. It controls various inducible inflammatory genes such as TNF-alpha, IL-1 beta, and IL-6 [57]. Inhibiting the NF-kB might be crucial therapeutically. A study was conducted by Campbell et al. [58], where insufficient p50 subunits in the animal models were more affected by collagen-induced arthritis. NF-kB is a dimer with p50 and p65 subunits in the cytoplasm and its activation is acquired for the transcriptional induction of MMP-1, as their promoters contain canonical binding sites for NF-kB [59].

c) Prostaglandin E2 and Cyclooxygenase2 (COX2)

The small potent inflammatory lipid mediators, prostaglandins (PG), have been reported to increase in RA patients' synovial fluid and synovial membrane. They are generated by nearly all nucleated cells from the essential fatty acids (EFAs) and are available in most organs and tissues. Their production is typically low in normal tissues but increases shortly due to acute inflammation from the association to leukocytes, leading to pro-inflammatory and anti-inflammatory reactions [60].

PGE2, a product of PGH2 is the main prostaglandins generated by macrophages, synovial fibroblasts and chondrocytes which induced by trauma and pro-inflammatory cytokines IL-1β, IL-6 and TNF-α. It has inhibitory response on NF-κB through ERK-dependent and independent pathways in RA-synovial fibroblast [60].

Prostaglandins are produced when arachidonic acid (AA) is released from diacylglycerol in the plasma membrane via phospholipase-A2 (plpA2) and is delivered into the cyclooxygenase pathway [61]. In the pathway, the AA is processed by cyclooxygenase (COX), prostaglandin endoperoxide H synthase (PGHS), and prostaglandin synthase enzymes, thus inducing the prostaglandins (PG) production. During the sequence, cyclooxygenase (COX) catalyzed the AA to bioactive prostaglandin (PGH2). Next, the PGH2 is catalyzed by specific PGE synthases (PGES) into bioactive PGs that are PGE2, PGF2α, PGD2, PGI2 (prostacyclin), and TXA2 (thromboxane), while PGE synthase (PGES) catalyzed the PGE2, it also activates the G-protein-coupled PGE receptors (EP) which are EP1, EP2, EP3 and EP4. In RA, the EP4 receptor plays a pro-inflammatory role in the disease pathogenesis [62].

Prostaglandin E synthase (PGES) has been classified into 3 forms including microsomal PGES-1 (mPGES-1), microsomal PGES-2 (mPGES-2) which are membrane-bound enzymes; and cytosolic PGES (cPGES). In inactive RA, the mPGES-1 is minimally expressed, and the initiation is controlled under COX-2 expression [60]. COX-2-derived PGE2 is known to have a role in tissue repair, fever, pain, inflammation, and cancer. In recent studies of gene targets, they found that mPGES-1 served as a novel target for anti-cancer and anti-inflammatory drugs [64]. The mPGES-2 is synthesized as a Golgi membrane-associated protein and associated with PGE2 generation by binding of COX-1 and COX-2. Cytosolic PGES (cPGES) is not committed in any pro-inflammatory stimuli but it is binding to COX-1 to stimulate immediate PGE2 released [60].

The cyclooxygenases catalyzed the first two steps in prostaglandins (PGs) biosynthesis, and have 2 major isoforms including COX-1 and COX-2. They are commonly used and targets for nonsteroidal anti-inflammatory drugs, thus explaining their functions in pain, tumorigenesis, and inflammation [65]. Both isoforms are encrypted by different genes and have definite patterns of expression. COX-1 is regularly expressed in various types of tissues. It is important to perform essential metabolic functions for the survival of PGs. While the expression of COX-2 is induced by stimuli or inflammatory mediators such as mitogens, cytokines, growth factors, bacterial endotoxins, and tumour promoters, further incorporated in the development of cancer and various pathogenesis such as apoptosis, angiogenesis, immune surveillance, cell differentiation, invasion and metastasis [60]. The COX activity inhibition is needed to restrain the PGs synthesis in inflammation cases such as RA [66], but the inhibition of COX-1 and COX-2 production by traditional NSAIDs frequently related to side effects including gastrointestinal bleeding, due to both isozymes suppression [60].

d) The inflammatory cytokines (TNF-α, IL-1β and IL-6)

The cytokines are synthesized by nearly all cells [67] and promote RA autoimmunity by regulating chronic inflammatory synovitis which leads to adjacent joint tissue destruction. The term ‘cytokine’ is originally from two Greek words combination that are “cyto” (cell) and “kine” (move) [68]. The categorization of cytokines is not practicable due to the simultaneous pleiotropic effect, but to understand the entire mechanism in RA pathogenesis. Cytokines have four categories; pro-inflammatory, inflammatory cytokines mainly in joints, anti-inflammatory, and natural cytokine antagonists [69].

Increased proinflammatory cytokines are identified in joint fluids and synovium of RA

patients including tumor necrosis factor-α (TNF-α) and interleukin (IL)-1 [8]. By targeting the endothelium, both IL-1 and TNF initiate the inflammatory mediators which act as endothelial adhesion molecules inducers during the emigration process into tissues [67]. They act locally and can affect the system such as being involved in cardiovascular disease and osteoporosis [69], also can cause inflammation, fever, tissue destruction and to be the worst, shock and deathv[67]. The inflammatory cytokines are mostly the pro-inflammatory cytokines that can be detectable in the joints of RA patients. They include IL-1, TNF-alpha, IL-15, IL-16, IL-17, IL-18, interferon (IFN)-γ, and granulocyte macrophage-colony stimulating factor [69]. The anti-inflammatory cytokines such as IL-4, IL-10, IL-13, and transforming growth factor (TGF) suppress or block the intensity of inflammatory cascade by inhibiting the generation of IL-1, TNF, vascular adhesion molecules and also chemokines, including IL-8 [67]. An adequate amount of anti-inflammatory cytokines concentrations causes an imbalance in the local area and inefficiency in mediating the counter-regulatory action across the dominant pro-inflammatory cytokines [69], thus representing the inflammation in RA. The natural cytokines antagonists are known as IL-1 receptor antagonists (IL-1ra), IL-18 binding protein, soluble type 2 IL-1 receptor, and soluble TNF receptor (sTNF-RI). They are involved in the mechanism of self-limiting or self-controlling by the immune system, but their observance levels in fluid and synovial tissues are inadequate and the present knowledge about them is not entirely known [69].

During the pathogenesis of RA, it is cytokines play a main role. Cytokines function in synergy to sustain their generation and increase the inflammatory process. Furthermore, they communicate with each other at the level of production such as IL-17 elevating the TNF and IL-1 production by monocytes. Together TNF, IL-17, and IL-1 trigger stromal cells to generate IL-6, thus these cytokines combination produce synergistic or additive effects. The synergistic effects between TNF, IL-17, and IL-17 occurred in variety cell types including synoviocytes, chondrocytes, osteoblasts, or myoblasts [72].

e) Tumour Necrosis Factor-alpha (TNF-α)

TNF released by activated fibroblasts and macrophages induced the release of additional chemokines, prostaglandins, proteases, and growth factors, together with the activation of neutrophils, B cells, and endothelial cells [34]. TNF-α is the main cytokine that initiates inflammation and it is adequate to trigger chronic synovitis, cartilage destruction, and bone erosion [73]. The increased level of TNF-α in the synovium and in synovial fluid of RA patients shows its main action in inflammation and bone degradation [74].

The bioactivities of TNF-α (as in Figure 3) start when it binds to and activates two distinct receptors, TNF-receptor 1 (TNFR1) and TNF-receptor 2 (TNFR2). Both receptors belong to the TNF superfamily receptors and initiate two different intracellular signalling pathways to the transcription of the gene. TNFR1 is exhibited in the body by nearly all cells including the entire lymphoid system, while TNFR2 is mainly expressed in immune cells and mediates the restricted biological reactions but their functional consequences of signalling are not well identified. TNFR1 is universally expressed by all human tissues, has a wide role in the activation of NF-kB, and is the TNF-Alpha major signaling receptor. The adaptor protein TNFR1-associated death domain (TRADD) is recruited by TNFR and causes the accumulation of various complexes. TNFR1-complex II activates the caspase-8 thus inducing the apoptosis. The TRADD protein also activates IkB kinase (IKK) through receptor-interacting protein (RIP) by recruiting the TNF receptor-associated factor (TRAF2). The absence of a death domain in TNFR2 makes it incapable of directly initiating apoptosis. TNFR2 recruits the TNFR-associated factor 2 (TRAF2) to form TNFR2-complex I. This complex activates mitogen-activated protein kinase (MAPKs),

nuclear factor-κB (NFκB), and protein kinase B that initiate inflammation, tissue degeneration, cell proliferation, and cell survival process [72].

TNF regulates downstream cytokines, including IL-6 or IL-1 [72]. Starting in early 1990s, various studies by Feldman and Maini in the 1990s proved that TNF is a main mediator of inflammation which presenting joint damage [34]. The blocking of TNF-α with antibodies in synovial cells from RA patients also significantly decrease the IL-1, IL-6, IL-8, and GM-CSF generation, thus showing higher effect on the inflammation itself compared to the blockade of other high concentration of cytokines present in synovial fluids [75].

f) Interleukin-1-Beta (IL-1β)

As both TNF-α andIL-1β are one of the main proinflammatory cytokines demonstrated to contribute to RA, both cytokines are pleiotropic which act on different cell types with various biological effects even though the mechanism of it still not yet fully understood [73]. The pleiotropic pro-inflammatory cytokines, IL-1 are cause various diseases, including RA, and are released generally by macrophages, monocytes, and dendritic cells [69]. The three IL-1 family consist of IL-1α, IL-1β, and IL-1 receptor antagonist (IL-1Ra). IL-1α is not actively secreted by cells, thereby being released during cell necrosis. IL-1β is an essential part of the host defence mechanism. It is transferred out from the cell where it acts locally or enters the blood circulation. It also secreted upon inflammatory signals during pro-inflammatory activity at the tissue level, leading to vasodilation or activation of innate immune cells such as neutrophils.IL-1β acts on T cells by promoting the differentiation of Th17 and also augments the production of T and B-lymphocytes, prostaglandin E, and the proliferation of fibroblasts [73]. IL-1Ra is articulate in nearly all tissues and the main role is to restrict the uncontrolled activation of IL-1R1 which is involved in IL-1β-mediated inflammation [69]. IL-1α and IL-1β stimulate the biological effects while IL-1Ra is an endogenous inhibitor that inhibits the biological actions of the previous two. The binding of IL-1Ra to Interleukin 1 receptor, type 1 (IL-1R1) and Interleukin 1 receptor, type 2(IL- 1R2) does not produce signal transduction because it does not induce the IL-1R accessory protein-interacting domain which is crucial for signal transduction. Plus, it reduces the biological effects of these cytokines by competitively antagonizing the binding of IL-1α and IL-1β, thereby diminishing cytokines' biological effects. The IL- 1R2 acts as a decoy receptor, and binding of IL-1 to this receptor does not induce cell activation due to the very short cytoplasmic domain that is incapable to induce signals [26] IL-1α and IL-1β utilized the same receptor and triggered cellular responses by stimulating only a few number of IL-1RI. The ligand binding of IL-1α and IL-1βto IL-1R1 is enhanced by IL-1R accessory protein (IL-R1AcP). The fully activeIL-1R1 binds to the adaptor protein of myeloid differentiation primary response gene 88 (MyD88) that further activates the protein kinase IL-1receptor-associated kinase (IRAK4) . IRAK1 is then activated and phosphorylates by the activated IRAK4. IRAK1 is associated with TRAF6, thus causing the activation of TRAF6 and interacts with a preformed complex ofTAK1-TAB1-TAB2. The activated TAK1 will activate the NFκB pathway via IKK activation and MAPK pathways via MAPKK activation, thus resulting in altered gene expression.

Both IL-1α and IL-1β are engaged in inflammatory reactions but only IL1B has been found in joint tissues of RA patients [76]. The immune cells shift to the inflammatory site in synovium and communicate with regional synoviocytes, and mesenchymal cells, and this leads to the pro-inflammatory cytokines assembly such as IL-1β and IL-6 [69]. High levels of IL-1 are observed in the synovial fluid and membrane in RA joints [26, 69]. IL-1 also stimulates the production of RANKL and MMP, incorporated in osteoclastogenesis which leads to the destruction

of bone and cartilage degradation [69]. Based on the study by Kay & Calabrese [26] which investigated the efficacy of anakinra, a recombinant human IL- 1Ra (r-metHuIL-1ra) to block IL-1 utilization, the blocking of IL-1 has been showed significantly reduce the symptoms and clinical signs of RA, contrast with placebo. A study by Bergström, et al. [77] also shows the inhibition of cytokine interleukin-1β (IL-1β) by methotrexate can inhibit the FLS proliferation from the primary FLS samples of RA patients.

g. Interleukin-6 (IL-6)

Interleukin-6 (IL-6) is released by varieties of cell like T cells, B cells, monocytes, fibroblasts, osteoblasts, endothelial cells, keratinocytes, mesangial cells, and some tumor cells [78]. IL-6 is one of the most expressed cytokines in the rheumatoid synovium [54] and their dysregulation persistent production plays a main part in the characteristic's development of RA [24].

IL-6 promotes B-cell differentiation into plasma cells and produces immunoglobulins. B-cell depletion therapeutic efficacy shows the effects of B-cell activity towards synovial inflammation and joint damage in RA. Neutrophils that express membrane-bound IL-6R are activated by IL-6. The activated neutrophils produce proteolytic enzymes and reactive oxygen intermediates thus destroying tissue and joint damage in RA patients. T lymphocytes are influenced by IL-6 to proliferate and differentiate into TH-17 cells and further produce IL-17 [54, 62].

IL-6 can be activated through both transmembrane IL-6 receptor (tIL-6R) and soluble IL-6 receptor (sIL-6R) (based on Figure 4). The tIL-6R is expressed only on restricted cells such as hepatocytes and some leukocytes, while gp130 is expressed on numerous types of cells. When binding to IL-6R, the complex of IL-6 and tIL-6R associates with signal-transducing molecule gp130 and develops the activation of downstream signaling actions via Janus Kinase (JAK) in the cells of the target and the activation is known as classic signaling pathway. The IL-6 and sIL-6R complex can also bind to gp130 producing the activation of a signalling cascade known as a trans-signalling pathway, which means the sIL-6R has a similar affinity to IL-6 same as tIL-6R. It was suggested that classic signalling is required for regenerative or anti-inflammatory activities, while trans-signalling is required for proinflammatory actions. The inhibition of IL-6 binding leads to disruption of the JAK/STAT system followed by the damping of IL-6 functions in RA including inflammatory and bone-destructive functions. These involve the process of Th17 differentiation, osteoclast differentiation, acute phase response, and matrix metalloproteinases (MMPs) [24, 54].

There is a relationship between the destruction of articular cartilage and MMPs when it is found that IL-6 and CRP correlate with proMMP-3 production in patients with early RA [79], indicating a link between IL-6 and proteinase activity. The blocking of IL-6 trans-signalling by a variant soluble gp130 molecule brings clinical improvement of systemic arthritis, based on the study by Nowell et al. [80] which studied the key role of trans-signalling in RA and used the murine experimental arthritis model. The presence of sIL-6R complex when binding with IL-6 also increased the VEGF levels in cultured synovial fibroblasts of RA patients, proving that high levels of VEGF are correlated with RA disease activity and the appliance of anti-IL-6R antibodies has been significantly lower down the VEGF concentration[81].

Cellular Signalling Pathway in RA

a) The nuclear factor-ΚB (NF-ΚB)

The existence of activated NF-KB transcription factors has been displayed in human arthritic joints, cultured synovial fibroblasts, and the animal joints by experimentally induced RA. The nuclear factor-ΚB (NF-ΚB) pathway activation in the synovial cells of RA patients induces the multitude of responsive genes transactivation that influence the inflammatory phenotype which involves the TNF-α from macrophages, matrix metalloproteinases from synovial fibroblasts and

chemokines that build up the immune cells to inflamed pannus [82].

The events leading to the NF-ΚB transcription factors activation include the so-called 'classical' or 'canonical' pathway. The result of 'canonical' NF-ΚB pathway activation which involves heterodimers of p50/p65 suggests that NF-KB may be one of the main regulators of inflammatory cytokine production in RA, based on the existence of both p50 and p65 in the nuclei of cells lining the macrophages and synovial membrane [83]. The action of NF-KB is tightly regulated at multiple levels due to its capability to control the expression of various genes. The three main players in the pathway are the NF-KB transcription factors, inhibitory IΚB proteins (IΚB, inhibitor of NF-ΚB), and IΚB kinase (IKK) complex, which is a kinase that phosphorylates IΚBs [59].

An important form of the NF-ΚB response is the formation of dimers that are bound to and inhibited by the IΚBs [82]. The NF-KB transcription factor family in mammals resides of five proteins, p105/p50 (NF-ΚB1), p100/52 (NF-KB2), p65 (RelA), RelB, and c-Rel that collaborate to create a distinct transcriptionally active homo- and heterodimeric complexes [59]. Three of the family (p65, RelB, and c-Rel) contain carboxy-terminal transactivation domains (TAD). TAD allows the specific dimer to act as an activator or a repressor that cooperates with general transcription factors and co-activators. For example, the presence of a TAD in p65 allows the heterodimer of p50 and p65 to activate the gene transcription. In contrast, the homodimers p50 with no TAD are competing for p50/p65 binding to the NF-KB consensus sequence, which acts as transcriptional repressors [84].

In common resting cells, the NF-kB dimers are associate with one of the prototypical IkB proteins, including IkBα, IkBβ, and IkBγ which regulate their cytosolic localization [59]. Both IκBα and IκBβ hide the nuclear localization arrangement on the p50/p65 heterodimer by binding to NF-κB, and further inhibit the entry into the nucleus. IKK complex phosphorylates and degrades IκBα and unmasked the nuclear localization signal thus leads to the active dimer translocation to the nucleus [59]. The IKK complex function in the canonical pathway is to phosphorylate IΚBα and IΚBβ, following by marking them for degradation through the ubiquitin/proteasome pathway. The three main subunits covers the canonical IKK complex are IKK1 (known as IKKα), IKK2 (known as IKKβ) and NF-KB essential modulator (NEMO, known as IKKγ) [85]. IKK2 is the most significant to RA, for its catalytic activity is more fundamental for phosphorylation of IΚBα by the IKK complex [86].

In the resting state, the dimers are bound with an inhibitory protein (IkB) to prevent the nuclear translocation of NF-KB dimers. The process starts when unknown molecules trigger the reaction together with the surface of T-cell receptors (TCRs) and endogenous or exogenous ligands for the toll-like receptor family (TLR ligands). In the synovium, the resident macrophages are activated, and this leads to IkB phosphorylation by the IKK complex, which is further degraded by the proteasome. This process releases NF-ΚB dimers and their translocation into the nucleus such as p50/p65 causing the pro-inflammatory cytokines and chemokines expression. Inflammation occurs and large numbers of immune cells are infiltrated into the synovium. FLS (fibroblast-like synoviocytes) synthesize abundant NF-ΚB-induced genes in response to TNF-α or IL-1, together with chemokines that cause more inflammatory infiltrates and MMPs that develop joint destruction [82].

b) The Mitogen-activated protein kinases (MAPKs)

Protein kinases are recognized as the enzymes that link covalently to the side chain of specific proteins inside cells; either serine, tyrosine, or threonine [87]. Mitogen-activated protein kinases (MAPKs) belong to the protein kinases family and are regulated by phosphorylation. It catalyzed

the substrate proteins' phosphorylation including other protein kinases, cytoskeletal proteins, transcription factors, and phospholipases [88].

MAPKs are composed of 2 others eventually activated kinases, MAPK kinases (MKKs) and MAPK kinase kinases (MKKKs). In response to different stimuli from the environment, MKKKs are selectively activated. MKKKs phosphorylate and activate specific MKKs, and further selectively phosphorylate specific MAPKs. The phosphates that were transferred to the protein substrate by MAPK during phosphorylation were removed by protein phosphatases [87].

In mammals, MAPKs involved the extracellular signal-regulated kinases (ERKs) and 2 stress-activated protein kinase (SAPKs) families; p38 and c-jun N-terminal kinase (JNK). All of this category of MAPKs has been expressed in the synovial tissue of RA. Much research has focused on MAPK inhibitors due to their implication as the key regulator of pro-inflammatory cytokines production such as IL-1, IL-6, and TNF [89].

The extracellular signal-regulated kinases (ERKs) play their role in the maintenance phase of disease by promoting the formation of pannus. They also play their part in cell division control, in which ERK1 and ERK2 are engaged in the regulation of meiosis, mitosis, and post-mitotic functions in differentiated cells, thus the inhibitors of these enzymes have been studied as anticancer agents [90]. The ERK is found to be activated in synovial fibroblasts and mononuclear cell infiltrates in synovial tissue of RA patients. Therefore, in synovial fibroblasts, ERK is activated during stimulation with IL-1, TNF, and fibroblast growth factor (FGF) [89].

The p38 MAPKs are stimulated by inflammatory cytokines together with other stimuli such as stresses, hormones, and ligands for G protein-coupled receptors in immune cells. They affect human diseases such as autoimmunity and asthma due to their function as key regulators of inflammatory cytokine expression [90].

The JNKs are identified as stress-activated protein kinases based on their activation in response to protein synthesis inhibition [91]. They increased the transcription activity by binding and phosphorylating the DNA-binding protein c-Jun. C-Jun is a main regulator of gene expression and one of the Activator protein-1 (AP-1) transcription complex components [92]. AP-1 regulates various cytokine genes and is activated in return to all stimuli including environmental stress, radiation, and growth factors [93]. JNKs are also important in ruling the apoptosis. Their inhibition elevates the chemotherapy-induced inhibition of tumor cell growth thus indicating they may present as a molecular target for the cancer treatment [90]. IL–1–induced collagenase gene expression involving the JNK-MAPK pathway in synoviocytes and joint arthritis shows that JNK is a relevant therapeutic target for RA [94].

c) NF-ĸB and MAPK Relationship

Various studies show the relationship between NF-KB and MAPK to control inflammation-related gene expression in cooperative ways. Studies conducted in human osteoarthritis (OA) chondrocytes and chondrosarcoma cells reveal that TNF-α or IL-1β induce NF-KB and MAP kinases to mediate the RNA/protein expression of MMP1, MMP3, and MMP-13. Both NF-KB and ERK MAPK are activated and mediate MMP-1 gene expression induced by IL-1 in rabbit synovial fibroblasts [89]. These suggest a promising therapeutic approach target to lessen articular cartilage degradation by MMPs in arthritis by the inhibition of TNF-α and IL-1β transduction [57].

Conclusion

There are various cell types involved in RA pathogenesis have been discussed in this review. Despite the rapid expansion of new members' identification associated with the pathogenesis in recent years, RA remains a complex disease with unknown aetiology beyond a single treatment plan. Recently, it has been discovered that RA is associated with anti-carbamylated protein (anti-CarP) antibodies, also known as anti-

homocitrulline antibodies. Elevations in anti-CarP antibody titres have the potential to worsen existing conditions and accelerate the loss of bone mass. Anti-modified protein antibodies (AMPA), sclerostin, osteoclast and osteoblast co-regulators exert effector functions on immune cells and on bone resorbing osteoclasts, thereby facilitating bone loss. The initiation of innate immune cells develops adaptive immunity, which results in autoantibody production. It is reasonable that fibroblast-like synovial cells (FLS) will remain identified as a significant participator in pathogenesis and might be ultimately expressed as a main therapeutic target. The NF-kB pathway controls the production of pro-inflammatory cytokine and leukocyte recruitment, while MAP kinases play a critical role in key cellular processes that are important for immune system homeostasis. However, further studies on the mediators and sequence of inflammatory cascade should be done to advance of our understanding the damaging part of inflammation which dominates the protective role in RA.

Acknowledgement
The authors thank members of Pharmacology and Toxicology Unit, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia.
Funding source
This research was supported by Fundamental Research Grant Scheme from the Ministry of Higher Education, Malaysia (FRGS/2/2018/SKK10/UPM/02/4).

Conflict of interest
None

Authors contributions
NSN- draft the preliminary manuscript, literature search, study conception and design.
YYK, SFMT, ZNMZ and MNH- supervisory in animal study, in vitro experiments, anti-inflammatory experiments, literature search, review, and edits.

Table 1. The examples of RA-associated genes *(Adapted from Fox[15])*

Gene	Functions
HLA-DR (shared epitope)	Antigen presentation, lymphocyte activation
PTPN22 (protein-tyrosine phosphatase non-receptor type 22)	Regulation of T cell receptor signalling
PADI4 (peptidyl arginine deiminase 4)	Post-translational change of arginine to citrulline
CCR6 (chemokine receptor 6)	Affinity of Th17 cells to inflammation sites
STAT4 (signal transducer and activator of transcription 4)	Signalling downstream of cytokine receptors

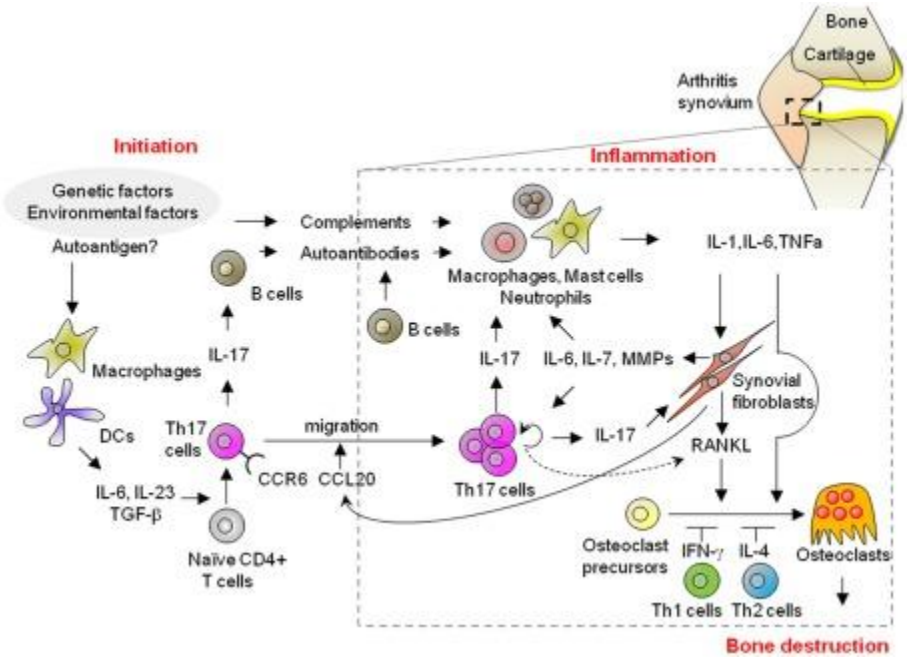

Figure 1. Stages in RA: The mechanism of initiation, inflammatory, and bone destruction.

The RA development is assisted by the role of various types of cell including lymphocytes, innate immune cells, osteoclasts and synovial fibroblasts. In RA synovium, macrophages and synovial fibroblasts stimulate the increased levels of the proinflammatory cytokines IL-1, IL-6, and TNF-α. These proinflammatory cytokines directly and indirectly generate the other proinflammatory cytokines and chemokines along with matrix-degrading enzymes, thus developing cytokine "storm" in the inflamed synovium. The interplay between CD4+ T-cells and mesenchymal cells in joints also play important role in the inflammation and bone destruction phases *(Adapted from Komatsu & Takayanagi[20] with permission*).

Table 2. Three mechanisms in synovial hyperplasia (*Adapted from Adam Mor*[28])

	Mechanism	Process
1.	**Hyperproliferation of FLS**	Several growth factors are overexpressed in the rheumatoid joint including; a) Platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF) b) MLS secrete transforming growth factor-h (TGF-h) which is overexpressed in RA synovium.
2.	**Decreased apoptosis**	RA-SF express high levels of Bcl-2 which is a proto-oncogene on chromosome 18q that inhibit apoptosis via inhibition of caspase activation.
3.	**Decreased senescence**	Senescence occurs when the ability of aging cells to continue to divide is limited but it is still alive and enter a permanently non-proliferative state.

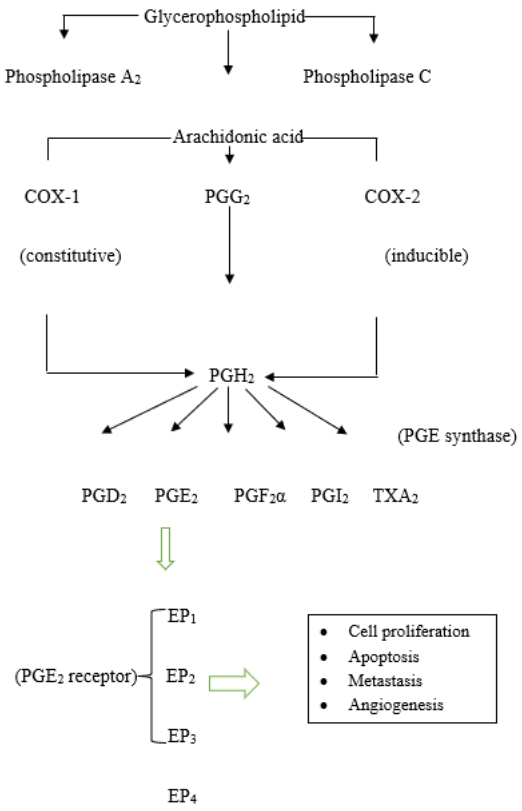

Figure 2. The conversation of arachidonic acid to PGE_2 in the biosynthesis of PGE_2 and the involvement of COX-2 in the disease development.

Arachidonic acid is released by phospholipase A2 from the membrane phospholipid. Enzymatic reactions by COX-1 and COX-2 converts the Arachidonic acid to prostaglandin (PG)-G2 and further the conversion into PGH2. The PGE2 is converted from PGH2 which catalysed by PGE2 synthase (PGES) with other PG isoforms. PGE2 is the major prostanoid among them and display various biologic activities via its EP receptors. (*Adapted from Urade[63] with permission).*

Table 3. The main roles of cytokines in *(Adapted from Nalbant & Birlik[69])*

Cytokines	Functions
1. Pro-inflammatory cytokines	
TNF-alpha	Autocrine stimulator and paracrine inducer of other inflammatory cytokines. Stimulate fibroblasts to express adhesion molecules such as intracellular adhesion molecule 1 (ICAM-1). Induces IL-1 production. (Vasanthi et al., 2007)
IL-1	Up-regulate the expression of CAMs. Stimulate production of chemokines. Stimulate synoviocytes and chondrocytes to release MMPs and other proteinases. Trigger the differentiation of osteoclasts. Activate mature osteoclasts to erosion. Stimulates TNF production.
2. Inflammatory cytokines	
TNF-alpha & IL-1	(as the above)
IL-6	Stimulate neutrophil migration. Inducing neovascularization, infiltration of inflammatory cells, and synovial hyperplasia Induce cartilage degeneration by producing matrix metalloproteinases (MMPs) (Hashizume & Mihara, 2011)
IL-15	Recruit and activate T cells. Activate neutrophils. Promotes synovial cytokine (including TNF) and chemokine. (Baslund et al.,[70])
IL-16	Anti-inflammatory effect by regulation of Tregs. Activate expression of adhesion molecules and recruit leukocytes to involved joints (Yoshida & Tanaka,[24]).
IL-17	Increase local chemokine production.

	Increase IL-6 production. Promotes the effect of IL-1b, TNF-a and IFN-g (Nalbant & Birlik,[69]).
IL-18	Increase the production of fibroblast-like synoviocytes and chondrocytes (Dai et al.[71]).
IL-21	Activate TH17 cells.
IFN-gamma	Immune modulation (both protection and activation).
Granulocyte macrophage – colony stimulating factor (GM-SF)	Promotes existing RA.
3. Anti-inflammatory cytokines	
IL-10	Inhibit Th1 cell activity by suppressing IFN-γ expression. Protection against cartilage destruction combination with IL-4.
IL-4	Increased level in synovial fluid during only synovial inflammation.
IL-13	Synergistic or inhibitory roles during the arthritis with IL-10, IL-21R, galectin-3 and TGFβ.
IL-20	Regulates osteoclast differentiation.
4. Natural cytokines antagonist	
IL-1 receptor antagonist (IL-1ra)	Low levels of IL-1 receptor antagonist (IL-1ra) causes erosive disease in patients.
Soluble type 2 IL-1 receptor	Cause competitive inhibition by binding interleukin-1α (IL1A), interleukin-1β (IL1B) and interleukin 1 receptor antagonist (IL1Ra).
Soluble TNF receptor (sTNF-RI)	It is not well-known; possible effect is to cause cleavage of TNF alpha.
IL-18 binding protein	Protect against the joint destructive effect by binding IL-18 in RA.

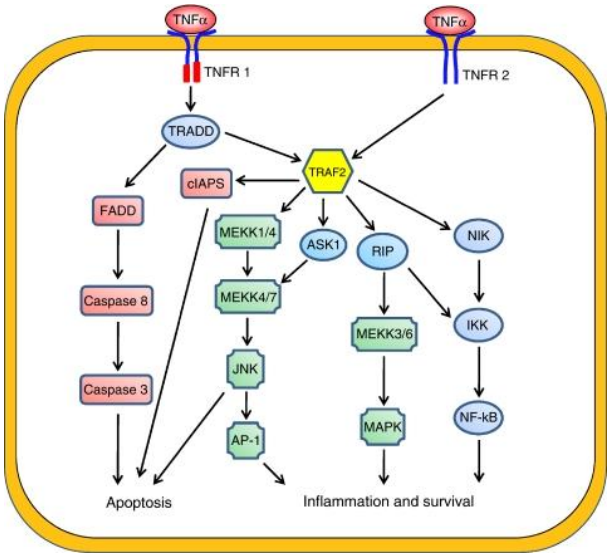

Figure 3. The activities of TNF initiated by the binding and the activation of two different receptors, TNF- receptor 1 (TNFR1) and TNF-receptor 2 (TNFR2) *(Adapted from* Noack, & Miossec[72] *with permission).*

Table 4. TNF-α action on different cells in rheumatoid arthritis *(Adapted from Vasanthi et al.,[75])*

Cell type	TNF-α action
Macrophages	Increases proliferation and production of cytokine.
Activated T-cell	Enhances proliferation and increases interleuken (IL)-2 receptor.
B-cell	Increases proliferation and differentiation.
Synovial lining cell	Induces proliferation, induces synthesis of IL-1, granulocyte monocyte-colony stimulating factor (GM-CSF), stromelysin, collagenase and prostaglandins.
Endothelial cells	Induces expression of intracellular adhesion molecule 1, vascular cell adhesion molecule 1 (VCAM-1), endothelial leucocyte adhesion molecule-1 (ELAM-1) and IL-8.

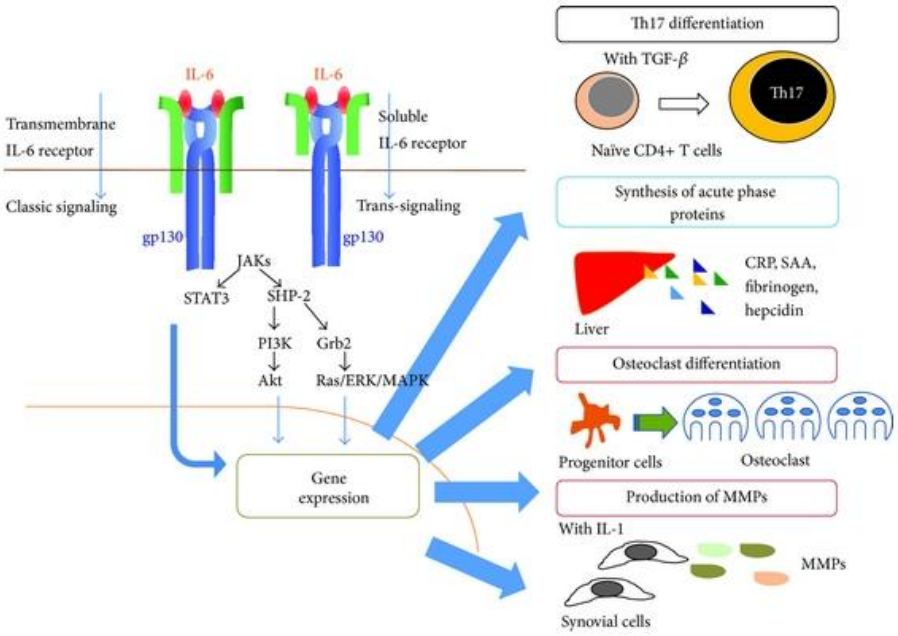

Figure 4. IL-6 signalling pathway. The pleiotropic activity of IL-6 begins with gp130 by binding to transmembrane or soluble IL-6 receptor. This activates the IL-6 signalling pathway and the STAT3 transcriptional factors initiate numerous gene expressions, producing cell differentiation or proliferation (JAKs, Janus kinases; STAT3, signal transducer and activator of transcription 3; SHP-2, SH2 domain-containing tyrosine phosphatase 2; PI3K, phosphoinositol-3 kinase; Grb2, growth factor receptor-bound protein 2; ERK, extracellular signal-regulated kinase; MAPK, mitogen activated protein kinase; Akt, protein kinase B; TGF-β, transforming growth factor beta; CRP, C-reactive protein; SAA, serum amyloid A; MMPs, matrix metalloproteinases) *(Adapted from Yoshida & Tanaka,[24] with permission)*

Table 5. The NF-κB transcription factors (RA, rheumatoid arthritis; TAD, transactivation domain)

(Adapted from Simmonds & Foxwell,[82])

Protein	Gene	Function
p105/p50	NFKB1	Canonical pathway p105 constitutively processed to p50 p50/p65 form activating heterodimers p50/p50 form inhibitory homodimers Found in RA synovium
p100/p52	NFKB2	Non-canonical pathway p100 processing to p52 tightly regulated
p65	RfgGHELA	Canonical pathway p50/p65 activating heterodimers Contains TAD Found in RA synovium
RelB	RELB	Contains TAD
c-Rel	REL	Contains TAD

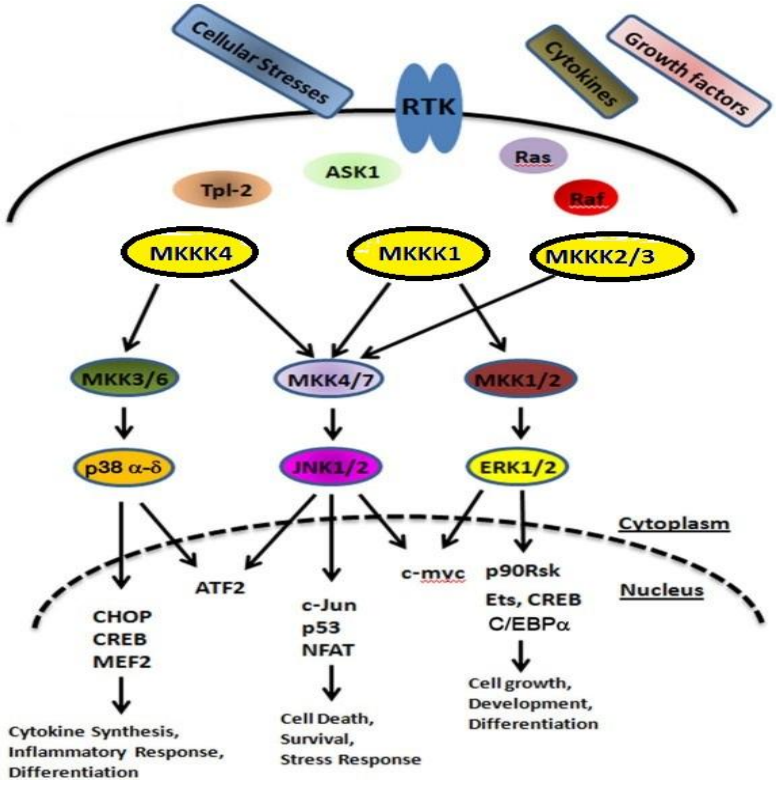

Figure 5. Mammalian MAPK signalling cascades: simplified diagram depicting the MAPK signaling network. MAP kinase pathways involved 3 basic parts that initiated by a signal from an external stimulus. MKKKs activates MKKs by dual phosphorylation which then activates a MAPK by the same mechanism. MKKKs, RAFs, and Tpl2 initiate the basic ERK pathway which activates MKK1 and MKK2, which in turn activates the multiple substrate by ERK1 and ERK2 phosphorylation. MKKKs activate MKK3 and MKK6 and activate 4 isoforms of p38. In the JNK pathway, these MKKKs activate MKK4 and MKK7, followed by JNK1 activation. All the pathways of p38, JNK and ERK further activate multiple transcription and translation factors *(Adapted from Munshi & Ramesh,[95] with permission).*

References

[1]. Senthelal, S., Li, J., Ardeshirzadeh, S., Thomas, M. A. Arthritis. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024.

[2]. Chen, Jian, Wu, H., Dai, M.-M., Li, H., Chen, J.-Y., and Hu, S.-L. Identification and distribution of four metabolites of geniposide in rats with adjuvant arthritis. *Fitoterapia*, 2014; *97*, 111–121.

[3]. Vysakh, A., Ratheesh, M., Rajmohanan, T. P., Pramod, C., Premlal, S., Girish Kumar, B., and Sibi, P. I. Polyphenolics isolated from virgin coconut oil inhibits adjuvant induced arthritis in rats through antioxidant and anti-inflammatory action. *International Immunopharmacology*, 2014: *20*(1), 124–130.

[4]. Dadoun, S., Zeboulon-Ktorza, N., Combescure, C., Elhai, M., Rozenberg, S., Gossec, L., and Fautrel, B. Mortality in rheumatoid arthritis over the last fifty years: systematic review and meta-analysis. Joint Bone Spine, 2013: 80(1), 29-33.

[5]. Almoallim, H., Al Saleh, J., Badsha,H., Ahmed,H. M., Habjoka, S., Jeanine A. Menassa, J. A., El-Garf, A. A Review of the Prevalence and Unmet Needs in the Management of Rheumatoid Arthritis in Africa and the Middle East. *Rheumatology and Therapy.* 2021; 8(1): 1–16.

[6]. Szeremeta, A., Jura-Półtorak, A., Zoń-Giebe, A., Olczyk, K., Komosińska-Vassev, K. TNF-α Inhibitors in Combination with MTX Reduce Circulating Levels of Heparan Sulfate/Heparin and Endothelial Dysfunction Biomarkers (sVCAM-1, MCP-1, MMP-9 and ADMA) in Women with Rheumatoid Arthritis. J Clin Med. 2022: 20;11(14):4213.

[7]. Olivares Martínez, E., Hernández Ramírez, D. F., Núñez-Álvarez, C. A., and Cabiedes, J. Citrullinated proteins in Rheumatoid Arthritis. *Reumatología Clínica (English Edition)*, 2011: *7*(1), 68–71.

[8]. Al-Rayes, H., Arfin, M., Tariq, M., and Al-Asmari, A. The role of TNF-α and TNF-β gene polymorphism in the pathogenesis of Rheumatoid Arthritis. *Reviews in Health Care*, 2013: 4(1), 29-53.

[9]. Carmona, L., Cross, M., Williams, B., Lassere, M., and March, L. Rheumatoid arthritis. *Best Practice & Research. Clinical Rheumatology*, 2010: *24*(6), 733–745.

[10]. Hyndman, I. J. Rheumatoid arthritis: past, present and future approaches to treating the disease. *International Journal of Rheumatic Diseases*. 2017; 20: 417–419

[11]. Deane, K. D. Can rheumatoid arthritis be prevented? *Best Practice & Research Clinical Rheumatology*, 2013: *27*(4), 467–485.

[12]. Tan, L.K., Too, C. L., Diaz-Gallo, L. M., Wahinuddin, S., Lau, I. S., Heselynn, H., Nor-Shuhaila, S., Gun, S. C., Eashwary, M., Mohd-Shahrir, M. S., Ainon, M. M., Azmillah, R., Muhaini, O., Shahnaz, M., Alfredsson, L., Klareskog, L., Padyukov, L. The spectrum of association in HLA region with rheumatoid arthritis in a diverse Asian population: evidence from the MyEIRA case-control study. *Arthritis Res Ther.* 2021:30;23(1):46.

[13]. Tobón, G. J., Youinou, P., and Saraux, A. The environment, geo-epidemiology, and autoimmune disease: Rheumatoid arthritis. *Journal of Autoimmunity*, 2010: *35*(1), 10–14.

[14]. Mousavi, M. J., Jamshidi, A., Chopra, A., Aslani, S., Akhlaghi, M., and Mahmoudi, M. Implications of the noncoding RNAs in rheumatoid arthritis pathogenesis. *Journal of Cellular Physiology*, 2019: *234*, 335–347.

[15]. Fox, D. A. Etiology of Rheumatoid Arthritis: A Historical and Evidence-Based Perspective. In *Clinical Management of the Rheumatoid Hand, Wrist, and Elbow* , 2016 (pp. 13–19).

[16]. Chang, K., Yang, S. M., Kim, S. H., Han, K. H., Park, S. J., and Shin, J. I. Smoking and rheumatoid arthritis. *International journal of molecular sciences*, 2014: *15*(12), 22279-22295.

[17]. Ola Kilsgard, Pia Andersson, Martin Malmsten, Sara L. Nordin, Helena M. Linge, Mette Eliasson, Eva So¨renson, Jonas S. Erjefa¨lt, Johan Bylund, Anders I. Olin, and Ole E. Sørensen Peptidylarginine Deiminases Present in the Airways during Tobacco Smoking and Inflammation Can Citrullinate the Host Defense Peptide LL-37 , Resulting in Altered Activities. *Am J Respir Cell Mol Biol*, 2012: *46*(2), 240–248.

[18]. Jose U. Scher, Walter A. Bretz, and S. B. A. Periodontal Disease and Subgingival Microbiota as Contributors for RA Pathogenesis: Modifiable Risk Factors? *Curr Opin Rheumatol*, 2015: *26*(4), 424–429.

[19]. Scher, J. U., Sczesnak, A., Longman, R. S., Segata, N., Ubeda, C., Bielski, C. and Huttenhower, C. Expansion of intestinal Prevotella copri correlates with enhanced susceptibility to arthritis. *elife*, 2013: *2*, e01202.

[20]. Komatsu, N., and Takayanagi, H. Inflammation and bone destruction in arthritis: Synergistic activity of immune and mesenchymal cells in joints. *Frontiers in Immunology*, 2012: *3*, 1–11.

[21]. Deane, K. D. Preclinical Rheumatoid Arthritis (Autoantibodies): An Updated Review. *Current Rheumatology Reports*, 2014: *16*(5), 1–16.

[22]. Gierut, A., Perlman, H., and Pope, R. M. Innate Immunity and Rheumatoid Arthritis. *Rheum Dis Clin North Am.*, 2011: *36*(2), 1–24.

[23]. Choy, E. Understanding the dynamics: pathways involved in the pathogenesis of rheumatoid arthritis. *Rheumatology*, 2012: *51*(suppl_5), v3-v11.

[24]. Yoshida, Y., and Tanaka, T. Interleukin 6 and rheumatoid arthritis. *BioMed Research International*. 2014: https://doi.org/10.1155/2014/698313

[25]. Schett, G., and Gravallese, E. Bone erosion in rheumatoid arthritis: mechanisms, diagnosis and treatment. *Nature Reviews Rheumatology*, 2012: *8*(11), 656–664.

[26]. Kay, J. The role of interleukin-1 in the pathogenesis of rheumatoid arthritis. *Rheumatology*, 2004: *43*(suppl_3), iii2–iii9.

[27]. Kinne, R. W., Stuhlmüller, B., and Burmester, G. R. Cells of the synovium in rheumatoid arthritis. Macrophages. *Arthritis research & therapy*, 2007: *9*(6), 224.

[28]. Matsuda, K., Shiba, N., Hiraoka, K. New Insights into the Role of Synovial Fibroblasts Leading to Joint Destruction in Rheumatoid Arthritis. *Int J Mol Sci.* 2023: 8;24(6):5173.

[29]. Bertrand J., and Hubert J. Overview. In: Lammert E., Zeeb M. (eds) Metabolism of Human Diseases. 2014: Springer, Vienna.

[30]. Bondeson, J. The Role of Synovial Macrophages in Rheumatoid Arthritis and Osteoarthritis: Its Implications for Radiosynovectomy. In *Local Treatment of Inflammatory Joint Diseases* 2015: (pp. 31-48). Springer, Cham.

[31]. Szekanecz, Z., and Koch, A. E. Macrophages and their products in rheumatoid arthritis. *Current Opinion in Rheumatology*, 2007: *19*(3), 289–295.

[32]. Kennedy, A., Fearon, U., Veale, D. J., and Godson, C. Macrophages in synovial inflammation. *Frontiers in Immunology*, 2011: *2*, 1–9.

[33]. Laria, A., Lurati, A., Marrazza, M., Mazzocchi, D., Re, K. A., and Scarpellini, M. The macrophages in rheumatic diseases. *Journal of Inflammation Research*, 2016: *9*, 1–11.

[34]. Zamri, F., de Vries, T. J. Use of TNF Inhibitors in Rheumatoid Arthritis and Implications for the Periodontal Status: For the Benefit of Both? *Frontiers Immunology*. 2020: 23:11:591365.

[35]. Bugatti, S., Vitolo, B., Caporali, R., Montecucco, C., and Manzo, A. B cells in rheumatoid arthritis: from pathogenic players to disease biomarkers. *BioMed research international*, 2014.

[36]. Gutcher, I., and Becher, B. APC-derived cytokines and T cell polarization in autoimmune inflammation. *The Journal of clinical investigation*, 2007: *117*(5), 1119-1127.

[37]. Alunno, A., Manetti, M., Caterbi, S., Ibba-Manneschi, L., Bistoni, O., Bartoloni, E. and Gerli, R. Altered immunoregulation in rheumatoid arthritis: the role of regulatory T cells and proinflammatory Th17 cells and therapeutic implications. *Mediators of inflammation*, *2015*.

[38]. Alzabin, S., and Williams, R. O. Effector T cells in rheumatoid arthritis: lessons from animal models. *FEBS letters*, 2011: *585*(23), 3649-3659.

[39]. Castanheira, F. V., and Kubes, P. Neutrophils and NETs in modulating acute and chronic inflammation. *Blood*, 2019: *133*(20), 2178-2185.

[40]. Navegantes, K. C., Gomes, R. D. S., Aparecida, P., and Pereira, T. Immune modulation of some autoimmune diseases : the critical role of macrophages and neutrophils in the innate and adaptive immunity. *Journal of Translational Medicine*, 2017: 1–21.

[41]. Paoliello-Paschoalato, A. B., Marchi, L. F., Andrade, M. F. D., Kabeya, L. M., Donadi, E. A., and Lucisano-Valim, Y. M. Fcγ and complement receptors and complement proteins in neutrophil activation in rheumatoid arthritis: contribution to pathogenesis and progression and modulation by natural products. *Evidence-Based Complementary and Alternative Medicine, 2015*.

[42]. Edwards, S. W. *Biochemistry and Physiology of the Neutrophil*. Cambridge University Press. 2005.

[43]. Bhattacharya, S. Reactive oxygen species and cellular defense system. In *Free radicals in human health and disease* 2015: (pp. 17-29). Springer, New Delhi.

[44]. Rosas, C. E., Correa, L. B., and Henriques, M. D. G. Neutrophils in Rheumatoid Arthritis: A target for discovering new therapies based on natural products. *Role of Neutrophils in Disease Pathogenesis,* 2017: *5*, 89-118.

[45]. Nagy, G., Koncz, A., Telarico, T., Fernandez, D., Érsek, B., Buzás, E., and Perl, A. Central role of nitric oxide in the pathogenesis of rheumatoid arthritis and sysemic lupus erythematosus. *Arthritis research & therapy*, 2010: *12*(3), 210.

[46]. Förstermann, U., and Sessa, W. C. Nitric oxide synthases: Regulation and function. *European Heart Journal*, 2012: *33*(7), 829–837.

[47]. Hirai, Y., Migita, K., Honda, S., Ueki, Y., Yamasaki, S., Urayama, S., and Eguchi, K. Effects of nitric oxide on matrix metalloproteinase-2 production by rheumatoid synovial cells. *Life Sciences*, 2001: *68*(8), 913–920.

[48]. Farrell, A. J., Blake, D. R., Palmer, R. M., and Moncada, S. Increased concentrations of nitrite in synovial fluid and serum samples suggest increased nitric oxide synthesis in rheumatic diseases. *Annals of the rheumatic diseases*, 1992: *51*(11), 1219-1222.

[49]. Dervisevic, A., Babic, N., Huskic, J., Sokolovic, S., Nakas-Icindic, E., and Causevic, L. Concentration of nitric oxide in saliva of patients with rheumatoid arthritis. *International Journal of Collaborative Research on Internal Medicine & Public Health*, 2012: *4*(7), 0-0.

[50]. Firestein, G. S. Kelley ' s Textbook of Rheumatology , Ninth Edition. *Kelley's Textbook of Rheumatology*, 2012: *9*, 1630.

[51]. Yoshida, M., Sagawa, N., Itoh, H., Yura, S., Korita, D., Kakui, K. and Fujii, S. Nitric oxide increases matrix metalloproteinase-1 production in human uterine cervical fibroblast cells. *Molecular human reproduction*, 2001: *7*(10), 979-985.

[52]. Chou, L. W., Wang, J., Chang, P. L., and Hsieh, Y. L. Hyaluronan modulates accumulation of hypoxia-inducible factor-1 alpha, inducible nitric oxide synthase, and matrix metalloproteinase-3 in the synovium of rat adjuvant-induced arthritis model. *Arthritis research & therapy*, 2011: *13*(3), R90.

[53]. Itoh, Y. *Metalloproteinases in Rheumatoid Arthritis : Potential Therapeutic Targets to Improve Current Therapies*. *Matrix Metalloproteinases and Tissue Remodeling in Health and Disease: Target Tissues and Therapy* 2017: (1st ed., Vol. 148). Elsevier Inc.

[54]. Srirangan, S., and Choy, E. H. The role of Interleukin 6 in the pathophysiology of rheumatoid arthritis. *Therapeutic Advances in Musculoskeletal Disease*, 2010: *2*(5), 247–256.

[55]. Hannemann, N., Jordan, J., Paul, S., Reid, S., Baenkler, H.-W., Sonnewald, S., and Bozec, A. The AP-1 Transcription Factor c-Jun Promotes Arthritis by Regulating Cyclooxygenase-2 and Arginase-1 Expression in Macrophages. *Journal of Immunology* 2017: *(Baltimore, Md. : 1950)*, 1601330.

[56]. Chambers, M., Kirkpatrick, G., Evans, M., Gorski, G., Foster, S., and Borghaei, R. C. IL-4 inhibition of IL-1 induced Matrix Metalloproteinase-3 (MMP-3) expression in human fibroblasts involves decreased AP-1 activation via negative crosstalk involving of Jun N-terminal Kinase (JNK). *Experimental Cell Research*, 2013: *319*(10), 1398–1408.

[57]. Guo, Q., Jin, Y., Chen, X., Ye, X., Shen, X., Lin, M., Zeng, C., Zhou, T., Zhang, J. NF-κB in biology and targeted therapy: new insights and translational implications. *Sig Transduct Target Ther.* 2024: 9, 53. https://doi.org/10.1038/s41392-024-01757-9

[58]. Campbell, I. K., Gerondakis, S., O'Donnell, K., and Wicks, I. P. Distinct roles for the NF-κB1 (p50) and c-Rel transcription factors in inflammatory arthritis. *The Journal of clinical investigation*, 2000: *105*(12), 1799-1806.

[59]. Oeckinghaus, A., and Ghosh, S. The NF- B Family of Transcription Factors and Its Regulation. *Cold Spring Harbor Perspectives in Biology*, 2009: *1*(4), a000034–a000034.

[60]. Fattahi, M. J., and Mirshafiey, A. Prostaglandins and Rheumatoid Arthritis. *Arthritis*, *2012*, 1–7.

[61]. Ricciotti, E., and FitzGerald, G. A. . NIH Public Access. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 2011:*31*(5), 986–1000.

[62]. Wang, H., Ye, M., Yu, L., Wang, J., Guo, Y., Lei, W., and Yang, J. Hippocampal neuronal cyclooxygenase-2 downstream signaling imbalance in a rat model of chronic aluminium gluconate administration. *Behavioral and Brain Functions*, 2015: *11*(1), 1–12.

[63]. Urade, M. Cyclooxygenase (COX)-2 as a potent molecular target for prevention and therapy of oral cancer. *Japanese Dental Science Review*, 2008: *44*(1), 57–65.

[64]. Thorén, Staffan, Rolf Weinander, Sipra Saha, Caroline Jegerschöld, Pär L. Pettersson, Bengt Samuelsson, Hans Hebert, Mats Hamberg, Ralf Morgenstern, and P.-J. J., Hebert, H., Hamberg, M., Morgenstern, R., & Jakobsson, P. J. Human microsomal prostaglandin E synthase-1: Purification, functional characterization, and projection structure determination. *Journal of Biological Chemistry*, 2003: *278*(25), 22199–22209.

[65]. Rouzer, C. A., and Marnett, L. J. Cyclooxygenases: structural and functional insights. *Journal of Lipid Research*, 2009: *50*(Supplement), S29–S34.

[66]. Turull, a, and Queralt, J. Changes in prostaglandin E2 (PGE2) levels in paw exudate, stomach and kidney of arthritic rats: effects of flosulide. *Prostaglandins & Other Lipid Mediators*, 2001: *66*(1), 27–37.

[67]. Dinarello, C. A. Proinflammatory cytokines. *Chest*, 2000:*118*(2), 503–508.

[68]. McInnes, I. B., and Schett, G. Cytokines in the pathogenesis of rheumatoid arthritis. *Nature Reviews Immunology*, 2007: *7*(6), 429–442. https://doi.org/10.1038/nri2094

[69]. Nalbant, S., and Birlik, A. M. Cytokines in Rheumatoid Arthritis (RA). In *New Developments in the Pathogenesis of Rheumatoid Arthritis, Edited by Lazaros I. Sakkas* 2016: (p. 162). https://doi.org/10.5772/65893.

[70]. Baslund, B., Tvede, N., Danneskiold-Samsoe, B., Larsson, P., Panayi, G., Petersen, J. and Parren, P. W. Targeting interleukin-15 in patients with rheumatoid arthritis: A proof-of-concept study. *Arthritis & Rheumatism*, 2005: *52*(9), 2686-2692.

[71]. Dai, S. M., Shan, Z. Z., Xu, H., and Nishioka, K. Cellular targets of interleukin-18 in rheumatoid arthritis. *Annals of the Rheumatic Diseases*, 2007: *66*(11), 1411–1418.

[72]. Noack, M., and Miossec, P. Selected cytokine pathways in rheumatoid arthritis. *Seminars in Immunopathology*, 2017: *39*(4), 365–383.

[73]. Alsousi, A. A., Siddiqui, S., and Igwe, O. J. Cytokine-mediated Differential Regulation of Cyclooxygenase-2 , High Mobility Group Box 1 Protein and Matrix Metalloproteinase-9 Expression. *J. Clin. Exp. Pharmacol*, 2017: *7*(4).

[74]. Xu, S. TNF inhibitor therapy for rheumatoid arthritis (Review). *Biomedical Reports*, 2012: 177–184.

[75]. Vasanthi, P., Nalini, G., and Rajasekhar, G. Role of tumor necrosis factor-alpha in rheumatoid arthritis: A review. *APLAR Journal of Rheumatology*, 2007: *10*(4), 270–274.

[76]. Scanzello, C. R., Umoh, E., Pessler, F., Diaz-Torne, C., Miles, T., DiCarlo, E., and Crow, M. K. Local cytokine profiles in knee osteoarthritis: elevated synovial fluid interleukin-15 differentiates early from end-stage disease. *Osteoarthritis and Cartilage*, 2009: *17*(8), 1040–1048.

[77]. Bergström, B., Carlsten, H., and Ekwall, A. K. H. Methotrexate inhibits effects of platelet-derived growth factor and interleukin-1β on rheumatoid arthritis fibroblast-like synoviocytes. *Arthritis research & therapy*, 2018: *20*(1), 1-8.

[78]. Guzmán, C., Hallal-Calleros, C., López-Griego, L., and Morales-Montor, J. Interleukin-6: A Cytokine with a Pleiotropic Role in the Neuroimmunoendocrine Network. *The Open Neuroendocrinology Journal*, 2010: *3*, 152–160.

[79]. Roux-Lombard, P., Eberhardt, K., Saxne, T., Dayer, J. M., and Wollheim, F. A. Cytokines, metalloproteinases, their inhibitors and cartilage oligomeric matrix protein: relationship to

radiological progression and inflammation in early rheumatoid arthritis. A prospective 5-year study. *Rheumatology (Oxford, England)*, 2001:*40*, 544–551.

[80]. Nowell, M. A., Williams, A. S., Carty, S. A., Scheller, J., Hayes, A. J., Jones, G. W., and Jones, S. A. Therapeutic Targeting of IL-6 Trans Signaling Counteracts STAT3 Control of Experimental Inflammatory Arthritis. *The Journal of Immunology*, 2009: *182*(1), 613–622.

[81]. Nakahara, H., Song, J., Sugimoto, M., Hagihara, K., Kishimoto, T., Yoshizaki, K., and Nishimoto, N. Anti-interleukin-6 receptor antibody therapy reduces vascular endothelial growth factor production in rheumatoid arthritis. *Arthritis and Rheumatism*, 2003: *48*(6), 1521–1529.

[82]. Simmonds, R. E., and Foxwell, B. M. Signalling, inflammation and arthritis: NF-κB and its relevance to arthritis and inflammation. *Rheumatology*, 2008: *47*(5), 584–590.

[83]. Park, M. H., and Hong, J. T. Roles of NF-κB in cancer and inflammatory diseases and their therapeutic approaches. *Cells*, 2016: *5*(2).

[84]. Natoli, G. Tuning up inflammation: How DNA sequence and chromatin organization control the induction of inflammatory genes by NF-κB. *FEBS Letters*, 2006: *580*(12), 2843–2849.

[85]. Shambharkar, P. B., Blonska, M., Pappu, B. P., Li, H., You, Y., Sakurai, H., and Lin, X. Phosphorylation and ubiquitination of the IκB kinase complex by two distinct signaling pathways. *The EMBO Journal*, 2007: *26*(7), 1794–1805.

[86]. Mbalaviele, G., Sommers, C. D., Bonar, S. L., Mathialagan, S., Schindler, J. F., Guzova, J. A., and Hu, Y. A Novel , Highly Selective , Tight Binding IκB Kinase-2 (IKK-2) Inhibitor : A Tool to Correlate IKK-2 Activity to the Fate and Functions of the Components of the Nuclear Factor- κB Pathway in Arthritis-Relevant Cells and Animal Models. *The Journal of Pharmacology and Experimental Therapeutics*, 2009: *329*(1), 14–25.

[87]. Johnson, L. N., and Lewis, R. J. Structural basis for control by phosphorylation. *Chemical reviews*, 2001: *101*(8), 2209-2242.

[88]. Roux, P. P., and Blenis, J. ERK and p38 MAPK-Activated Protein Kinases : a Family of Protein Kinases with Diverse Biological Functions ERK and p38 MAPK-Activated Protein Kinases : a

Family of Protein Kinases with Diverse Biological Functions. *Microbiology and Molecular Biology Reviews : MMBR*, 2004: *68*(2), 320–344.

[89]. Thalhamer, T., McGrath, M. A., and Harnett, M. M. MAPKs and their relevance to arthritis and inflammation. *Rheumatology*, 2008: *47*(4), 409–414.

[90]. Johnson, G. L., and Lapadat, R. Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. *Science*, 2002: *298*(5600), 1911-1912.

[91]. Cargnello, M., and Roux, P. P. Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases. *Microbiology and molecular biology reviews*, 2011: *75*(1), 50-83.

[92]. Brandt, B., Abou-Eladab, E. F., Tiedge, M., and Walzel, H. Role of the JNK/c-Jun/AP-1 signaling pathway in galectin-1-induced T-cell death. *Cell death & disease*, 2010: *1*(2), e23-e23.

[93]. Gazon, H., Barbeau, B., Mesnard, J. M., and Peloponese Jr, J. M. Hijacking of the AP-1 Signaling Pathway during Development of ATL. *Frontiers in microbiology*, 2018: *8*, 2686.

[94]. Han, Z., Boyle, D. L., Chang, L., Bennett, B., Karin, M., Yang, L., and Firestein, G. S.c-Jun N-terminal kinase is required for metalloproteinase expression and joint destruction in inflammatory arthritis. *Structure*, 2001: *108*(1), 73–81.

[95]. Munshi, A., and Ramesh, R. Mitogen-Activated Protein Kinases and Their Role in Radiation Response. *Genes & Cancer*, 2013: *73104*(4), 401–408.