CHAPTER I

INTRODUCTION

1.1 Statement of the problem

Fibroblast-like synovioal cells are key essential role in this pathological process. Induced-Fibroblast-like synovioal cells produced pro-inflammatory which involve the inflammation lead to the damage of associated joints especially rheumatoid arthritis (RA). It is a chronic autoimmune disease and progressive articular destruction that cause pain, stiffness, loss of mobility, inflammation and erosion in the joint. Activation of FS is induced by proinflammatory cytokines as well as cytokine independent pathway and stimulated exogenous retroviral element such as lipopolysacchalide (LPS). It is a major component of the outer membrane of gram-negative bacteria cell walls. The activation of FS by LPS through Toll-like receptor-4 (TLR-4) leads to the high transcription of gene and effecter molecules involve in inflammation especially hyaluronan (HA). Production of HA in RA is uncontrolled, often leading to enormous amounts of HA in affected joints while in normal joints only a fine layer of HA covers and protects joint surfaces. Furthermore, LPS encourage inflammatory cytokine production such as interleukin-1β (IL-1β) and IL-1β motivate HA production via up-regulate the expression of HASs (Hyaluronan syntheses) gene. Possibly, the relation of LPS, IL-1β and HASs is mainly mechanism which associated rheumatoid inflammation.

One problem that impedes experiment progress is growth rate of FS cell. According to its slow growth, the study lasts a lot of time and cost. Thus, we hope that using cell line represent this cell. It might more convenience to study about mechanism and metabolism of this disease. Yamazak T. *et al*. used SW982 to study the effect of cytokines on matrix metalloproteinases (MMPs) gene expression that involved RA condition however the effect of LPS on HAS1, 2 and 3 gene expressions haven't been reported before.

Treatment for rheumatoid arthritis aim to reduce joint inflammation, so relieve pain and prevent or slow joint damage. There are many different pharmacological agents used in the treatment of rheumatoid arthritis: non-steroidal anti-inflammatory agents (NSAIDs) and disease modifying anti-rheumatic drugs (DMARDs). However, the both drugs have many side effects such as gastrointestinal discomfort, nausea, vomiting, loss of appetite, abdominal pain, skin rashes and allergic manifestation. Therefore, the several previous reports studied alternative therapies to fewer side effects, relieve pain and symptom other than pain. Preliminary studies revealed the anti-inflammatory effect of Plai extracts which could inhibit edema in carrageenan-induced rat paw including the hexane and ethanol extracts of Plai possesses a potent chondroprotective activity using the cartilage explants model stimulated with Retinoic acid (RetA) or Interleukin 1β

Plai, *Zingiber cassumunar* Roxb., is a Thai herbal plant which has long been regarded by Thai massage therapists as one of those oils necessary to have in their kit to combat joint and muscle problem. Furthermore, the compounds isolated from hexane extract of the Plai rhizome can be exhibited anti-inflammatory activity especially cis-3-(2', 4’, 5’-trimethoxyphenyl)-4-{(E)- 2''',4''',5'''- trimethoxylstyryl}-cyclohex-1-ene (compound C). Hence, the goal of this study was to investigate the effect of compound C on LPS-induced hyaluronan synthase gene expression and hyaluronan synthesis in a human synovial fibroblast SW982 cell line. The results of this study will bring about alternative pharmacological agent for arthritis disease.

1.2 Literature review

1.2.1 Synovial fibroblast and Synovial fibroblast cell line (SW982 cell line)

Synovial membrane is composed of a form of connective tissue that lines the cavity of a synovial joint and produces the synovial fluid; it lines all internal surfaces of the cavity except for the articular cartilage of the bones (Figure1). Normal synovial tissue consists of two anatomically distinct layers: a surface layer (intima, or synovial lining), and an underlying layer (subintima). The intima, in direct contact with the intra-articular cavity, is 20–40 μm thick, one to three cells deep, loosely organized, avascular, and not supported by a basement membrane. The subintima is even more loosely organized, consisting of a meshwork of connective tissue interspersed with cells and blood vessels (1). In the normal intima and subintima, two cell types predominate: macrophage-like (type A) synoviocytes (MLS) (2, 3), and FLS (type B synoviocytes). Two-thirds of native synoviocytes are FLS. FLS are bipolar, spindle-shaped cells with prominent secretory machinery, including extensive endoplasmic reticulum, regular ribosomal arrays, and well-developed Golgi apparati (1). Their nuclei are pale, with several nucleoli (4).

Synovial fibroblast cell line (SW982 cells line), a soft tissue sarcoma that most often occurs around leg or arm joints, has a 50% rate of metastasis. SW982 cell line are greatly facilitate for study of RA because these cells are more advantage than primary synovial fibroblasts such as SW982 cells are easy to obtain and they grow fast in culture, including non ending with a senescence phase (5).

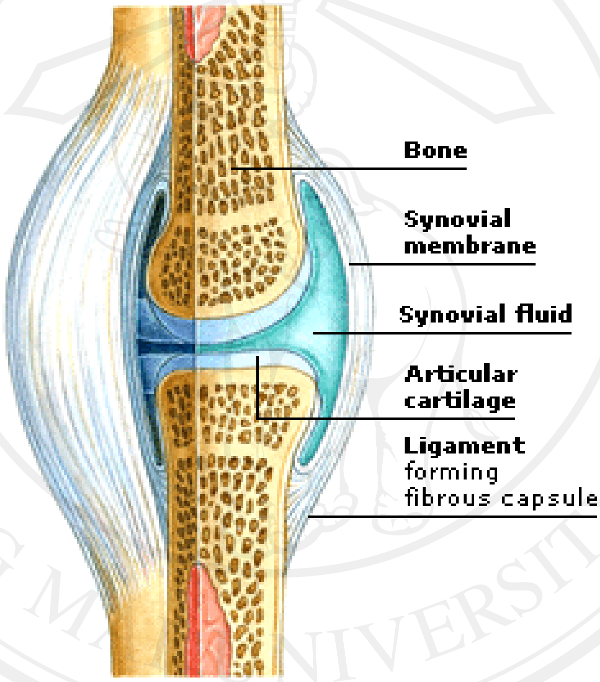

Figure 1. Architecture of typical synovial joint. All free-moving joints, such as the finger, hip, knee, and elbow joints, are called synovial joints and have a similar structure. The synovial membrane that lines the joint produces a fluid that lubricates movement. The bone ends are covered by a layer of articular cartilage, which is smooth and so minimizes friction. The joint is kept in place by a fibrous capsule, which encases the joint completely (6).

1.2.2 Function and metabolism of synovial fibroblast

Synovial fibroblasts (FLS) play critical roles in normal joint homeostasis. Mature intimal FLS secrete high levels of long-chain, polymeric hyaluronan into joint cavity, which has both lubricating and immunomodulatory properties. Intimal FLSs also produce the glycoprotein lubricin, as well as plasminogen activator. Lubricin contributes to joint space viscosity. Plasminogen activator may prevent fibrous adhesions in the joint, promoting normal movement (7). Moreover, FLS secrete multiple connective tissue components, including fibronectin, type IV and type VI collagen, laminin, chondroitin proteoglycans, and fibrillin. Therefore, FLS participate in the maintenance of the joint capsule (8).

A related FLS function is control of synovial fluid volume. Expanded synovial fluid volumes increase mechanical stress on intimal FLS, reducing hyaluronan production via a negative feedback loop. Low hyaluronan concentrations may then decrease oncotic pressure in the joint, encouraging restoration of reduced synovial fluid volumes. In contrast, joint effusions in arthritis represent a failure of synovial fluid volume control, with friction forces stimulating excess hyaluronan production, and retention of plasma dialysate in the synovial cavity (9).

One hallmark of RA is synovial hyperplasia. The intimal layer expands to a depth of 15 or more cells, primarily owing to increase in FLS number. Although the subintima becomes infiltrated with a variety of inflammatory cells, the FLS population in this region is also over expanded (10). In addition, RA FLS are hyperproliferation (11), and several studies suggest that RA FLS divide more rapidly than cells from normal or osteoarthritic joints (12). It is well known that primary mammalian cells in culture have a finite replicative life span and eventually enter a state of senescence in which they remain metabolically active but cease to proliferate and routinely obtaining RA-derived synovial tissue samples is difficult including the study lasts a lot of time and cost. Thus, if we can find other cells better than this cell, it might more convenience to study about mechanism and metabolism of this disease. From previous report indicate that human synovial fibroblast cell line (human synovial sarcoma cells; SW982 cells) are useful tool for the study of expression of inflammatory cytokine and matrix metalloproteinases (MMPs) gene expression that involved process of rheumatoid arthritis (13). In addition, it has been suggested that inhibition of the transcriptional factors nuclear factor (NF)-KB and activator protein (AP)-1 by dexamethasone (DEX), have long been considered the most effective treatment for RA, in SW982 is a promising approach for the treatment of inflammation (14).

1.2.3 Rheumatoid arthritis (RA)

Rheumatoid arthritis is a chronic inflammatory disorder that mainly affects the various diarthrodial joint in patient body. It affects about 1% of the population, in a female/male ratio of 2.5/1. The disease can occur at any age, but it is most common among those aged 40–70 years, its incidence increasing with age. The predominant symptoms are pain, stiffness, and swelling of peripheral joints. The clinical course of the disorder is extremely variable, ranging from mild, self-limiting arthritis to rapidly progressive multisystem inflammation with profound morbidity and mortality (Figure 2) (15).

In RA the white blood cells move from bloodstream into joint tissue. Joint fluid may increase, and the white cells are found in the fluid as well. The white cells in the joint tissue and fluid produce may substances, including antibodies and other molecules, which lead to the joint damage and the sick feeling that occurs in people with rheumatoid arthritis. Inflamed joints may be warm, swollen, tender, often red and painful or difficult to move. These physical signs of arthritis are due to inflammation of the lining of joint and tendons in a layer of tissue that is call synovium. The cells of the immune system within the synovium appear active and capable of causing tissue damage. If this inflammation persists or does not respond well to treatment, destruction of nearby cartilage, bone, tendons and ligament can follow. This leads to deformity and disability that can be permanent (Figure 3) (16).

Although the pathogenesis of RA remains incompletely understood, the previous suggested that cause of RA that it occurs individual genetic predisposition, environmental factors and infectious agent such as micro-organism especially gram-negative bacteria. It motivates dysregulated immune responses lead to hyperplasia and hyperproliferation synoviocytes and over produced synovial fluid that prominent of RA (17).

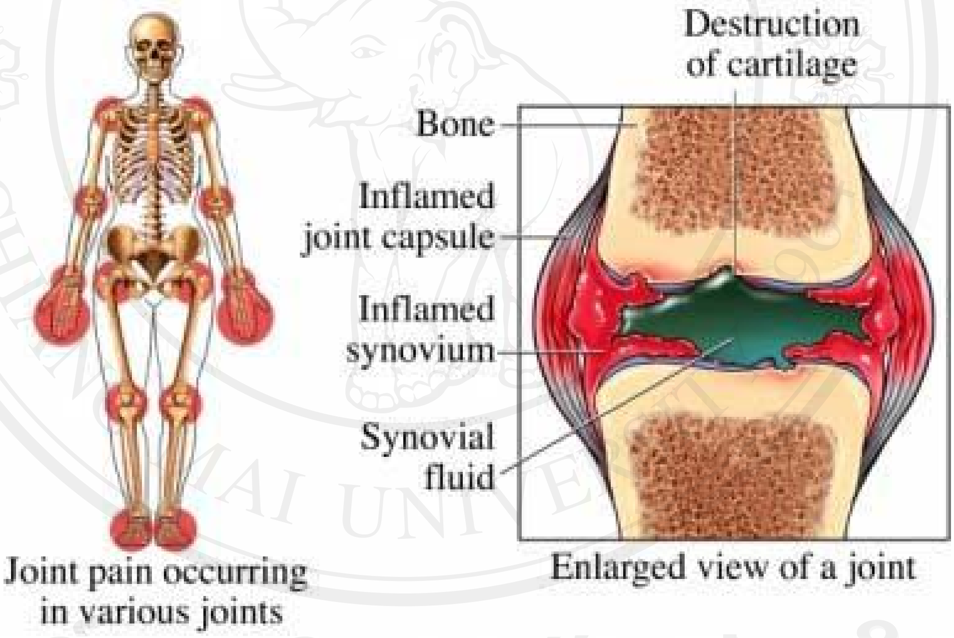

Figure 2. Architecture of rheumatoid arthritis (RA) (18)**.**

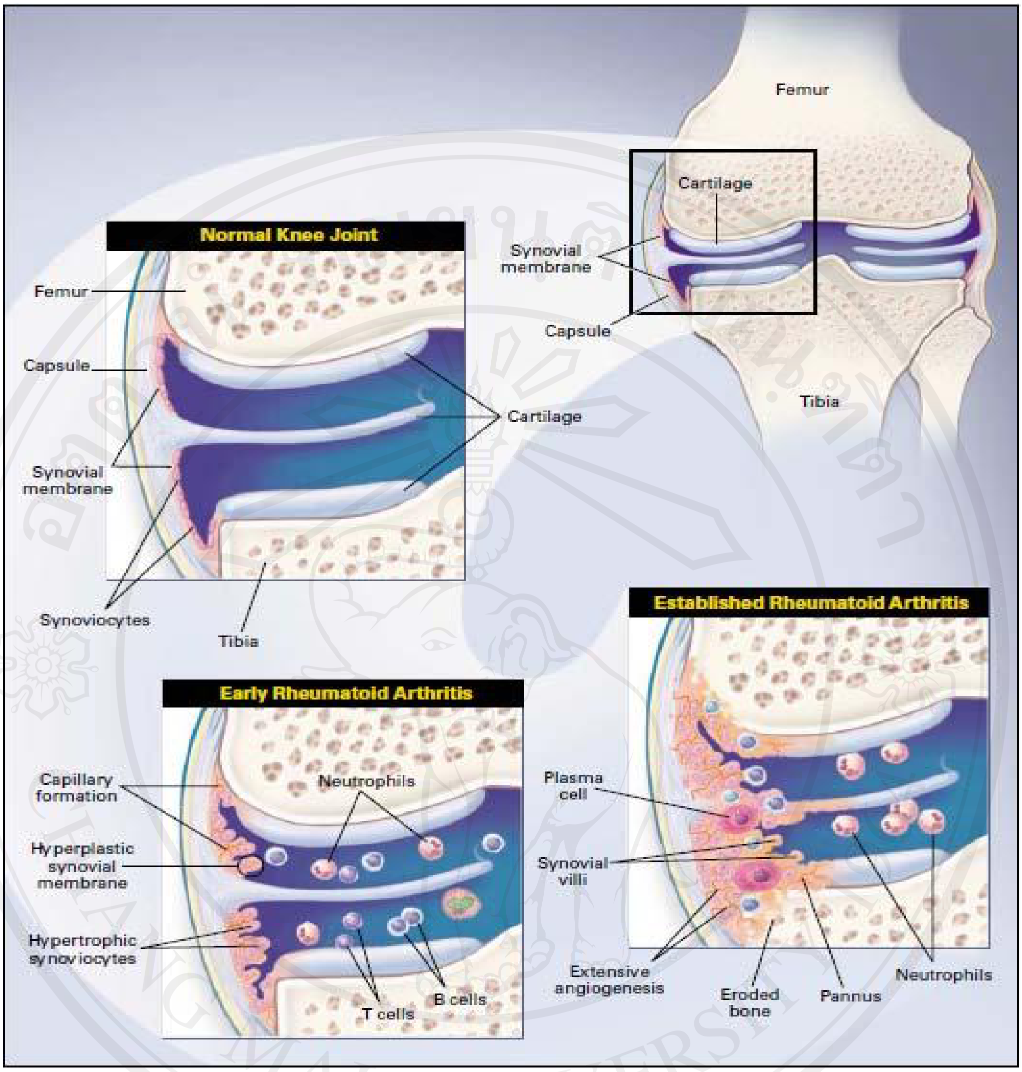

Figure 3. Pathogenesis of Rheumatoid Arthritis. In the normal knee joint, the synovium consists of a synovial membrane (usually one or two cells thick) and underlying loose connective tissue. Synovial-lining cells are designated type A (macrophage-like synoviocytes) or type B (fibroblast-like synoviocytes). In early rheumatoid arthritis, the synovial membrane becomes thickened because of hyperplasia and hypertrophy of the synovial lining cells. An extensive network of new blood vessels is formed in the synovium. T cells (predominantly CD4+) and B cells

(some of which become plasma cells) infiltrate the synovial membrane. These cells are also found in the synovial fluid, along with large numbers of neutrophils. In the early stages of rheumatoid arthritis, the synovial membrane begins to invade the cartilage. In established rheumatoid arthritis, the synovial membrane becomes transformed into inflammatory tissue, the pannus. This tissue invades and destroys adjacent cartilage and bone. The pannus consists of both type A and type B synoviocytes and plasma cells (19).

1.2.4 Hyaluronan (HA)

A major component of synovial joint fluid is hyaluronan (HA). HA is a high-molecular weight, highly anionic glycosaminoglycan (GAG). It is composed of repeating alternating units of glucuronic acid and N-acetylglucosamine, all connected by β-linkages, $[\text{-}\beta\ (1, 4)\text{ - GlcUA-}\beta\ (1, 3)\text{-GlcNAc-}]_n$ that can reach 10^7 Da in molecular size. Hyaluronan is the simplest of the GAGs, the only one not covalently linked to a core protein, not synthesized by way of the Golgi pathway, and the only non-sulfated GAG (Figure 4) (20).

A single enzyme is now recognized as being able to synthesize HA, dual-headed transferases that utilize alternately the two UDP-sugar substrates, UDP-glucuronic acid and UDP-Nacetylglucosamine (Figure 5).

The HA cytoplasmic product is extruded by an unknown mechanism through the plasmamembrane into the extracellular space, permitting unconstrained polymer growth. There are three synthase genes in the mammalian genome, coding for Hyaluronan synthase (HAS) -1, -2, and -3, each producing a different size polymer

,but (21) three gene products are similar in amino acid sequence and molecular structural characteristics. HAS3 is responsible for the synthesis of low molecular mass hyaluronan (< 2 x 10^5) while HAS1 and HAS2 are responsible for the synthesis of high molecular weight hyaluronan (HMW-HA) (>2 x 10^6). Though the synthesis is differentially regulated, the major controls for steady-state levels of HA deposition appears to occur at the level of catabolism (Figure 6) (22).

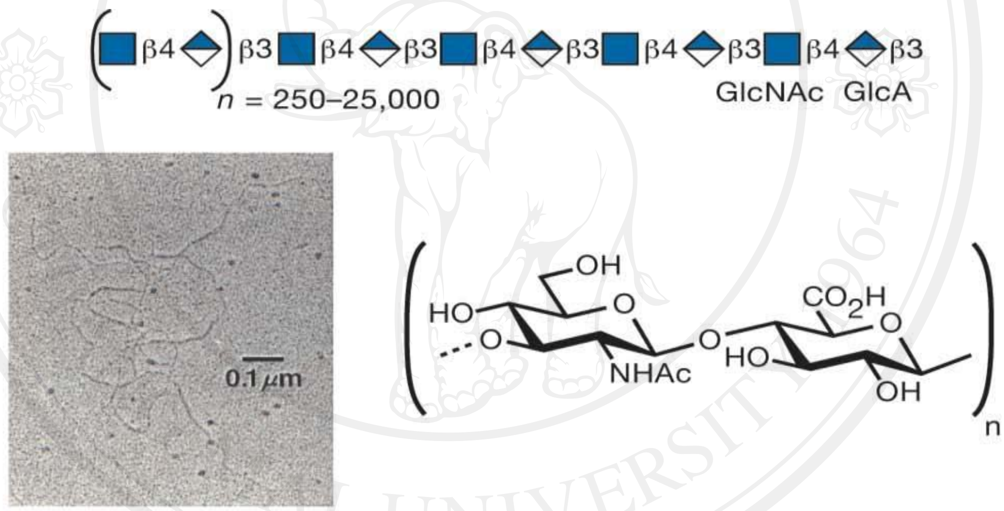

Figure 4. Hyaluronan structure. Hyaluronan consists of repeating disaccharides composed of *N*-acetylglucosamine (GlcNAc) and glucuronic acid (GlcA). It is the largest polysaccharide found in vertebrates, and it forms hydrated matrices (23).

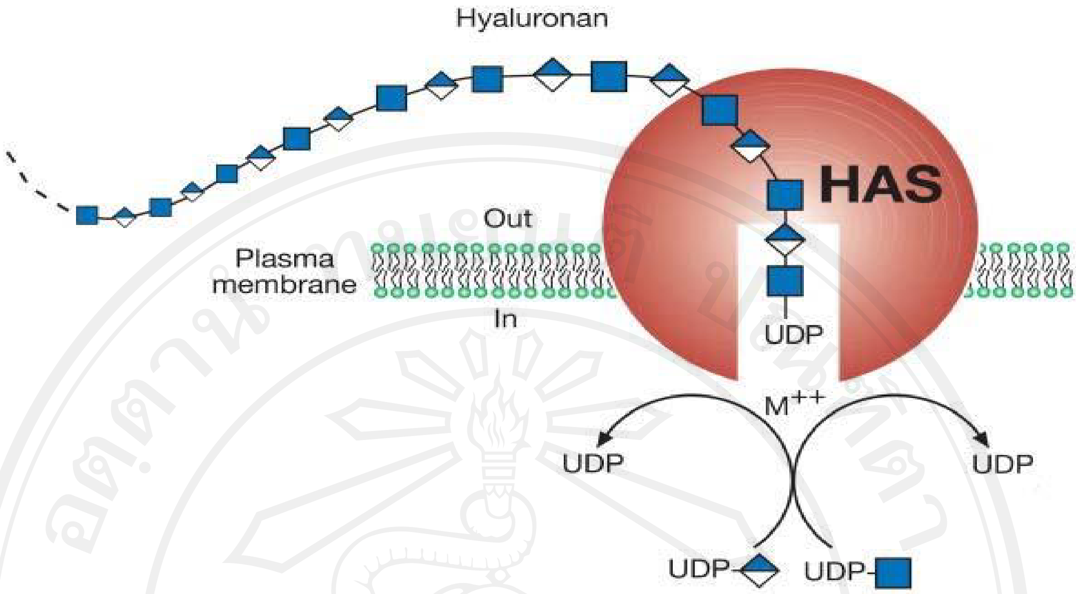

Figure 5. Hyaluronan biosynthesis by hyaluronan synthase (HAS); occurs by addition of sugars (*N*-acetyl-glucosamine and glucuronic acid) to the reducing end of the polymer. M^{++} refers to a metal is cofactor (23).

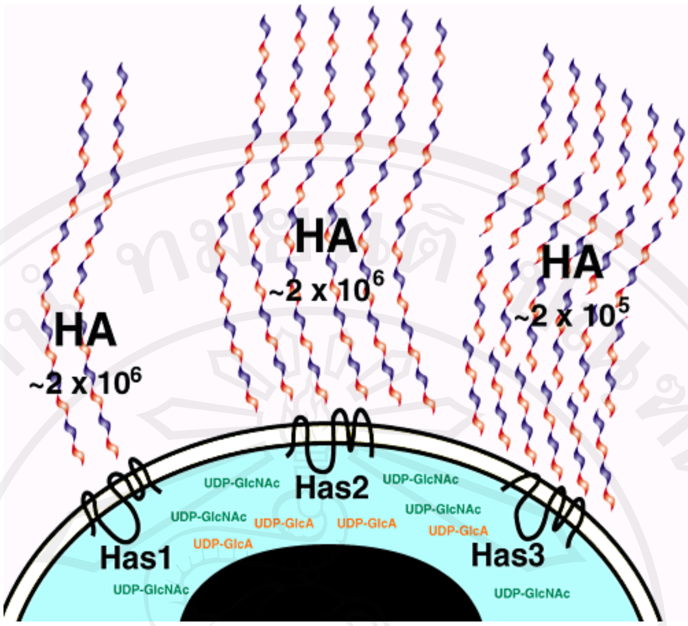

Figure 6. Function of Hyaluronan synthesis. Regulation of hyaluronan amount and chain length by expression of a specific HAS protein. Biochemical characterizations of the vertebrate HAS enzymes expressed in mammalian cell culture have revealed similarities and differences between the respective mammalian hyaluronan synthase enzymes. The differences are depicted in this figure. HAS1 produces small amounts of high-molecular-weight hyaluronan. HAS2 produces significantly more high-molecular-weight hyaluronan. HAS3 is the most active of the hyaluronan synthases, yet produces low-molecular-weight hyaluronan chains (48).

HA is a major component of extracellular matrices, particularly in tissue with rapid cell proliferation and migration. The interaction of HA with various HA-binding protein and cell-surface receptor plays important roles in regulating fundamental cell behaviors such as cell adhesion, migration, and differentiation, and its physiological roles are markedly dependent on its size (24-26).

High molecular weight HA (HMW-HA) is a major component of synovial fluids and the extracellular matrix (ECM) of many tissues, including those in the joint (27). It physically acts as a viscous lubricant for slow joint movements, such as walking, and as an elastic shock absorber during rapid movements, such as running. HMW-HA has a variety of biologic effects on cells in vitro, such as inhibiting the synthesis of prostaglandin E2 and the release of arachidonic acid (28, 29), and down-regulating the expression of TNF-α, IL-8 and iNOS (30). Several researches demonstrated that HMW-HA could suppress the degradation of cartilage matrix induced by fibronectin fragments and cytokines *in vivo* (31, 32). Moreover, it could relieve joint pain by masking free never ending organelles in animal experiments (33, 34). Hence, it is suggested that HMW-HA is an indispensable component in the maintenance of joint homeostasis (35).

Recently, the glycosaminoglycan HA has also been identified as an inducer of TLR4 activation, capable of causing the release of pro-inflammatory cytokines. It has been suggested that trauma can release small molecular weight HA fragments (sHA) from the extracellular matrix, thus potentially presenting HA as an intrinsic signal of inflammation (36-40). Taylor *et al*. have found that hyaluronan fragments generated enzymatically and LPS induced overlapping, but distinct, patterns of gene expression in macrophages. It is known that the TLR-4 response to LPS is dependent on a number of accessory molecules including CD14. The response to hyaluronan in Taylor's study was dependent on TLR-4 but not CD14, and rather involved CD44. This suggests that the TLR-4 response to hyaluronan fragments involves a unique receptor complex distinct from the LPS–TLR binding complex (Figure 7) (41). These agreed with previous study which found that Hyaluronan fragment produced as a results from TLR-2 and TLR-4 regulated expression of gene involved in inflammation in macrophages. HMW-HA will prevent lung injury during inflammation and ultimately promoting repair. Whether hyaluronan species generated within the joint can activate TLR signaling is unknown, but appears worthy of investigation (Figure 8) (42).

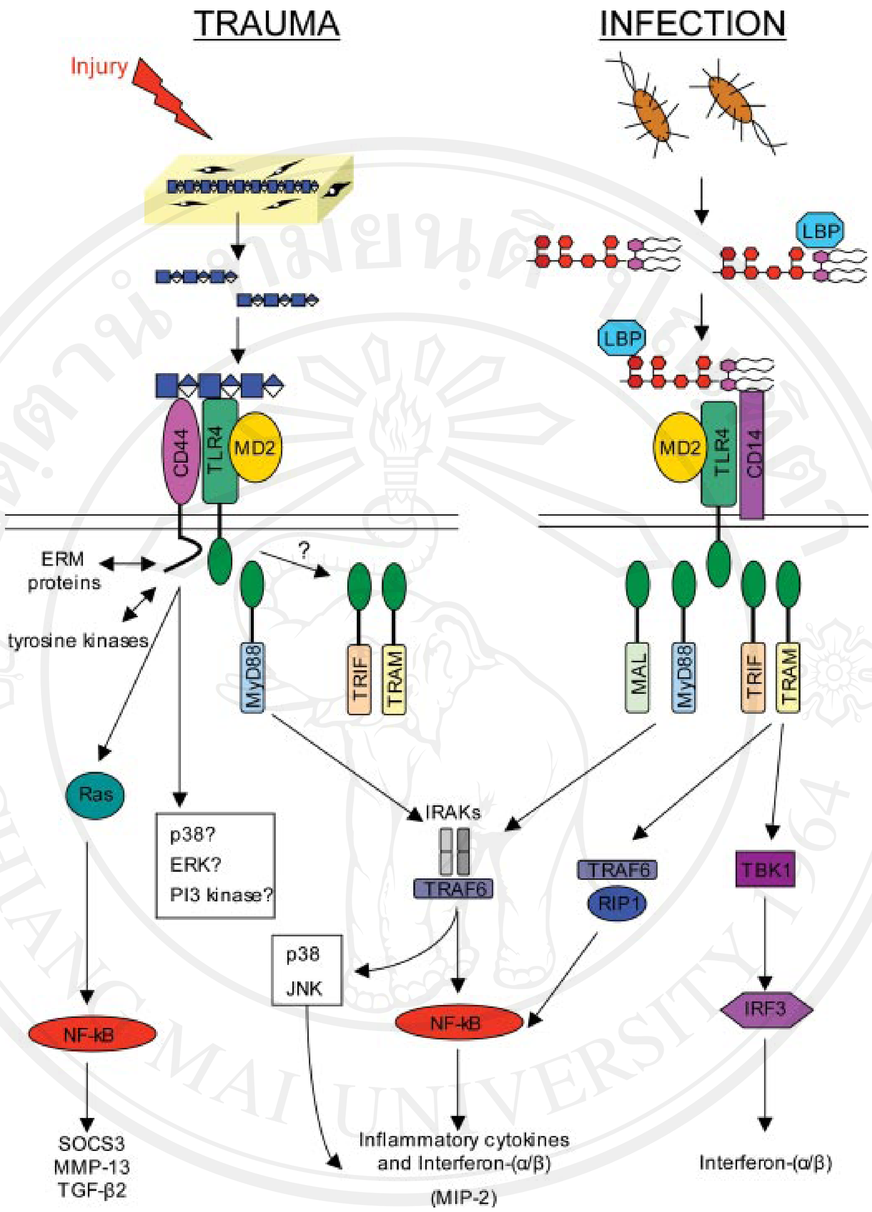

Figure 7. Model of proposed HA signaling. Injury leads to extracellular matrix breakdown, including the degradation of HA and resulting oligosaccharides. Lower molecular weight HA signals through TLR4, requiring coaccessory molecules MD-2 and CD44. In contrast, LPS requires CD14 and LPS binding protein to signal through TLR4 (41).

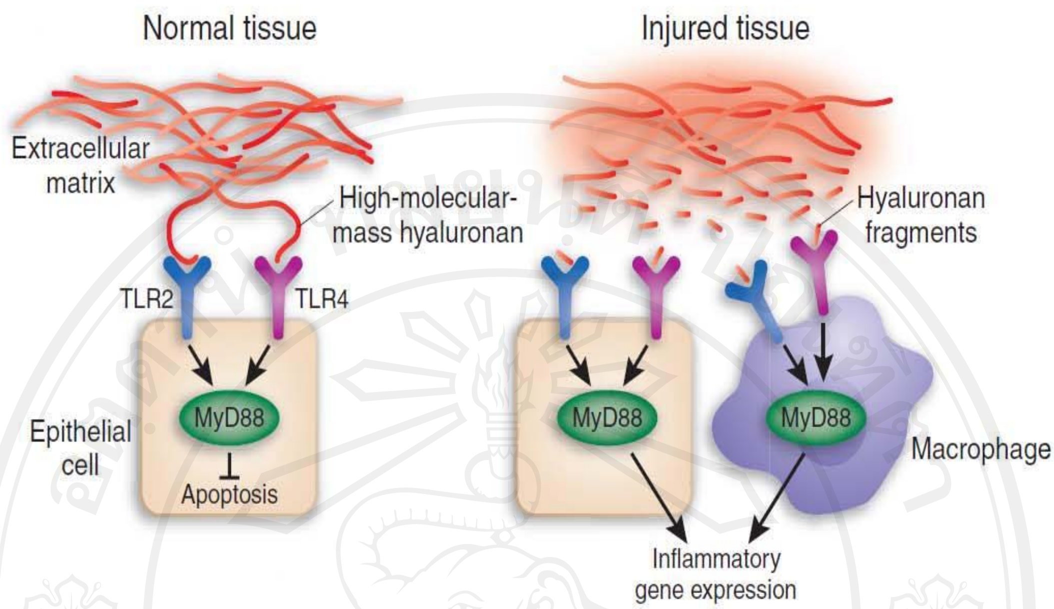

Figure 8. TLR4 act as key sensors of the extracellular matrix component hyaluronan in the lung. (a) HMW-HA is sensed by TLR-4 in epithelial cell, maintaining the integrity of the epithelium by preventing apoptosis. (b) If hyaluronan fragment are generated, the balance shifts away from HMW-HA. Inflammation will result, again through TLR-4. The status of hyaluronan in the lung is therefore sensed by the TLR system, with HMW-HA protecting tissue and probably allowing for repair, whereas hyaluronan fragment will provoke inflammation and cause injury. Altering the balance may have therapeutic utility in the treatment of lung inflammation (42).

1.2.5 Lipopolysaccharide (LPS)

The bacterial endotoxin, Lipopolysaccharide (LPS), is a major component of the outer membrane of gram-negative bacteria (Figure 9). It is composed of a hydrophilic polysaccharide moiety, which is covalently linked to a hydrophobic lipid moiety (Lipid A) LPS from most species is composed of three distinct regions: the O-antigen region, a core oligosaccharide and Lipid A (Figure 10) (43).

LPS elicits a wide variety of pathophysiological effects, such as endotoxin shock, tissue injury, and death (44). LPS do not act directly against cells or organs but through activation of immune system, especially the monocytes and macrophages, thereby enhancing immune responses. These cells release mediators, such as tumour necrosis factor, several interleukins, prostaglandins, colony stimulating factor, platelet activating factor and free radicals (45, 46). From many previous reports found that synovial cells are markedly activated by this immune mediators result in enhance inflamed synovium and over produced abnormal synovial fluid that involve in process of RA inflammation (47-53).

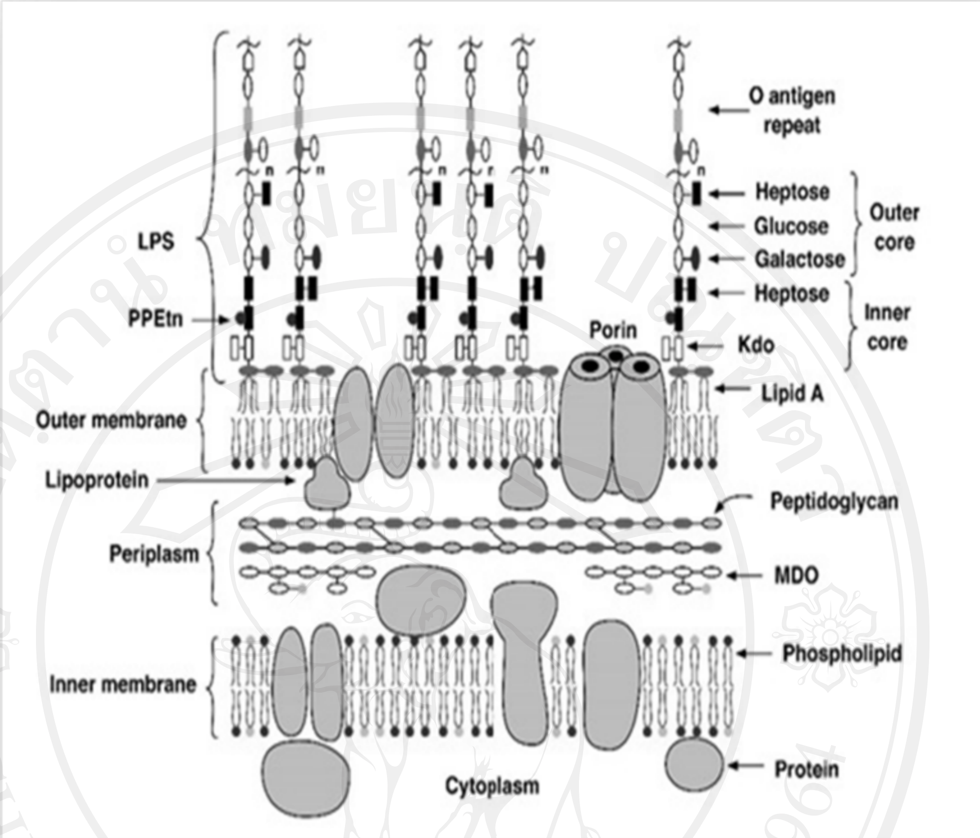

Figure 9. Molecular model of the inner and outer membranes. Geometric form: ovals and rectangles represent sugar residues, as indicated, whereas circles represent polar head groups of various lipids. Abbreviation: PPEtn (ethanolamine pyrophosphate); LPS (lipopolysaccharide); Kdo (2-keto-3-deoxyoctonic acid) (43).

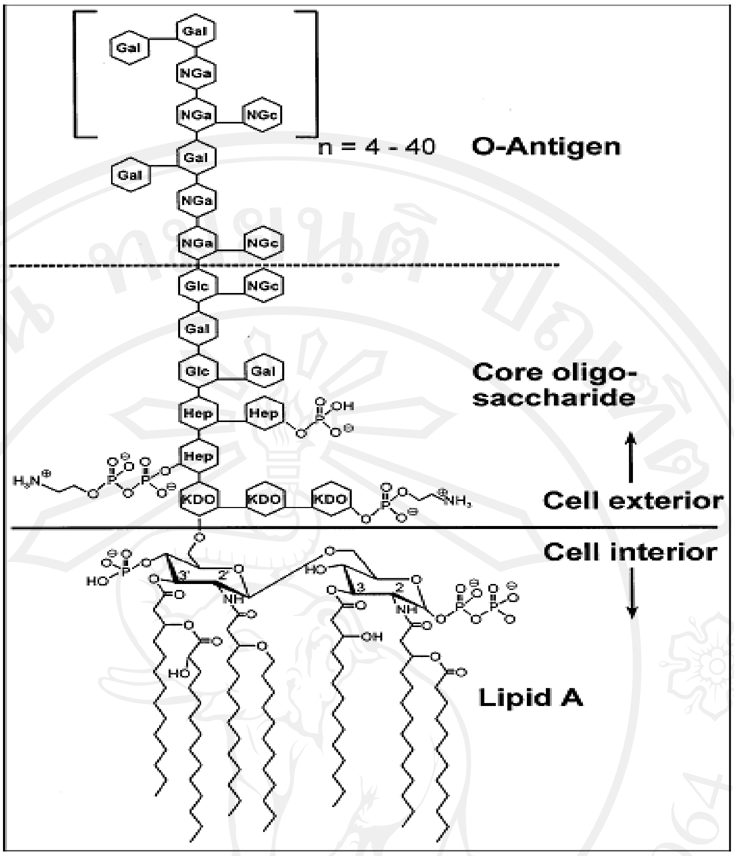

Figure 10. Chemical structure of endotoxin from *E. coli* O111:B4 (Hep) L-glycerol-D-manno heptose; (Gal) galactose; (Glc) glucose; (KDO) 2-keto-3-deoxyoctonic acid; (NGa) N-acetyl- galactosamine; (NGc) N-acetyl-glucosamine (43).

1.2.6 Toll-like receptor signaling

Toll-like receptor (TLR) family now consists of more than 13 members, each detecting distinct pathogen-associated molecular pattern (PAMPs), such as viruses, bacteria, protozoa and fungi (54).

These microbial components include bacterial lipopolysaccharide (LPS; TLR-4 ligand), lipoproteins (TLR-2 ligand), flagellin (TLR-5 ligand), bacterial CpG DNA (TLR-9 ligand), viral single-stranded RNA (TLR-7 ligand) and viral double-stranded RNA (TLR-3 ligand) (Figure 11) (55).

Among the PAMPs, lipopolysaccharide (LPS) is known to be a potent inducer of proinflammatory cytokines, such as interleukin (IL)-1β, tumor necrosis factor-α (TNF-α), IL-6, and IL-12, and inflammatory effector substances, such as prostanoids, leukotrienes, and nitric oxide, in monocytes (56). The first host protein involved in LPS recognition is LPS-binding protein (LBP) where it recognizes and forms a high-affinity complex with the lipid A moiety of LPS. The primary sequence of LBP appears to that of aiding LPS to dock at the LPS receptor complex by initially binding LPS and then forming a ternary complex with cluster of differentiation 14 (CD14), thus enabling LPS to be transferred to the LPS receptor complex composed of TLR-4, Myeloid differentiation primary response gene (MyD88), Myeloid differentiation protein 2 (MD-2) and Toll/IL-1 receptor (TIR) domain (Figure 12) (57, 58). From the evidence that is emerging, the TLR4-mediated response to LPS can be divided into two categories: an early MyD88-dependent response; and a delayed MyD88-independent response (59-63). Ultimately, these lead to the activation of mitogen-activated protein kinases (MAPKs) and nuclear factor (NF)-κB signaling pathway that cause the transcription of inflammatory mediators (Figure 13). This mediators found in RA condition can adversely affect the type B synoviocyte and lead to the synthesis of Hyaluronan (HA) with abnormal molecular weight (64).

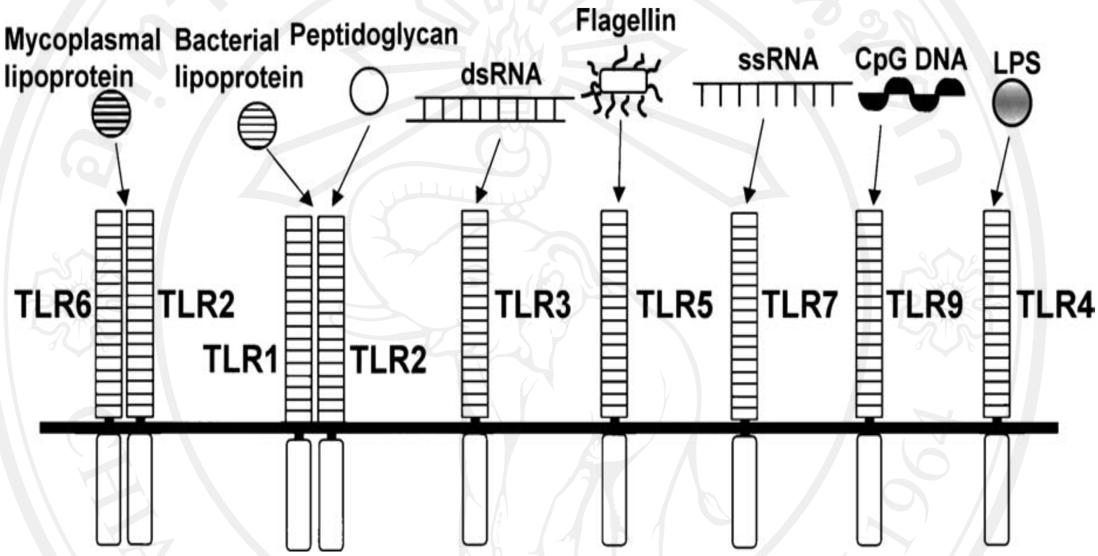

Figure 11. Pathogen-associated molecular patterns and TLRs. Microbial components such as LPS, peptidoglycan, and dsRNA are recognized by TLRs. Among TLRs, recognition of PAMPs by TLR1 and TLR6 requires heterodimerization with TLR2 (54).

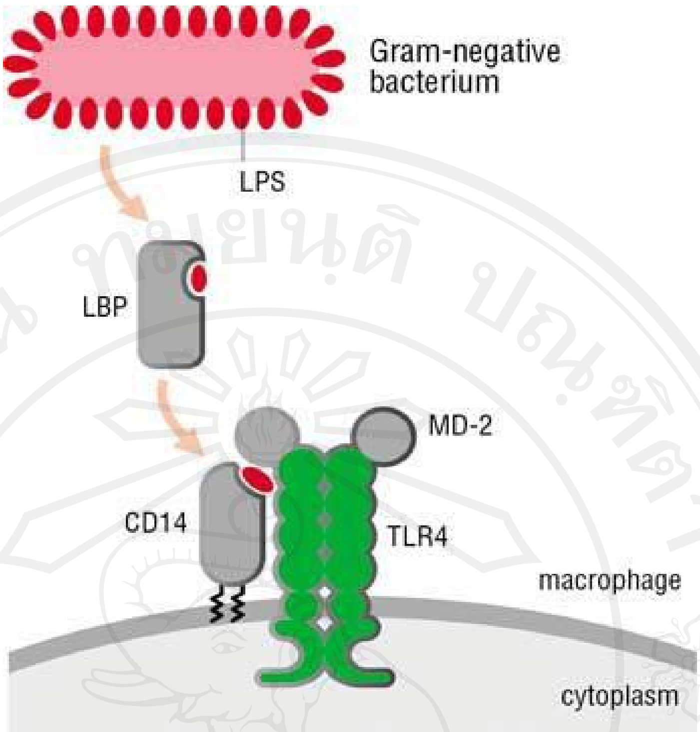

Figure 12. Recognition of bacterial lipopolysaccharide by innate immune cells. Lipopolysaccharide (LPS) monomers are extracted from bacterial membranes by the serum protein LPS-binding protein (LBP) which transfers the LPS monomer to a lipid-binding site on CD14 in the membrane of phagocytes. CD14 promotes the binding of LPS to the TLR4–MD-2 complex, which signals to the cell interior. In the absence of CD14, TLR4–MD-2 can still function with some forms of LPS or with much higher LPS concentrations (65).

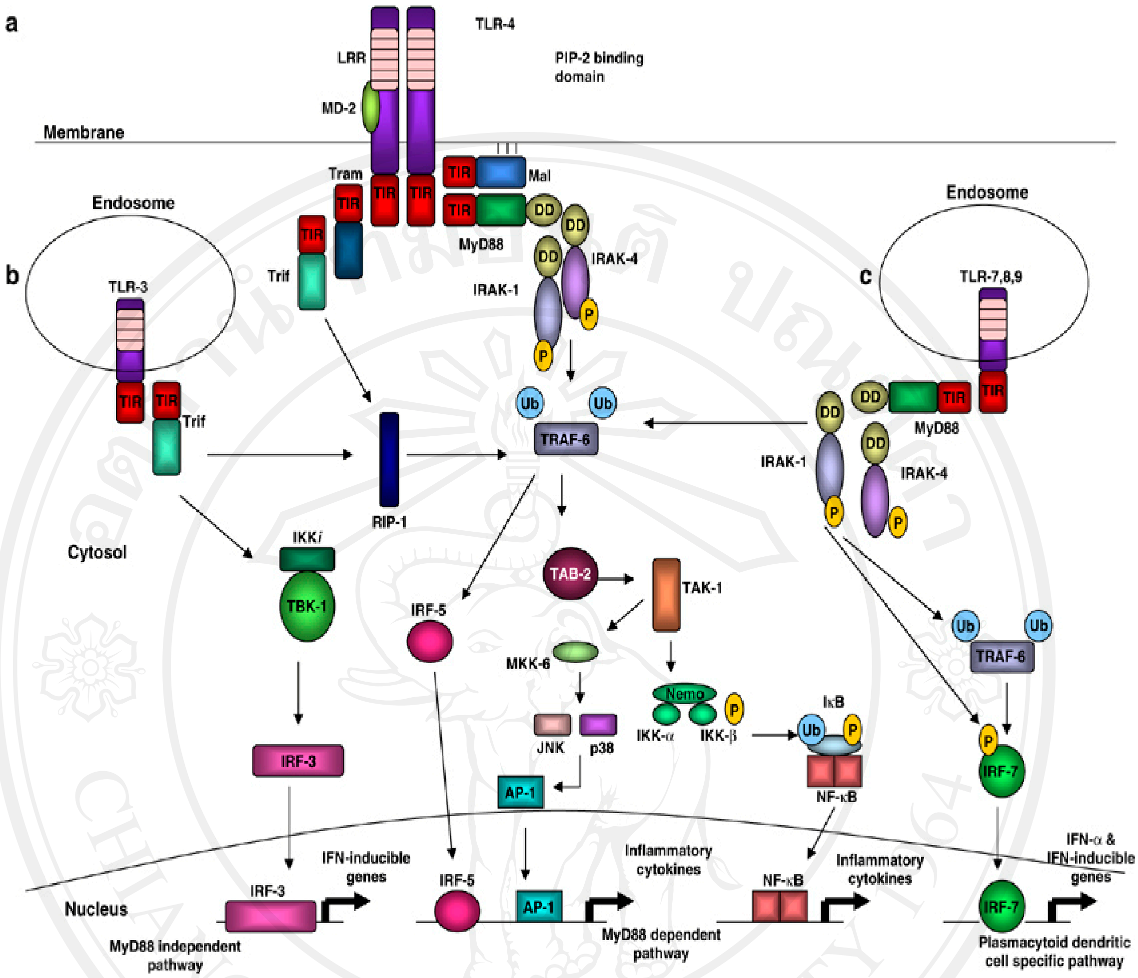

Figure 13. The three signaling pathways involved in Toll-like receptor (TLR) signalling. (**a**) The MyD88-dependent pathway: This pathway is used by all TLRs except TLR3. It is initiated in the case of TLR4 by the TIR domain of Mal binding the TIR domain of TLR4, which has dimerized after ligand binding. Mal recruits MyD88, which binds IRAK4 and -1. IRAK1 is phosphorylated by itself and IRAK4 and leaves the membrane to activate TRAF6. After TRAF6 is ubiquitinated, it interacts with TAB2 to activate TAK1. TAK1 activates the IKK complex and IκB is phosphorylated, ubiquitinated and degraded allowing NF-κB to translocate to the

nucleus to produce proinflammatory cytokines. TAK1 also activates MKK6 which in turn activates JNK and p38 leading to AP-1 activation and the production of proinflammatory cytokines. TRAF6 can also activate IRF5. (**b**) The MyD88-independent pathway or the TRIF-dependent pathway: This pathway is used by TLR3 and with TLR4 by binding TRAM at the membrane. This pathway begins with the activation of TBK-1 leading to the activation of IRF3, a transcription factor that translocates to the nucleus to produce IFN-inducible genes. Alternatively RIP1 is activated by TRIF and this feeds into the MyD88 pathway by activating TRAF6. (**c**) The alternative MyD88 pathway: This only occurs in plasmacytoid dendritic cells with the activation of TLR7 and -9 leading to the activation of TRAF6 through MyD88, IRAK4 and -1. This results in the activation of IRF7 which translocates to the nucleus to produce IFN-α and IFN-inducible genes (66).

1.2.7 Interleukin-1β

The interleukin-1 (IL-1) family of cytokines consists of structurally and functionally related molecules with pleiotropic activities involved in immune and inflammatory responses. IL-1β, one member of this family, has been implicated in wide range of physiologic and pathologic processes (67).

Interleukin-1β (IL-1β)-converting enzyme (ICE), or caspase 1, is a novel cysteine protease that cleaves the 31-kD inactive cytoplasmic IL-1β precursor into active extracellular 17-kD IL-1 beta (68).

It was well know that LPS stimulated macrophage to release inflammatory cytokines, IL-1β through both NF-κB and MAPKs signaling pathway (Figure 14) (69). Abnormal IL-1β production which induced by LPS involved in pathogenesis of rheumatoid arthritis (RA) because IL-1β has been detected in synovial fluid of RA patients and increased level of IL-1β in serum or plasma of patients with active disease (70). Additionally, several reports have shown that a number of IL-1β is ability to increase the expression of HASs mRNA in difference cell culture types. The obvious consequence of this stimulation is a marked increment in HA production (24, 71, 72, 73). However, the association between LPS, IL-1β and HA wasn't fully cleared.

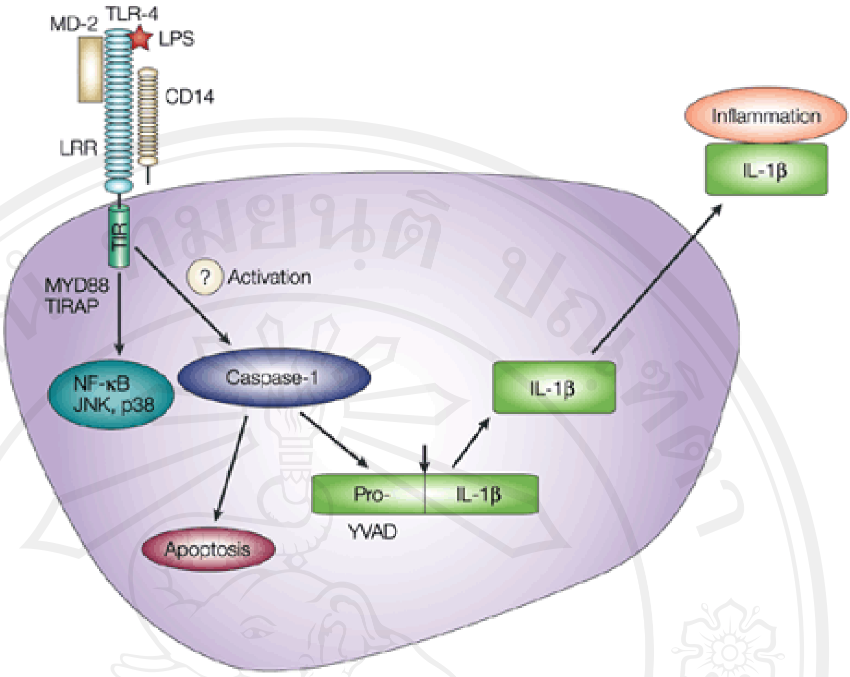

Figure 14. LPS induce IL-1β production. Recognition of lipopolysaccharide (LPS) by Toll-like receptor (TLR)-4 is aided by two accessory proteins known as CD14 and MD-2. TLR-4 contains extracellular leucine-rich repeats (LRRs) and an intracellular Toll/interleukin-1 (IL-1) receptor (TIR) domain, and transmits signals through the two TIR adaptor proteins MYD88 and TIRAP (Toll/IL-1 receptor domain-containing adaptor protein). This leads to the activation of the p38, c-Jun N-terminal kinase (JNK) and nuclear factor κB (NF-κB) signalling pathways. Caspase-1 is activated by an unknown mechanism, and then active caspase-1 cleaves the inactive precursor of IL-1β (pro-IL-1β) downstream from the aspartate residue 116 within the substrate-recognition sequence YVAD, thereby liberating the active form of IL-1β. Processing of IL-1β is associated with its release into the extracellular space, where it initiates the

inflammatory response. In certain situations, active caspase-1 can also trigger apoptosis through cleavage and activation of the downstream caspase-3 (74).

1.2.8 Drug used in treatment of rheumatoid arthritis

There are two general classes of drugs commonly used in the treatment of rheumatoid arthritis: non-steroidal anti-inflammatory agents (NSAIDs) and disease modifying anti-rheumatic drugs (DMARDs). NSAIDs have a short onset of action while DMARDs can take several weeks or months to demonstrate a clinical effect.

NSAIDs (such as diflunisal, sulidac, naproxen, diclofenac, celecoxib and piroxican) are major effect of these agents is to reduce acute inflammation thereby decreasing pain and improving function. NSAIDs inhibit the generation of prostaglandins by blocking cyclooxygenase enzymes, COX-1 and COX-2. Prostaglandins are mediators of inflammation and pain but also have important roles in maintenance of normal body functions including protection from stomach acid, maintenance of kidney blood flow, and contributing to platelet stickiness and vascular function. COX-2 selective inhibitors selectively block prostaglandins generated via COX-2 which have prominent roles in inflammation. The most common toxicity of NSAIDs is gastrointestinal disturbance which may clinically include burning, belching, or irritation, but which can represent irritation of the lining of the stomach, erosions, and even ulcerations that can result in bleeding. Because prostaglandins play a role in the regulation of the blood flow in the kidneys and maintenance of glomerular filtration, NSAIDs can also impair renal function in certain patients leading to salt retention, edema, and increased blood pressure. The patients at highest risk are those with fluid imbalances or with compromised kidney function (e.g., heart failure, diuretic use, cirrhosis, dehydration, and renal insufficiency). Selective COX-2 inhibitors may also increase cardiovascular risks by their effects on blood pressure and additional effects on vascular beds. Thus the use of this class of medications must into account their relative risks in an individual patient of gastrointestinal damage versus potential cardiovascular risk factors (75, 76).

DMARDs (such as methotrexate, sulfasalazine, leflunomide, etanercept, infliximab, antimalarials and gold salts) have an effect upon rheumatoid arthritis that is different and may be more delayed in onset than NSAIDs. The anti-inflammatory effects of DMARDs in rheumatoid arthritis appear to be related at least in part to interruption of possible effects on TNF pathways. DMARDs bind TNF in the circulation and in the joint, preventing interaction with cell surface TNF receptors thereby reducing TNF activity. The side effect of DMARDs is hepatic cirrhosis, interstitial pneumonitis, severe myelosuppression, stomatitis, oral ulcers, mild alopecia, hair thinning, and GI upset may occur and are related to folic acid antagonism (76, 77).

1.2.9 *Zingiber cassumunar* Roxb. and *cis*-3-(2', 4', 5'-trimethoxyphenyl)-4-{(E) - 2''', 4''', 5'''-trimethoxylstyryl}-cyclohex-1- ene (compound C)

Zingiber cassumunar Roxb., commonly known as Plai, is a medicinal herb belonging to the family Zingiberaceae which has been exploited for medicinal purposes in Thailand and Southeast Asia for centuries. The rhizome part of the herb has a yellow to green color with fleshy thick texture containing multiple sessile tubers. Leaf stems 1 to 1.5 m tall. Leaves distichous, oblong-lanceolate, 20 to 30 cm long and 2 to 8 cm wide, pubescent below; sheath glabrous or hairly. Inflorescences scapose; peduncle 8 to 30 cm long, with pubescent sheaths. Spike ovoid-ellipsoid: bracts greenish red, narrowly obovate or rhomboid, 2.5 to 3.5 cm long; bracteole shorter than bract, ovate, 3-dentate. Calyx truncate, glabrous. Corolla tube ca. 2.5 cm long, pale yellow, dorsal lobe cymbiform, lateral lobe linear-lanceolate. Labellum pale yellow, suborbicukar, apex emarginated, lateral lobe ovate-oblong, append-age slightly longer than anther; stamen pale yellow Ovary inferior, 3-celled. Fruit, small, globose capsule ca. 0.5-1 cm (Figure 15) (78).

The fresh rhizome of Plai is used in traditional Thai massage for muscle relaxant and joint pain (79). The rhizome of *Zingiber cassumunar* Roxb. is widely used in Thai traditional medicine for topical treatment of sprains, contusions, joint inflammations, muscular pain, abscesses and similar inflammation-related disorders. Interestingly, the compounds isolated from hexane extract of the Plai rhizome can be exhibited anti-inflammatory activity especially *cis*-3-(2', 4', 5'-trimethoxyphenyl)-4-{(E)- 2''',4''',5'''-trimethoxylstyryl}-cyclohex-1-ene (compound C) (Figure 16). It is member of the cyclohexane derivatives. From previous study suggest that compound C exhibited anti-inflammatory activity on 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced ear edema in rat however the effect of compound C on joint inflammation didn’t clearly (80-84).

Figure 15. Plai and its rhizomes (85).

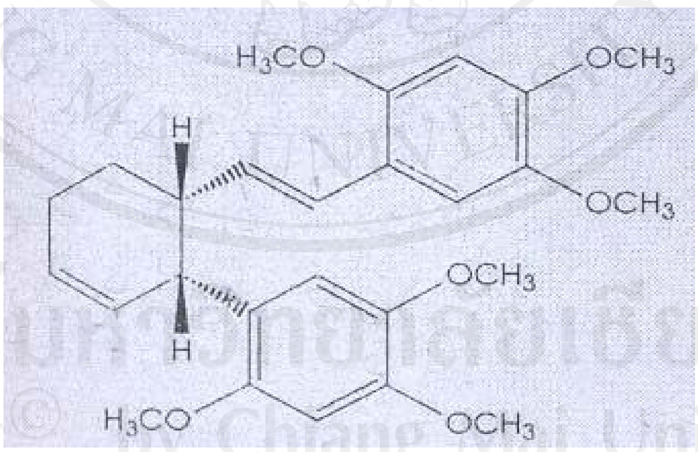

Figure 16. Structure of *cis*-3-(2', 4', 5'-trimethoxyphenyl)-4-{(E) - 2''', 4''', 5''' trimethoxylstyryl} cyclohex-1-ene (compound C) (83).

1.2.10 Objective

1. To compare the LPS-induced expression of hyaluronan synthase genes and HA synthesis between human synovial fibroblast SW982 cell line and primary human synovial fibroblast.
2. To investigate the effect of compound C on LPS-induced hyaluronan synthase genes expression and hyaluronan synthesis in human synovial fibroblast SW982 cell line.