CHAPTER II

LITERATURE REVIEWS

The immune system is a host defense system of the body against foreign antigens. This system consists of several interdependent cells and mediators that coordinately protect the body from pathogens and tumor cells. The immune responses of this system are divided into innate immunity and adaptive immunity. The innate immune response is the first line defense against invading pathogens. This response occurs rapidly within hours by the function of physical and chemical barriers as well as cells and mediators in the immune system to protect or eliminate the pathogens [6]. Cells and mediators of the innate immunity can activate or present these pathogens to both T and B lymphocytes to generate an adaptive immunity. Adaptive immunity is characterized by specificity for distinct molecules and an ability to "remember" and response more vigorously against repeated exposures to the same pathogens [7]. Macrophages are cells in the immune system that play important roles in both of innate and adaptive immune responses.

Macrophages are mononuclear phagocytes derived from pluripotent haematopoietic stem cells in the bone marrow. Their precursor cells are in myeloid lineage. They differentiate to monoblasts, pro-monocytes, and monocytes that circulate in the peripheral blood with an estimated half-life of 8-9 hours before migrating to various tissues and becoming mature cells as macrophages (Fig.1) [8]. Macrophages locating in organs and connective tissues have specific names to designate specific location such as alveolar macrophages in the lungs, histiocytes in connective tissues, Kupffer cells in the liver, microglia in neural tissue, and osteoclasts in bone [9]. Macrophages are professional phagocytes that can recognize, engulf and destroy many pathogens including bacteria, pathogenic protozoa, and fungi. They play an important role in host defense both in innate immune response and adaptive immune response and also in inflammation.

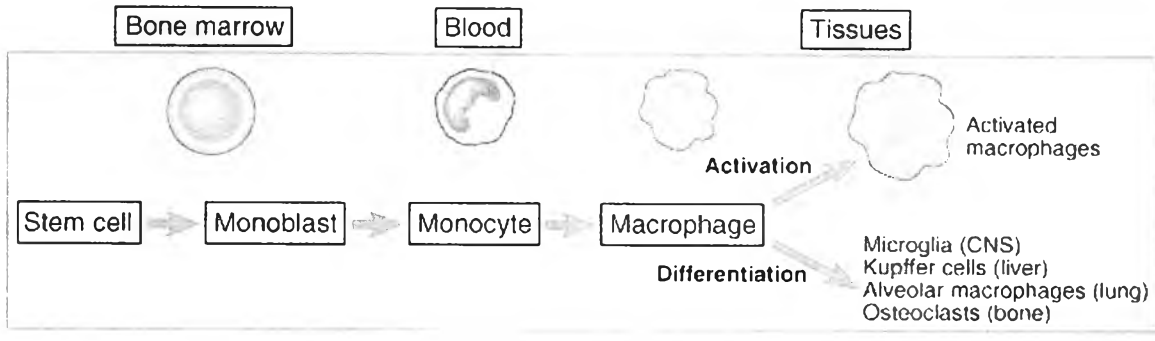

Figure 1: **Maturation of mononuclear phagocytes. Monocytes originate in the bone marrow from dividing precursor cells.** They then enter the peripheral blood, in which they leave it to become macrophages in the tissues. [10]

Recognition of microbes by macrophages

Macrophages, which broadly distribute throughout every organ, play important role innate immunity by recognizing invading pathogens and their component using a large variety of receptors called pattern-recognition receptors (PRRs). These receptors can recognize signature molecules of pathogens known as pathogen-associated molecular patterns (PAMPs), for example mannans in the yeast cell wall, formylated peptides and various bacterial cell-wall components such as lipopolysaccharide (LPS), lipopeptides, peptidoglycans and teichoic acids [11]. These molecules are considered to be a crucial component for the survival of the pathogen. PRRs are either cytosolic PRRs or membrane bound receptors on the cell surface of phagocytes as well as macrophages to mediate phagocytosis and contribute to the activation of pro-inflammatory pathways. Macrophages have several types of PRRs such as mannose receptors, scavenger receptors, receptors for opsonin, seven α-helical transmembrane/G protein-coupled receptors as well as Toll like receptors (TLRs) (Fig. 2).

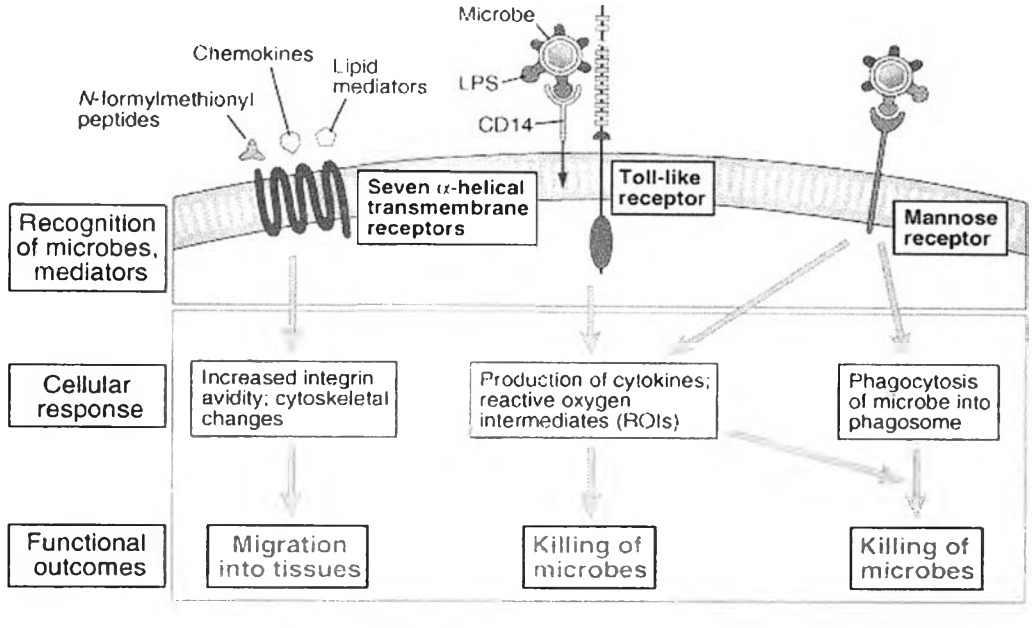

Figure 2: Receptors for recognition of macrophage [10]. Macrophages use distinct receptors to recognize various microbes, these receptors activate cellular response that function to stimulate inflammation and destroy microbes.

The mannose receptors (MR) and **scavenger receptors (SRs)** mediate phagocytosis of various pathogens by recognizing pathogen-associated molecular patterns (PAMP) of pathogens [12]. The MR is a PRR primarily present on the surface of macrophages and dendritic cells. It recognizes mannose, fucose, or *N*-acetylglucosamine which are not commonly found on mammalian glycoproteins but are often present on the glycoproteins of the surface of several pathogens [13]. This recognition leads to receptor activation and triggers phagocytosis of the pathogens. SRs mostly present on macrophages. They recognize modified forms of low-density lipoprotein (mLDL) such as oxidized LDL and a broad range of polyanionic ligands including polysaccharides and polyribonucleic acids [14]. These receptors can mediate different functions as endocytosis, phagocytosis and adhesion [15].

Receptor for opsonins, such as Fc-receptors and complement receptors that promote phagocytosis of microbes coated with a range of protein, including antibodies, complement proteins, and lectin, allowed them more visible to phagocytic cells. The

process of coating a microbe to phagocytosis is called opsonization, and substances that coat the microbes are opsonins, that aids in the process of phagocytic recognition [16-17].

The Seven α-helical transmembrane/G-protein couple receptors (GPCRs) are expressed on neutrophils, macrophage, and most other types of leukocytes, and they are specific for diverse ligand. These receptors have a function mainly to regulate the inflammatory response through stimulate adhesion and migration of the leukocyte to site of infection, also survival and activation of this cell [18]. An example of these receptors is N-formyl methionine receptors which recognize short peptides comprise *N*-formylmethionine [9]. N-formyl methionine is the first amino acid in bacterial proteins but it is not typically found in mammalian proteins Binding of *N*-formyl Met to its receptor on phagocytes promotes the motility, the chemotaxis, and phagocytosis of these phagocytes.

Toll like receptors (TLRs) are type I transmembrane receptor [19]. To date, there are 13 different TLRs identified in mammals [20]. TLRs recognize various PAMPs derived from components of the bacterial cell wall such as LPS from gram-negative (recognized by TLR4), peptidoglycan from gram-positive bacteria (recognized by TLR2) [21]. Individual TLRs recognize distinct microbial components and lead to phagocyte activation, production and release of inflammatory cytokines such as type I interferons (IFN-α and IFN-β) and proinflammatory cytokines, as well as immune response against pathogens.

Macrophage activation

Various endogenous and exogenous stimuli (e.g., phagocytosis of foreign antigen particles, cytokines from activated TH1 cells, mediators of the inflammatory response, and components of bacterial cell walls such as lipopolysaccharide (LPS)) are recognized by a wide variety of pattern-recognition receptors (PRRs) on macrophages. These stimuli can then turn macrophages into activated macrophages.

Activated macrophages have much more capacity than resting macrophages in their several functions. They increased in their phagocytic activity, ability to kill ingested microbes, synthesis of various mediators involve in host defense and inflammation including cytokines, chemokines, lipids mediators, reactive oxygen intermediates (ROI) and reactive nitrogen intermediates (RNI), ability to activate T cells, and release of various cytotoxic proteins that exert cytotoxic effects against viral infected cells and tumor cells. Activated macrophages also increase cell surface expression of class II MHC molecules, resulting them more effective as antigen-presenting cells (APCs) to activate T helper lymphocytes to initiate adaptive immune response [22].

LPS-activated macrophages

The best model for studying functions of activated macrophages in innate immunity or in inflammation is by activating the cells with bacterial lipopolysaccharide (LPS). LPS is the major component of the outer membrane of gram-negative bacteria. It is known as an endotoxin which is very potent stimulant of innate immunity in picomolar concentration [23].

LPS is a complex glycolipid that consists of three separate regions (fig. 3): lipid A, a core polysaccharide and an *O*-polysaccaride (or *O*-antigen repeats) [24-25]. Lipid A is an essential structural part of LPS that is responsible for the toxic effects of gram-negative bacteria infections. It is a potent stimulator of macrophage by activating several signal transduction pathways to promote phagocytosis and secrete various mediators including, nitric oxide, prostaglandin E_2 (PGE_2), and inflammatory cytokine such as TNF-α, IL-1, and IL-6 [26].

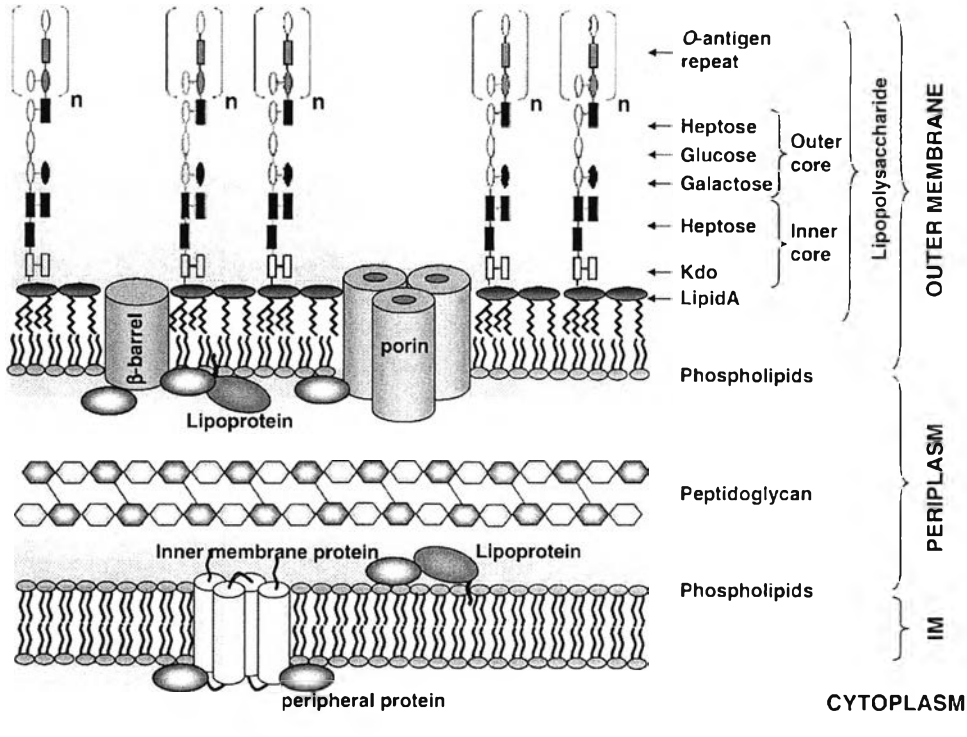

Figure 3: Structure of *Escherichia coli* cell envelope [24].

High concentration of LPS can induce over production of several inflammatory mediators that cause fever, increase heart rate, lead to septic shock and lung or kidney failure cause of death in humans [23]. The effects of LPS are mediated by interaction with toll like receptor-4 (TLR-4) [6].

Phagocytosis

Phagocytosis is the main cellular immune response of innate immunity against invading pathogens such as bacteria, parasites, as well as dead host cells by phagocytes as macrophages and neutrophils [27]. Macrophages express a variety of receptors for particle recognition such as mannose receptor and receptors for opsonins (Fc receptors and complement receptors) (Fig. 4) [28]. After binding to their particulate ligands, these receptors transmit intracellular signal to trigger phagocytosis of the particle and activate the phagocytes. The activated phagocytes destroy these internalized particles by generating several enzymes and free radicals to degrade them.

They also produce inflammatory cytokines, chemokine, as well as molecules utilized for antigen presentation to T cell [29].

Phagocytosis is a process of engulfing a foreign particles whose size exceeds about 0.5 μm. This process can be divided into 3 phases as follows: (Fig. 5)

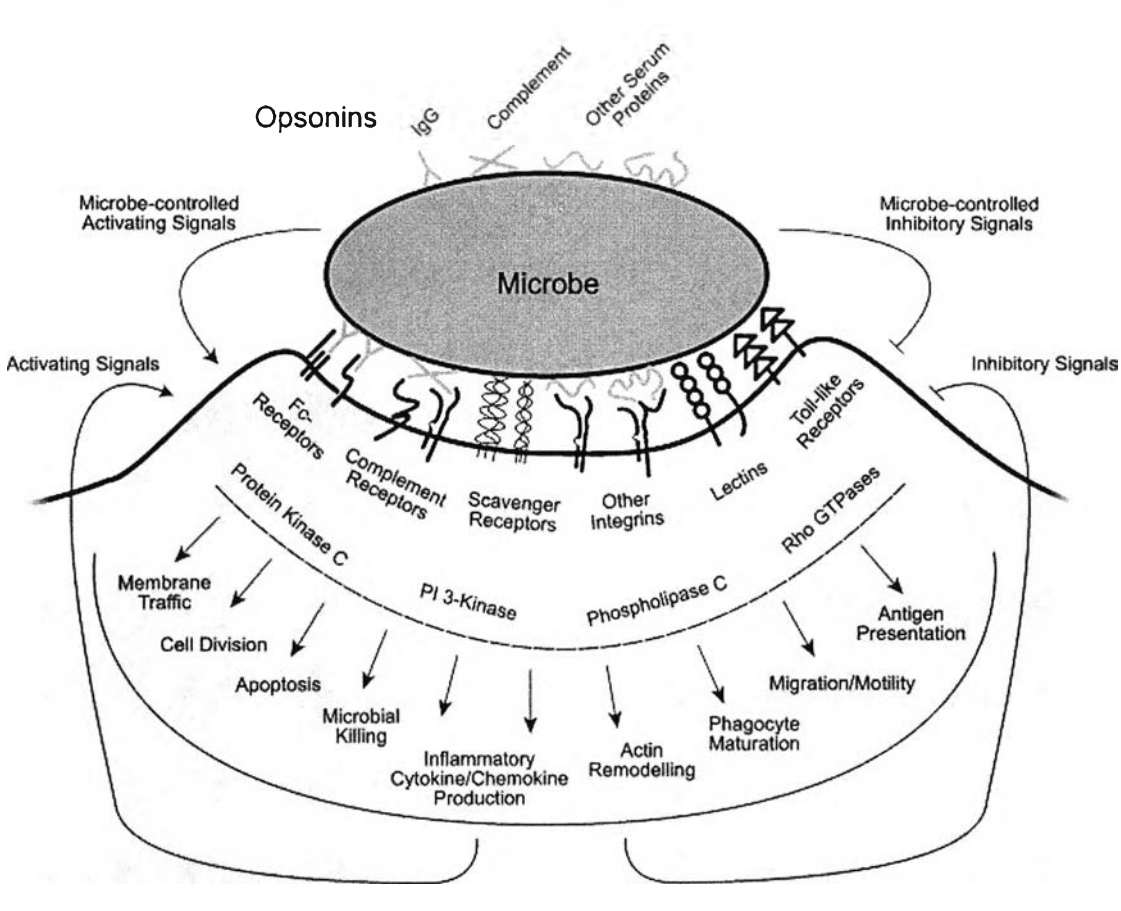

Figure 4: Receptors and signaling interactions during microbial phagocytosis of a phagocyte [29].

1) Attachment phase:

Phagocytosis is initiated by receptor recognizing of large particles such as inert beads, apoptotic cells, and microbe by PRRs of the phagocytic cells. These receptors including the mannose receptors that recognize on the surface of pathogen such as fucose residues of glycoproteins, glycolipid and mannose (e.g., β-glucan that is made

up by zymosan, which is a *Saccharomyces cerevisiae* yeas cell wall particle), the mannose receptors have been demonstrated to mediate phagocyosis of yeast and zymosan [14,29]. Opsonization which is the process of coating a particle greatly enhances efficiency in phagocytic recognition by receptor for opsonins, such as Fc-receptors that recognize antibody and complement receptors that recognize for C3 fragment.

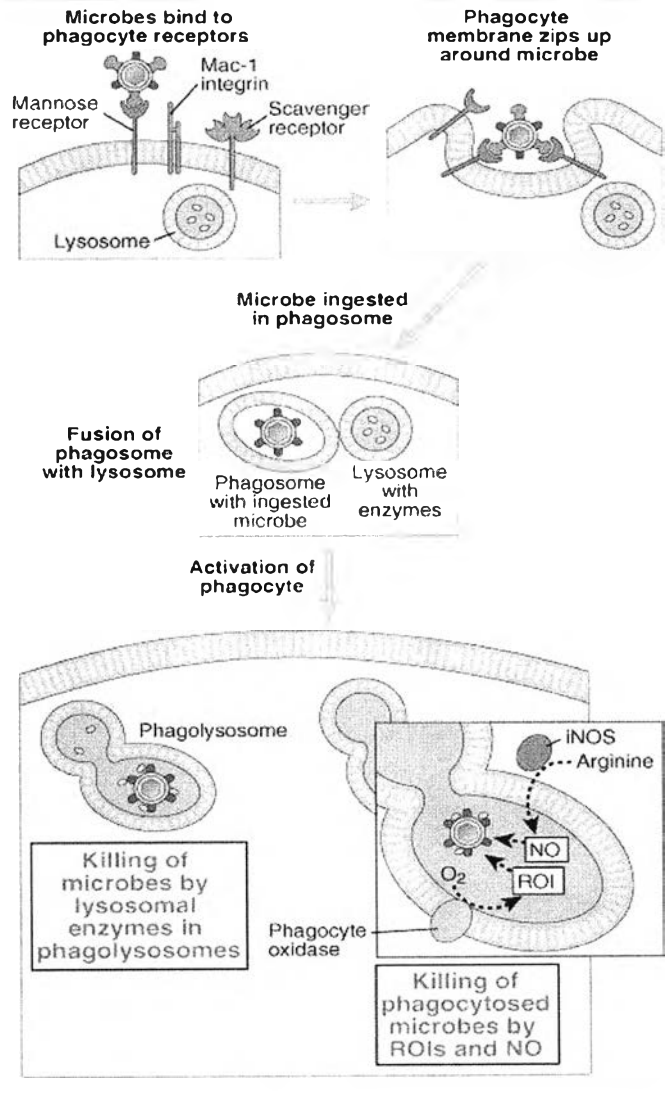

Figure 5: The process of phagocytosis and internalization of microbe [10]

2) Ingestion phase

Phagocytes extend plasma membrane called pseudopods closely to the surface of the attached particle. These pseudopods fuse around the particles and form a

phagosome. The phagosome continues to fuse with lysosome, forming a phagolysosome [29].

3) Killing and degradation phase

The killing and degradation of pathogens in the phagolysosome by enzymatic degradation after phagocytosis using either oxygen-dependent and –independent mechanisms [29]. Phagolysosomes contain microbicidal and proteolytic substances that can destroy particle without damaging the phagocytic cells. However, overproduction of these microbicidal substances by very potent stimuli may be released into the extracellular space and contribute to host tissue destruction or inflammation [30].

Oxygen-dependent degradation mechanism: In phagolysosome of activated macrophages, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase converts molecular oxygen (O_2) to reactive oxygen intermediates (ROIs), superoxide anion (O_2^-) and free radicals, which are toxic to the ingested microbes. Inducible nitric oxide synthase (iNOS) in activated macrophages generates high level of nitric oxide from L-arginine. NO at high levels is very rapidly oxidized to peroxynitrite ($OONO^-$) which induce microbial toxicity by nitrosating DNA and tyrosine residues and inducing lipid peroxidation [30-31].

Oxygen-independent degradation mechanisms: Activated macrophages also kill phagocytosed microbes in phagosome by lysosomal enzymes. These enzymes include various hydrolytic enzymes, antimicrobial peptides (e.g., defensins), and metabolic competitors (e.g., lactoferrin) [31].

The degradation of foreign proteins generates antigenic peptides in phagolysosome. These peptides are able to bind to class II major histocompatibility complex (MHC) molecules. These peptide–class II MHC complexes then presented at that the cell surface of macrophage to $CD4^+$ T lymphocytes. This leads to T cell activation to initiate an adaptive immune response [27]. Therefore, phagocytosis of phagocytes plays a role in both innate and adaptive immune responses.

Pro-inflammatory cytokines

Activated macrophages can generate a numerous cytokines involve in innate and adaptive immune responses as well as in inflammation. They are also a major source of pro-inflammatory cytokines including TNF- α, IL-1, IL-6 and IL-8. These cytokines exert a wide range of biological activities as follows;

-Tumor necrosis factor-alpha (TNF-α)

Tumor necrosis factors- alpha (TNF-α) is mainly produce by macrophages monocytes and T cells during infection and injury. It is a potent activator of macrophages to produce IL-1 and IL-6 [32]. TNF-α has a wide range of biological activities, including inflammation, cytotoxicity, tumoricidal activity either by necrosis or apoptosis induction, anti-viral activity, growth modulation, and induction of cellular differentiation [33-34]. It also plays a role in septic shock syndrome (e.g., fever and hypotension), tissue injury, cachexia, diabetes, etc. [35].

-Interleukine-1 (IL-1)

Interleukin-1(IL-1) is small protein with molecular weight of about 17.5 Kda. It is produced by monocyte, macrophages, endothelial cells, B cells, and activated T cells [30,36]. There are two forms of IL-1, IL-1α and IL-1β, with the same receptor and biological functions. Most of the IL-1 found in the circulation is IL-1β [37]. At low concentration, IL-1 functions as a mediator of local inflammation by increasing expression of surface molecules that mediate leukocyte adhesion (e.g., ligands for integrins). At high concentration, IL-1 which is released into the blood circulation can cause acute phase response. It induce fever by enhancing prostaglandin E_2 synthesis from vascular endothelium of the hypothalamus, acute protein synthesis, anorexia, somnolence, and cachexia [37-38]. IL-1 also associates with chronic inflammatory disease such as rheumatoid arthritis, and malignancies [38].

-Interleukine-6 (IL-6)

IL-6 is a glycoprotein with molecular weight 21-28 kDa [39]. It is produced by osteoblasts, monocytes, macrophages, T cells and synovial fibroblasts. The production of IL-6 is stimulated by various cytokines such as IL-1 and TNF-α, and bacterial product such as LPS [40]. It induces inflammatory symptoms (e.g., fever) and the production of acute-phase proteins from the liver similar to IL-1 [39]. Overproduction of IL-6 has been associated with acute and chronic inflammation (e.g., thyroiditis, rheumatoid arthritis), autoimmune disorder, proliferative disease, hematological disorder and bone and cartilage destruction (Fig. 6) [41,39].

IL-6 is not only a proinflammatory cytokine but also a maturation factor of B cell to become plasma cells. It also stimulates T cell proliferation and cytotoxic-T-cell differentiation [39].

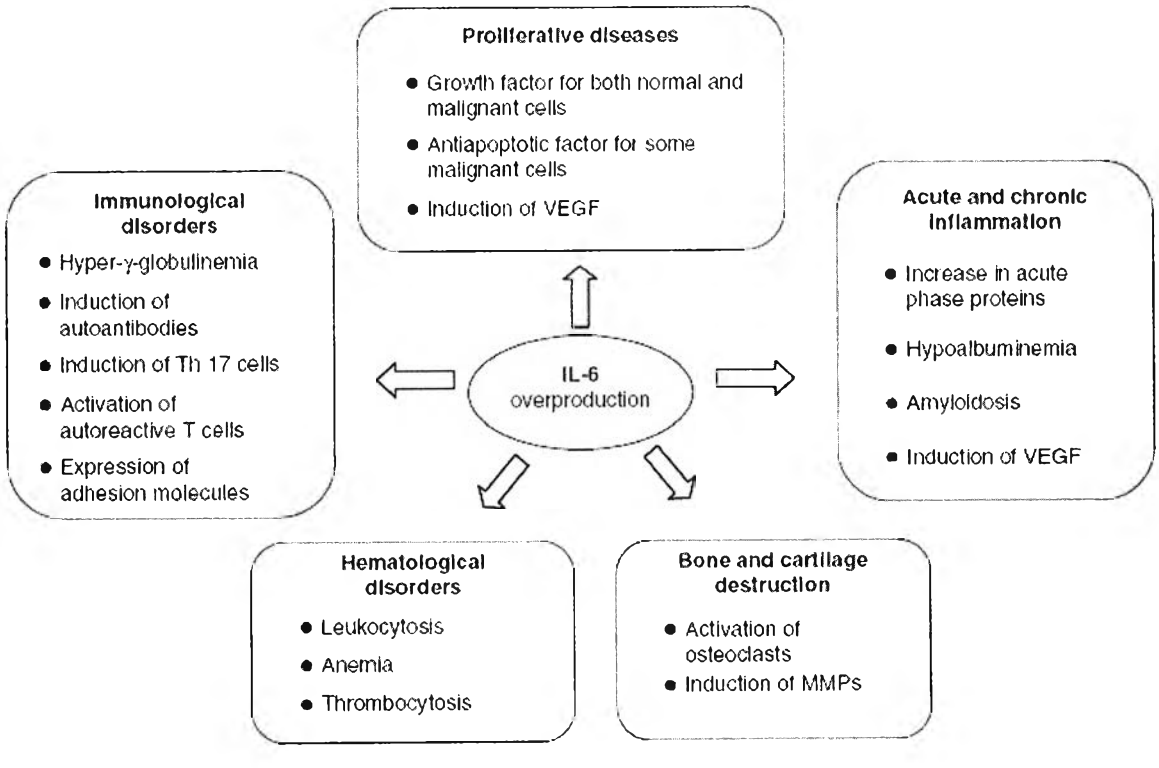

Figure 6: Overproduction of IL-6 is involved in the development of many diseases and disorders [39].

Nitric oxide (NO)

Nitric oxide (NO) is a soluble gas synthesized from L-arginine by nitric oxide synthases (NOSs). It plays essential role as a intercellular signaling molecule in several biological process. It also acts as an inflammatory mediator in inflammation. NO is synthesized from either one of three NOS isoforms, endothelial, neuronal and inducible NOSs (eNOS, nNOS and iNOS) [42].

nNOS and eNOS are constitutively produced in neuronal cells and vascular endothelial cells, respectively. They are calcium-dependent enzymes and generate low amount of NO in picomolar (pM) in short period of time for normal physiological functions (Fig. 7) [43-46]. iNOS is the third NOS isoform which is normally nor present in resting cells. It is an inducible enzyme which expresses only in activated phagocytes as macrophages and neutrophils. Pro-inflammatory cytokines (TNF-α, IL-1 and IL-6) and LPS can induce iNOS expression [47]. iNOS calcium-independently catalyzed the synthesis of NO in high concentration for a longer period of time than nNOS and eNOS (hours or days) depending on how long iNOS is present in the activated cells [47].

Production in high concentration of NO by iNOS is important as a host defense against invaliding pathogens. High concentration NO interacts with superoxide to become peroxynitrite free radical ($ONOO^-$) in activated macrophages. $ONOO^-$ is a potent cytotoxic agent. Large amount of NO acts as pro-inflammatory mediator and potent vasodilator. Overproduction of NO by iNOS can be harmful to the host tissue and associates with many inflammatory diseases such as atherosclerosis, rheumatoid arthritis, diabetes, septic shock, and multiple sclerosis [48].

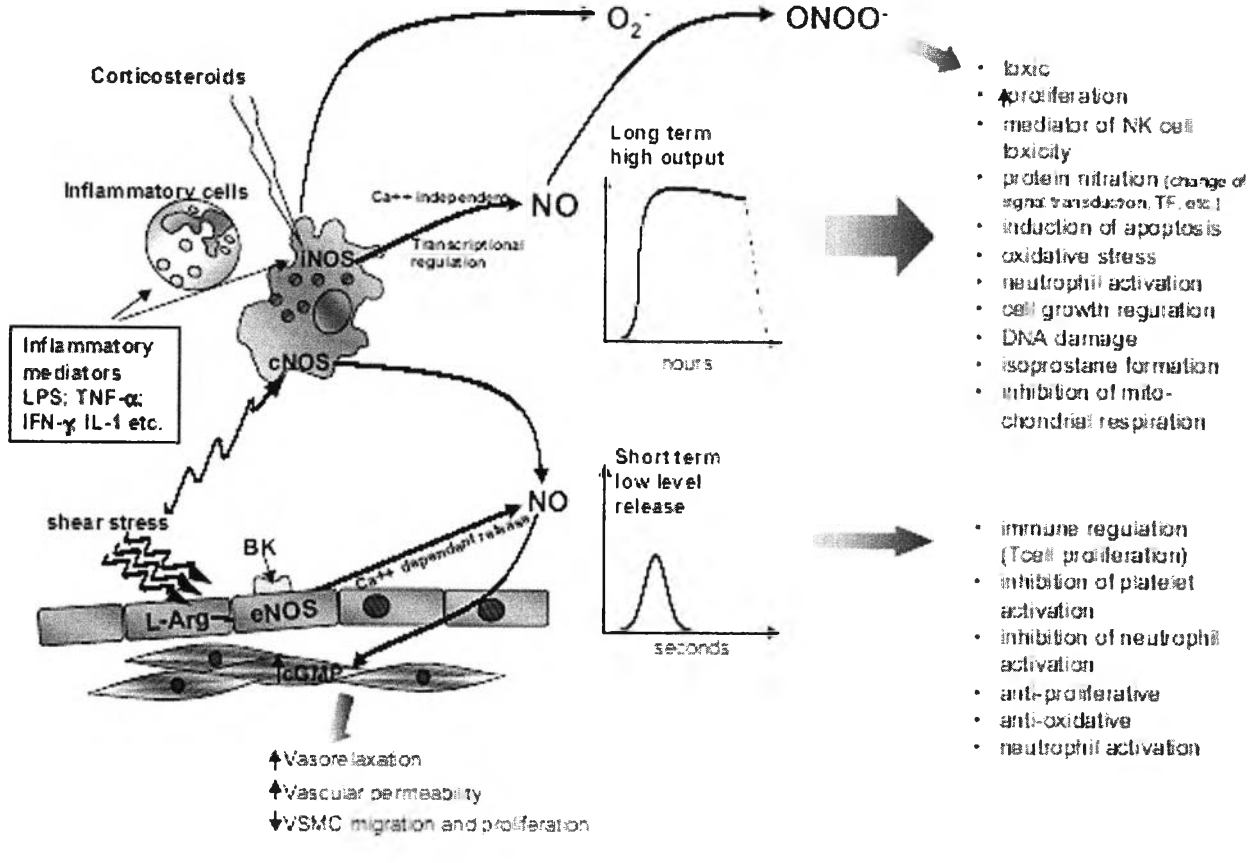

Figure 7: Effects of nitric oxide produced by constitutive or inducible nitric oxide synthase [43].

Prostaglandins (PGs)

Prostaglandins are lipid mediators derived from arachidonic acid (AA) which is a lipid component of membrane phospholipids. AA is released from plasma membrane phospholipids by phospholipase A2. It is turn into lipid metabolites by two pathways, lipoxygenase and cyclooxygenase pathways. AA is catalyzed by lipoxygenases to leukotrienes and lipoxin and by cyclooxygenases (COXs) to several prostaglandins [49]. There are two isoforms of COXs, COX-1 and COX-2. COX-1 is a constitutively enzyme that generates prostaglandins in physiological amount for normal functions of several tissues and organs [50]. COX-2 is an inducible enzyme that produces much larger amount of PGs for a longer time than COX-1 [50]. COX-2 expression is also induced in activated macrophages by various inflammatory mediators and LPS to generate large

amount of PGE_2 and $PGF_{2\alpha}$ which are potent inflammatory mediators. These PGs cause vasodilation, increase vascular permeability, induce edema and fever [8,51].

Overexpression of COX-2 has been associated with the pathogenesis of many diseases such as cancer, osteoarthritis, rheumatoid arthritis, Ischemia and Alzheimer's disease [49,52].

Inflammation

Inflammation is a fundamental process when tissues of the body response to harmful stimuli such as infection and tissue injury. There are four cardinal signs of inflammation, heat, redness, swelling, and pain. Inflammatory response is normally benefit to limit infection, causes tissue remodeling and repair, and maintains tissue integrity. However, dysregulated inflammation can cause harmful event such as septic shock from infectious inflammatory response [53].

Inflammation can be either acute or chronic inflammation. Acute inflammation is characterized by increasing blood flow and microvascular permeability along with the accumulation of fluid, leukocytes, and inflammatory mediators leading to a protein-rich fluid into the extravascular tissue to forming edema with pain [54]. After tissue injury, several inflammatory mediators, including chemokines, cytokines, vasoactive amines and eicosanoids are produced by endothelial cells, mast cells, macrophages and dendritic cells. These mediators play important roles in the inflammatory process to induce fever, vasodilation, synthesis of acute phase proteins, and leukocytosis [41]. Chronic inflammation develops from acute inflammation if the injurious stimuli still persist for a long time in weeks, months, or years [41].

Activated macrophage play a key role in chronic inflammation. They chronically produce many pro-inflammatory cytokines (TNF-α, IL-1 and IL-6), free radicals as superoxide and hydrogen peroxide, NO, and PGs which result in clinical symptoms of chronic inflammation such as anorexia, cachexia, fever, leukocytosis, and progression to diseases such as rheumatoid arthritis and atherosclerosis [41,55].

Anti-inflammatory drugs

Treatment of both acute and chronic inflammatory disorders is mostly by interrupting the synthesis or action of inflammatory mediators. Two major groups of anti-inflammatory drugs are steroids and nonsteroid anti-inflammatory drugs (NSAIDs).

Steroid drugs

Synthetic steroids such as dexamethasone, betamethasone, fludrocortisone, triamcinolone and prednisolone are derived from glucocorticoid hormone from adrenal cortex [56]. They are potent, highly effective anti-inflammatory drugs and widely used for treatment of several chronic inflammatory diseases such as asthma, chronic obstructive pulmonary disease (COPD), rheumatoid arthritis, inflammatory bowel disease and various autoimmune diseases [57].

Mechanisms of inflammatory actions

Steroid drugs can penetrate plasma membrane to bind to their cytosolic receptor in the cytosol and form steroid-receptor heterodimer complex. This complex acts as a transcription regulator by binding to a glucocorticoid response element (GRE) on the regulatory sequences of several genes and leading to either inhibit or stimulate these gene transcriptions. It inhibits expression of several pro-inflammatory cytokines and adhesion molecules but stimulates the expression of lipocortin-1 which blocks phospholipase A2 to release arachidonic acid from phospholipids.

Anti-inflammatory actions of steroids (Fig. 5)

-Increase expression of anti-inflammatory proteins

Steroid drugs suppress inflammation by increasing the synthesis of anti-inflammatory proteins. They increase the synthesis of lipocortin-1 which inhibits phospholipase A_2 (PLA_2) activity, resulting in inhibit the production of arachidonic acid. They promote the production of other anti-inflammatory proteins including IL-1 receptor antagonist which inhibits the binding of IL-1 to its receptor, secretory leukoprotease inhibitor which inhibits degradation of an inhibitor of NF-kB (IkB-α), and IL-10 which is a inhibitory cytokine [58].

-Decrease expression of pro-inflammatory proteins

Steroid drugs inhibit the transcription of many cytokines, chemokines and adhesion molecule that associated with inflammation response [58]. They also inhibit expression of iNOS and COX-2 which generate NO and PGs, respectively [58].

-Inhibit cell migration

Steroids inhibit migration of inflammatory cells such as macrophages and neutrophils to accumulate at the site of inflammation by decrease the expression of adhesion molecules involve in leukocyte migration. The anti-inflammatory effect of steroid by inhibition of these cells accumulation at the site of inflammation, thus reducing the acute sign of inflammation [58-59].

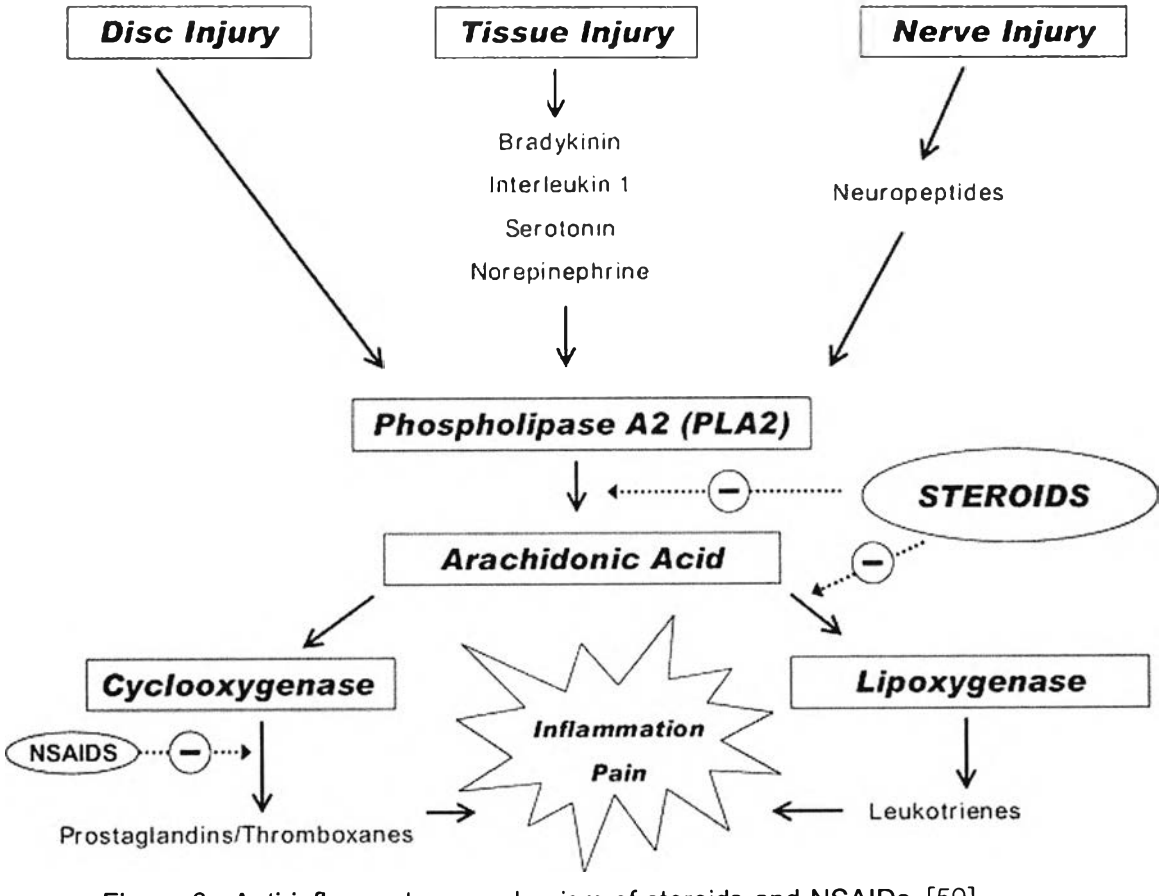

Figure 8: Anti-inflammatory mechanism of steroids and NSAIDs. [59]

Adverse effects of steroid drugs

Steroids cause several local and systemic side effects as presented in Table 1.

Table 1: Systemic and local side effects of steroids [59].

Endocrine	Adrenal suppression, hypercortisolism, cushingoid syndrome, hyperglycemia, precipitation of diabetes mellitus, immunosuppression, hypokalemia, amenorrhea, menstrual disturbances, growth retardation
Metabolic	Hyperglycemia, glucosuria, redistribution of fat, negative nitrogen balance, sodium and water retention
Cardiac	Hypertension, fluid retention, CHF, DVT
Musculoskeletal	Osteopenia/osteoporosis, avascular necrosis of bone, pathologic fracture, muscle wasting and atrophy, muscle and joint pain
Psychological	Mood swings, insomnia, psychosis, anxiety, euphoria, depression
Gastrointestinal	Ulcerative esophagitis, hyperacidity, peptic ulceration, gastric hemorrhage, diarrhea, constipation
Ocular	Retinal hemorrhage, posterior subscapular cataracts, increased intraocular pressure, exophthalmos, glaucoma, damage to optic nerve, secondary fungal and viral infection
Dermatologic	Facial flushing, impaired wound healing, hirsutism, petechiae, ecchymosis, hives, dermatitis, hyperpigmentation, hypopigmentation, cutaneous atrophy, sterile abscess
Nervous System	Headache, vertigo, insomnia, restlessness, increased motor activity, ischemic neuropathy, seizures
Other	Epidural lipomatosis, fever

Non-steroid anti-inflammatory drugs (NSAIDs)

NSAIDs are anti-inflammatory agents which inhibit COXs. Most of them inhibit both COX-1 and COX-2 activities. There are two classes of NSAIDs, non-selective COX inhibitors and selective COX-2 inhibitors.

Non-selective COX inhibitors such as aspirin, ibuprofen and indomethacin, inhibit both COX-1 and COX-2 enzyme [60]. The major problem of drugs in this class is gastrointestinal tract irritation and ulceration of the stomach lining. These drugs inhibit COX-1 which synthesizse cytoprotective prostaglandins in the stomach [61].

Selective COX-2 inhibitors such as celecoxib, etocoxib and lumiracib, selectively inhibit COX-2 enzyme. Drugs in this class have been replaced nonselective COX inhibitors to reduce GI side effects, but they are found to increase the risk of cardiovascular side effects [62].

Other anti-inflammatory agents

Cytokine inhibitors are the other group of anti-inflammatory agents used to treat chronic inflammatory diseases such as rheumatoid arthritis. They inhibit pro-inflammatory cytokine activities. Anakinra is a recombinant human IL-1 receptor antagonist (IL-1ra) which acts as a decoy receptor to neutralize IL-1. There are also two types of TNF-α inhibitors which are also used to treat rheumatoid arthritis. They are

soluble TNF-α receptor (etanercept) and monoclonal antibodies against TNF- α (adalimumab and infliximab) [9]. The problems of these cytokine inhibitors are their side effects which include risk of infection, cancer, autoimmune diseases, parenteral routes of drug administration and high cost.

Derris reticulata Criab

Development of the novel anti-inflammatory agents is still in need in order to lessen the adverse effects and to maximize the therapeutic effect. Natural products from plants are one of the major sources for the drug development. Anti-inflammatory agents derived from medicinal plants make a large contribution to drug discovery up to now.

There are many traditional plants in Thailand that have been investigated for anti-inflammatory activity. The chloroform extract of a red rhizome of *Boesenbergia pandurata* showed anti-inflammatory activity in the TPA-inducer ear edema assay in rats [4]. The butanol extract of *Vitex peduncularis* Wall. inhibited both COX-1 and COX-2 enzyme in murine macrophage cells [4]. *Streblus asper* Lour or Koi in Thai inhibited iNOS and COX-2 expression in macrophages cells and inhibition of edema in carrageenan-induce paw edema in rats [63]. *Rhinacanthus nasutus* Kurz or Thong-pan-chung in Thai inhibited iNOS and COX-2 expression in macrophage cells [64]. Several medicinal plants in Thailand are still unexplored for their pharmacological properties which may be useful for clinical application in the future.

Derris reticulata Craib or "Cha-aem-nuea" is a plant in the Leguminosae family that widely distributes throughout Thailand. It is a climbing plant, alternative pinnately compound leaves, elliptic leaflet and acute, bouquet of flowers from stem, white-yellow petal, silique fruit, and solid seeds. In Thailand, the stem and root of *D. reticulata* are used as a sweetening agent, an expectorant, an anti-tussive, a remedy for throat diseases and as a tonic agent [3].

Figure 9: *D. retuculata* Craib

It has been identified that *D. reticulata* contains flavonoids as its major active compounds. These fiavonoids include lupinifolin, lupiwighteone, 2, 3-epoxylupinifolin, dereticulatin, 1-hydroxy-2,3-epoxylupinifolin, 4,5-dihydroxy-8-hydroxymethyl-6,6-dimethylpyrano [2,3:7,6] flavanone, and 2, 3-dihydroxylupinifolin [3,65]. Very few pharmacological activities of these flavonoids have been reported. Lupinifolin has been evaluated to inhibit Epstein–Barr virus early antigen activation induced by 12-O-tetradecanoylphorbol-13-acetate in Raji cells, without cytotoxicity, and demonstrate chemoprotective effect on mouse skin tumor promotion in vivo [66]. Pharmacological activities of the chemical constituents of plants in the genus *Derris* have been reported as follows:

Rotenoids are flavonoid glycosides from the root of *D. elliptica* have been used as fish poisons and insecticides [67]. Pomiferin is a flavonoid from *D. malaccensis* showed strong anti-fungal, anti-oxidant activities, and also cytotoxicity towards human cholangiocarcinoma cells (HuCCA-1) [65]. Isoflavonoids from stem of *D. scandens* had anti-inflammatory effect on inhibition production of eicosanoids and antibacterial effect both of gram-positive and negative bacterials [68]. Tannin, saponin, and triterpenoids isolated from *D. elliptica*, *D. indica*, and *D. trifoliata* have been found to have a broad spectrum of antibacterial activities [69].