FREE RADICAL, OXIDATIVE STRESS AND ITS ROLES ON INFLAMMATORY RESPONSE

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Abstract: Chronic inflammation contributes to the occurrence of various metabolic diseases and even cancer. *Chronic inflammation results from excessive response of free radicals. Free radicals are triggered by various agents and oxidation processes in the body as Reactive Oxygen Stress (ROS). The high level of free radicals (oxidants), without adequate intake of antioxidants will lead to oxidative stress conditions. Oxidative stress triggers persistent of inflammation. The formation of ROS is difficult to avoid because it can be generated from cellular metabolic processes. Therefore, we should intake adequate of antioxidants and avoid the various agents induces ROS in everyday life.*

Keywords *: ROS, Stress Oxidative, Inflammation*

INTRODUCTION

Inflammation describe as complex reaction occur when the body fight against infection, microbial invasion or tissue injury.[1] When inflammation occurs continuously lead to chronic condition. Chronic inflammation contributes to the occurrence of various metabolic diseases and even cancer. Chronic inflammation results from excessive response of free radicals as Reactive Oxygen Stress (ROS). ROS are a very reactive oxidizing agent and included of free radicals.[2] The body's physiological processes produce Reactive Oxygen Species (ROS) as a result of normal cellular metabolism.[3] Oxidation reactions are essential for some organisms to produce energy and fuel for biological processes, but uncontrolled oxygen production will form free radicals.[4]

The condition of high free radicals that failed to overcome by competent cells, will cause oxidative stress. Oxidative stress is a term used to describe the imbalance between free radicals and the ability of cells to detoxify, resulting in the destruction of proteins, lipids and DNA.[5] This condition could further potentially trigger some diseases such as cancer, rheumatoid arthritis, cirrhosis and atherosclerosis, as well as aging-related degenerative processes.[4]

Excessive free radical production induce to an inflammatory response characterized by mononuclear cell infiltration, tissue destruction and fibrosis. Chronic inflammation lead to pathological conditions.[6] The inflammatory process that occurs as a initiators of pathological conditions due to excessive free radical, will be reviewed in this article

A. FREE RADICALS

Free radicals are defined as unpaired electrons, paramagnetic in nature, unstable and highly reactive. There are many types of radical, but most of them derived from oxygen that known as Reactive Oxygen Species (ROS). In the field of biology, the main free radical types are divided into Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS).[7] Under certain stress conditions, oxygen becomes more active and forms ROS, superoxide radical anions (O_2^-), hydroxyl radicals (OH^-), peroxyl radicals (ROO^-), and non-free radical species such as H_2O_2, singlet oxygen ($_1O^2$)[8]. The most important RNS are nitric oxide (NO), nitrogen dioxide (NO_2), and peroxynitrite anions.[6]

Free radicals formed by reactive oxygen or nitrogen produced during body defense activities, and are produced from several environmental factors such as pollution, cigarettes and sunlight.[4] Free radicals come from both endogenous and exogenous sources. Endogenous free radicals can be derived from immune cells activation, inflammation, response of stress, excessive exercise, ischemia, infection, cancer and aging.[9]

The cellular source of ROS production comes from the phagosome of immune cells that involved in the killing of pathogens and peroxisomes that facilitate catabolic oxidation reactions. The three sources involved in the formation of ROS in cells are the endoplasmic reticulum, cell membranes and mitochondria.[10] ROS has function in immune defense, as described by Warnatsch *et al* (2017) cybb-deficient mice that lack of microbicidal phagocytes has impaired phagocytes and pathogen removal due to lack of ROS.[11]

Meanwhile high levels biomarkers of oxidative damage correlate with a higher risk of disease.[12] Reactive Oxygen Species (ROS) is a term used for the oxidants groups as well as free radicals. The formation of intracellular ROS consists mainly of superoxide radicals (O_2^-) and nitric oxide radicals (NO). Under normal physiological conditions, about 2% of the oxygen consumed by the body will be converted to O_2 through mitochondrial respiration, phagocytosis.[8] ROS can be interpreted as a more reactive and potentially oxygen-containing molecule than the oxygen molecule itself.[10]

Exogenous free radicals are the result of air and water pollution, tobacco smoking, alcohol consumption, exposure to heavy metals and certain drugs (cyclosporine, takrolimus), industrial solvents, cooking and radiation processes.[12] While several types of ROS sources from the environment are pesticides and chemicals in the environment. Pesticides are toxic because of their ability to form free radicals, subsequent peroxidation of biomolecules and changes in scavenger enzymes.[5] According to Bierben *et al* (2012) there are several sources of ROS from outside the body such as ozone exposure, hyperoxia, and ion radiation.[3] Tobacco cigarettes contain some oxidants and free radicals as well as organic compounds such as superoxide and nitric oxide. Inhaling tobacco cigarettes into the lungs can activate several endogenous mechanisms such as the accumulation of neutrophils and macrophages which can further increase oxidant damage. ROS can also be formed during UV light radiation, X-rays, and γ rays, produced during the reaction of metal catalysts, formed as pollutants in the atmosphere.[13]

According to Schumacker (2015) elevated of oxidants have been implicated to cancer, and ROS affect phenotypic behavior of cancer cells related to response of therapy. High level of ROS possible to damaging cellular process by attacking proteins, lipids, and DNA lead to pathological effects. Balancing of higher level of ROS can be helped with antioxidants.[14]

Antioxidant is a molecule that has ability to delaying or preventing the oxidation of other molecules. It consist of enzyme and non enzyme antioxidant. All cells in the body contain antioxidant enzyme that classified as endogenous antioxidant. Three major antioxidant enzymes are superoxide dismutases, catalases, and glutathione (GSH) peroxidases. Non enzymatic antioxidants mostly found in dietary or food (exogenous antioxidants), including ascorbic acid or vitamin C, glutathione, tocopherols and tocotrienols or vitamin E, and beta-carotene.[15]

Role of antioxidants is to neutralize and protects the cells against free radicals. Defense system of antioxidants by blocking the intial production of free radicals, scavengering oxidants, converting oxidants to less toxic compounds, blocking the secondary products of toxic metabolites or inflammatory mediators, blocking chain propagation of econdary oxidants, repairing the molecular injury induced by free radicals and enhancing endogenous antioxidants to defense of free radical.[15] Antioxidants are potent because they donate their own electrons to ROS so that neutralizing the negative effects latter. Antioxidants perform in three roles : a). Prevention by keeping formation of ROS to a minimum level for example desfferioxamine; b). Interception by scavenging ROS either by catalytic and non calatytic molecules for example ascorbic acid, alphatocopherol; c). Repair by repairing damaged target molecules for example glutathione.[8]

B. REACTIVE OXYEN SPECIES (ROS)

Reactive Oxygen Species are produced from oxygen molecules as a result of normal metabolism or called endogenous ROS. ROS is divided into two groups, namely free radical and non radical. The molecule contains one or more unpaired electrons then provides reactivity to a molecule called free radical.[3]

1. Source of Endogenous ROS

Low level of ROS is essential to physiological function such as gene expression, cellular growth and defense against infection, either as the stimulating agents to biochemical processes within the cell.[8] ROS also produced from immune response such as phagosytosis, inflammation, H_2O_2-MPO Halide system, and prostaglandine production.

Source ROS from inflammation

When two free radicals share unpaired electrons, a non-radical compound is formed. Superoxide anions (O_2^-) are formed by the addition of an electron to an oxygen molecule mediated by nicotine adenine dinucleotide phosphatase (NADPH) oxidase or xantine oxidase or via an electron transport system in the mitochondria. (NADPH) oxidase is found in polymorphonuclear leukocytes (PMN), monocytes, and macrophages. Those cells are potential cells as phagocytes.[3]

Acute inflammation begins with recruitment of immune cells especially PMN to the site of inflammation, further these cells engulf the invading pathogens known as phagocytosis. ROS generated by PMN at the site of inflammation then junction between endothelial cells are opening. At inflammatory sites, PMN is found to be abundant. NADPH oxidase indentified in phagocytes and endothelial cells which are central to inflammatory response. NOX1, NOX2 and NOX4 are the major isoforms of NADPH oxidase that are expressed in the vascular system and implicated in inflammation. NOX1 activity was increased in endothelial cells on angiogenesis stimulation.[16]

Source ROS from Phagocytosis system

Phagocytosis is the physiological event to defense from the pathological cells or non-self materials. Monocytes, neutrophils and dendritic cells are the professional phagocytes, whose numerous molecular bactericidal mechanism. Leukocytes increase consumption of molecular oxygen during phagocytosis, in a process reffered to as respiratory/ oxidative burst.[17] Respiratory burst is mediated by NADPH-oxidase followed by neutrophils activation then produces O_2^- and hydrogen peroxide (H_2O_2).[18] Respiratory burst of phagocytes as part of the mechanism bacteria and viruses elimination and denatured of foreign antigens.[19] When the phagocytosis progresses, ROS are produced by NOX in the small level of the phagosome. The major oxidant produced by Myeloperoxidase (MPO) is hypochlorous acid (HOCl), HOCl can attack any oxidizable group. It induced peroxidation of polyunsaturated lipids that belong to pathogens. Oxidation by HOCl will generated protein chloramines, which have cytotoxic actions, but also oxidize and inactive granule proteases. The reaction has been suggested enhance microbial killing.[20] Production of ROS in appropriate levels has been suggested to phagocytosis activation.

Source ROS from H2O2-MPO-Halide system

Microbicidal activity in phagocytes related to toxic oxygen-derived products such as Hydrogen peroxide (H_2O_2), HOCl, chloramines and $^*OH$. HOCl derived from H_2O_2. Oxidative modification by H_2O_2/MPO/halide system play role in inflammatory response.[21] Hydrogen peroxide (H_2O_2) can easily spread through the plasma membrane. Hydrogen peroxide is also produced by xantine oxidase, amino acid oxidase and NAD(P)H oxidase. The hydroxyl radical (OH^-) is formed from O_2^- which reacts with H_2O_2, where OH^- this is the most reactive ROS and is capable of destroying proteins, lipids and carbohydrates, as well as DNA. In addition, OH^- can play a role in initiating lipid peroxidation by taking electrons from polyunsaturated fatty acids.[13] O_2^- and H_2O_2 can damage a various biomolecules, also damage other enzymes lead to metabolic defects including auxotrophy for aromatic, branched-chain, and sulfur-containing amino acids. These reaction affect to damages biological molecules such as DNA, or even carbonylation of protein, in turn can eradicate microbial.[22]

Source ROS from prostaglandin

Production of ROS can derived by plasma membrane oxidation. Plasma membrane is site that generally exposed to

oxidizing environment. Free radicals can synthesized during changing arachidonic acid into its products, including prostaglandins.[23] The other endogenous sources of ROS include prostaglandin synthesis. Isoprostanes is the prostaglandin like substances synthesized by esterification of arachidonic acid.[24] There are many enzymes that produce ROS, such as NADPH oxidase, xanthine oxidase, mitochondrio, or even cyclooxygenase. Arachidonic acid is oxidized by cyclooxygenase 1(COX-1) or cycooxygenase 2(COX-2) into prostaglandin G2 (PGG2) and prostaglandin H2(PGH2) in cyclooxygenase pathway.[25]

Free radicals derived from oxygen are peroxyl radicals (ROO·*). The simplest form of the radical is a hydroperoxyl radical (HOO·*). Free radicals can trigger the lipid peroxidation chain reaction by separating the hydrogen atom from the methylene carbon side chain. The lipid radical then reacts with oxygen and produce peroxyl radicals, which initiate chain reaction and convert polyunsaturated fatty acids into lipid hydroperoxides. Lipid hydroperoxides are highly labile and readily converted into secondary products such as aldehydes and malondialdehyde (MDA).[3]

2. Role ROS in Physiological Events

According to Kunwar and Priyardarsini (2011), at low levels, ROS has normal physiological functions such as gene expression, cell growth, and defense against infection, sometimes ROS also acts as an agent that stimulates biochemical processes in cells.[8] ROS can also induce transcription factors, one of which is Mitogen Activated Protein Kinases (MAPKs). MAPKs are serine-threonine protein kinases that involved in signal transduction from cell surface to nucleus.[26] It can also act as a secondary messenger at developmental stages, prenatal and embryonic development in mammals, molecular biosynthesis such as thyroxine, prostaglandins, ROS are also required in the immune system, and play a role in inflammation.[8]

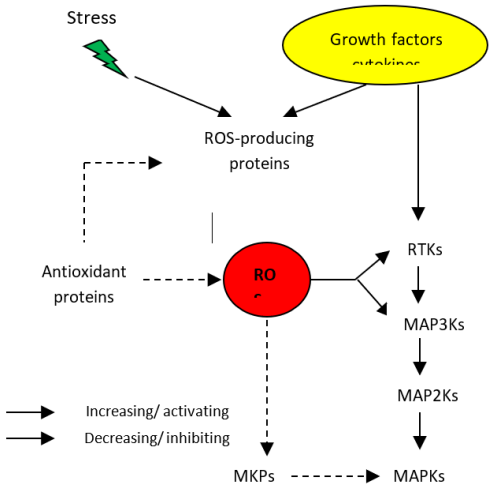

Figure 1. Activation of MAPKs Pathways mediated by ROS.[26]

ROS normally activated by various factors for examples growth factors, cytokines, and stress that can be removed by endogenous antioxidant. High leve of ROS than antioxidant capacity may induce oxidative modification of MAPK signling protein such as Receptor Tyrosine Kinases (RTKs) and MAPK kinase kinase (MAP3Ks) then affect to activation of MAPK.[27]

3. Role ROS in Immune System

ROS contribute to defense immune system both innate and adaptive responses. In pathogenic exposure, excessive ROS production as part of oxidative burst in phagocytic cells. Those accumulate in the inflammatory region as innate response to pathogen. Furthermore, ROS will be involved in adaptive immune responses because abundant production of ROS will continue by phagocytes, then increase the intracellular transduction cascade in T lymphocytes, consequently decreasing the activation threshold.[28]

The immune system and ROS are closely related. Based on several studies stated that ROS as a regulator in the immune system. There are three different ways which ROS affect the inflammatory responses (1) ROS derived from metabolic processes and ROS from neutrophils will induces inflammation. (2) T lymphocyte activity will be significantly decreased by monocytes associated with the presence of ROS. And (3) inflammation without T cell involvement can be derived activity by NOX2 derived from ROS.[16]

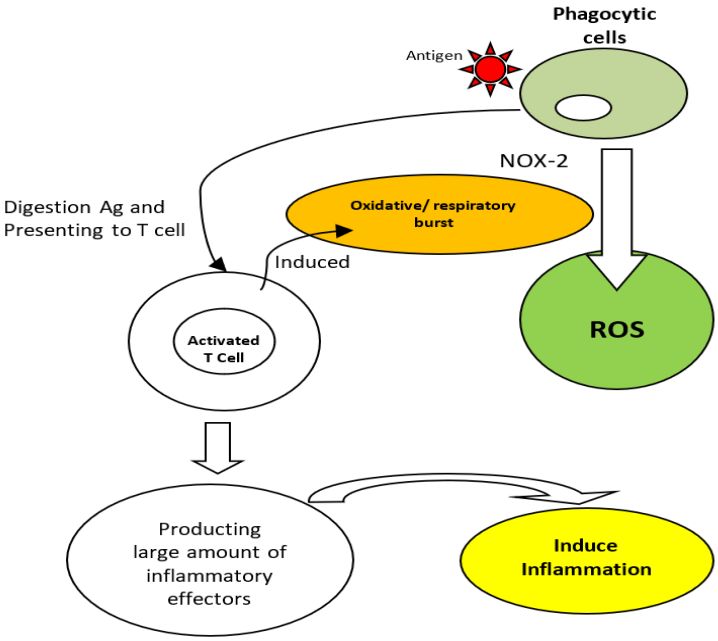

Figure 2. Production ROS Enhance Immune System.[26]

Exposure of antigen will induce phagocytes and lead to oxidative burst. Production of ROS derived from oxidative burst and phagocytosis as first line of immunity. Since fragment of pathogens might escape then may produce larger number of pathogen. Adaptive immunity may activated by phagocytic cell that engulf extracellular antigen, then presenting to T cells. This action affect to

activated T cells, producing large amount of inflammatory effectors, and induce oxidative burst then induce inflammation.[26]

4. ROS induces oxidative damage

Depending on its nature, ROS reactions with biomolecules such as lipids, proteins and DNA, produce some secondary radicals such as lipid radicals, sugar-derived radicals, amino acid radicals and tinyl radicals. Cell membranes are highly susceptible to damage and oxidation by ROS due to the high concentrations of unsaturated fatty acids and their lipid components.[8]

According to study from Wang *et al* (2013), lipid peroxidation can induced by production of ROS generated from toxic effect of lead administration to male rats. This condition lead to oxidative stress that induces significant changes in biochemicals parameters in serum.[30] Proteins in the body can suffer direct and indirect damage after interacting with ROS resulting in peroxidation, altered tertiary structures, proteolytic degradation, cross-linking of proteins and protein fragmentation. Another effect of ROS is it can interact with DNA and cause some types of DNA damage, such as DNA-base modification, single or double rupture of DNA, loss of purine, deoxyribose sugar deficiency, DNA-protein cross-linking and damage to DNA repair systems. Radicals hidroxyl (OH) are one inducer of DNA damage. Free radicals can produce peroxyl radical and subsequently can break double helix of DNA. The consequences of DNA damage include changes in genetic material resulting in cell death, mutagenesis, carcinogenesis and aging.[8] When ROS production exceeds to cellular antioxidant capacity it will continue to oxidative stress.[7]

C. OXIDATIVE STRESS

The term oxidative stress is a condition that illustrates the imbalance between free radicals and the ability of cells to detoxify free radicals resulting in the destruction of proteins, lipids and DNA.[5] According to Birben et al (2012), a change in the balance between oxidants and antioxidants is called oxidative stress.[3]

Some biomarkers for oxidative damage can be detected, moreover response of time to oxidative stress can predicted. Some biomarkers exhibit acute and chronic oxidative stress due to smoking, such as F2-isoprostanes that increase levels after smoking, allantoin is a biomarker produced by oxidative breakdown of urate.[12]

Oxidative stress contributes to several pathological conditions and diseases including neurological disorders, hypertension, ischemia / perfusion, diabetes, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, acute pulmonary disease, and asthma.[3] This condition could further potentially trigger some diseases such as cancer, rheumatoid arthritis, cirrhosis and artheroskeloris, aging-related degenerative processes.[4] According to Jyoti *et al* (2016) uncontrolled long-term oxidative stress will have implications for some inflammatory diseases such as hepatitis, stroke, retinal damage arthritis.[7] ROS is generally formed by inflammatory cells not only to help destroy pathogens, but also affect the inflammatory cell itself, altering the intracellular redox balance of in the cell and functioning as a molecule that gives signals in the regulation of inflammatory and immunomodulatory genes.[29]

D. INFLAMMATION

Inflammation is a host defense and immune response against foreign pathogens. There are 4 signs (Cardinal signs) that occur during acute inflammation such as heat (calor), redness (rubor), swelling (tumor), and pain (Dolor), and initiate loss function of tissue.[16] Inflammatory response is a natural immune response to infection and injury.[32]

Essentially, inflammation is an important mechanism for the destruction of intruder factors and the restoration of tissue structures. The acute phase of inflammation involves influx of granulocyte, neutrophils and mature monocytes or inflammatory macrophages.

Various mediators are released during inflammation. In the inflammatory process, mediators such as proinflammatory cytokines TNF-α, IL-1β and Vascular Endothelial Growth Factor (VEGF) plays an important role in this process. Prostaglandin is also the mediator of inflammatory molecules.[33]

In general, inflammation is a normal protective response to various cell and tissue damage, to destroy and eliminate injured or harmful cells and tissues, then continue to repairing of tissue. However, uncontrolled inflammatory responses will cause excessive cell and tissue damage, destruction of normal cells because chronic inflammation, implicate to various inflammatory diseases in humans, which is due to prolonged oxidative stress.[10]

Polypeptides known as cytokines are produced in response to inflammation, most of these cytokines have many functions. There are pleiotrophic molecules that have local or systemic effects. Among them IL-1, TNFα, IL-6, IL-11, IL-8 and chemokines, GCSF, GM-CSF play a role in meditating acute inflammatory reactions.[7] An important inflammatory process in the elimination of various pathogens is played by the neutrophil cell. These neutrophil recruitment are regulated by pro-inflammatory cytokines such as interleukin 1β (IL-1β). Some studies suggest there is a relationship between neutrophil presence and IL-1β production during bacterial infection, which means that neutrophils may play a role in modulating inflammation. When compared with other phagocyte cells, neutrophils produce higher ROS concentrations.[11]

According to Jyoti *et al* (2016), PMN recruitment including neutrophil, lymphocyte recirculation and monocyte involves adhesion and transmigration through blood vessel walls. Inflammatory responses are initiated by infiltration of leukocytes in the area of infection rapidly, then swallowing and attacking pathogens. During the inflammatory process, PMN will roll slowly through the blood vessel wall mediated by L, P and E selectin. Some cytokines and adhesion molecules such as TNF-α, IL-1 and VCAM-1 mediate this process.[7]

1. Acute inflammatory stage

Inflammation in this early stage normally begins with the nearest localized area, but depends on the severity of the infection or injury. Based on the time of inflammation, in the early stages or acute inflammation is the initial response to dangerous agents and recover quickly. When the threat has been overcome, the inflammatory response at this stage will stop immediately.[34] The acute inflammatory response is a series of tissue responses that occur within the first few hours after injury. In this phase occurs quick forming of plasma molecules resulting in the accumulation of antibodies, complement proteins, clotting factors, and other factors.[35]

2. Chronic inflammatory stage

Chronic inflammation occurs when there is persistence of threat, either an injurious agent or pathogen. The onset of chronic inflammation is characterized by the replacement of neutrophils with macrophages and other immune cells such as T cells. During chronic inflammation, granulomas are formed. Continues inflammation leads to increased cellular turnover that results in appearance of cells with high risk for cancer. In chronic inflammatory conditions it will associate in various diseases such as cardiovascular, metabolic diseases and neurodegenerative diseases, stroke and myocardial infarction.[34]

E. The Role of Oxidative Stress in Inflammation

Free radicals are key molecules that signal and contribute to initiation and progression during inflammation. During inflammation, ROS determines several stages and encourages the migration of inflammatory cells through endothelium barriers that help eliminate foreign mediators.[7] Inflammation is induced by various stimuli, and is the result of a balance of pro and anti-inflammatory cytokines. In the inflammatory response will be found several inflammatory mediators who should have initiated the formation of pro-inflammatory cytokines.

Some proinflammatory cytokines such as TNFα, IL-1β, IL-6, IL-8 are found in the circulation at the beginning of the inflammatory process. These cytokines are not produced without assist of mediators, especially free radicals derived from oxygen. These free radical mediators will help the formation of cytokines by modulating the nuclear factor (NFκB). NFκB is a group of proteins that bind to DNA as homo or heterodimers that activate cytokines or synthesize stress genes.[36]

Inflammasome is a newly discovered multi-oligomer protein such as Nod-Like Receptor Protein (NLRP) 3 and 6, NLR family CARD domain-containing protein (NLRC) 4 and Absent in Melanoma-2 (AIM2) that plays an important role in initiating and maintaining inflammation.[37] The formation of inflammasome in response to microbial or dangerous signals initiates the division of procaspase 1 into the first active enzyme Caspase-1 dividing into inflammatory cytokines IL-1β and IL-18.[38] Production of ROS by PMN and monocytes when exposed to NLRP3 activators regulates activation of oxidation-dependent transcription factors such as the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and protein-1 activator (AP-1) via mitogen-activated protein kinases (MAP kinases) and production of proinflammatory cytokines.[37]

Immune cells in phagocytosis also produce ROS. Phagocytes such as neutrophils play an important role in host defense against pathogenic microbes. The role of neutrophils as anti-microbial is due to the production of superoxide anions (O_2^-) that form other ROS and release proteins and antimicrobe peptides. The enzyme responsible for the production of O_2^- is called NADPH oxidase or respiratory burst oxidase. Activation of this enzyme in phagocytosis can be induced by a number of solute and particulate agents. This process depends on the phosphorylation of the P47phox protein.[39]

Phosphorylation and migration of p47phox to the leukocyte plasma membrane is essential for ROS production. Some pro-inflammatory cytokines such as TNF-α, GM-CSF and G-CSF are known to induce phosphorylation of p47phox and assist the formation of ROS.[7] The process shift of physiologic regulators into pathogenic conditions is identified in a ROS-related illnesses. Uncontrolled and excessive formation of ROS which result of oxidative stress, especially mitochondrial ROS (mtROS), will stimulate upregulation of inflammatory cytokines.[10]

Inflammatory triggers such as excessive ROS / RNS production in oxidative metabolism processes in some natural or artificial chemicals, are initiated by inflammatory processes resulting in the synthesis and secretion of proinflammatory cytokines. Activation of NF-κB / AP-1 and TNF-α plays an important role in the inflammatory process that eventually leads to several chronic diseases. Overproduction of oxidative stress will induce some severe cellular damage, for example in the part of the diabetic brain. Oxidative stress inducing diabetes will increase levels of proinflammatory

cytokines such as TNF-α, Il-6 and also upregulates inflammatory molecules such as Vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and NF-κB.[40]

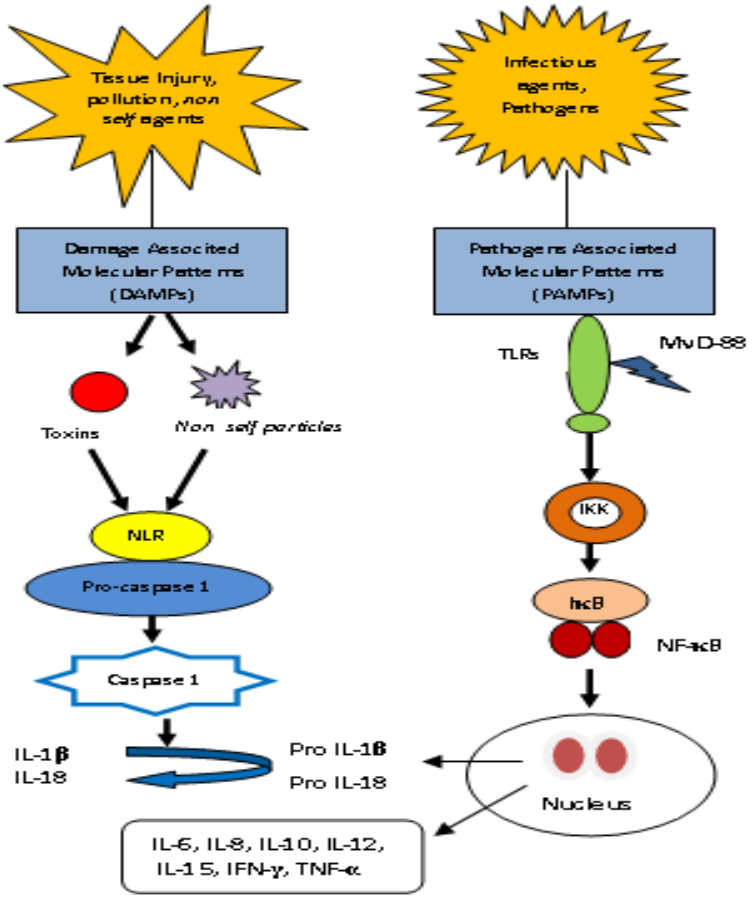

Figure 1. Production of Pro-Inflammatory Cytokines.[33]

Tissue injury, chemical material from pollution, non self antigen or even infectious agents such as pathogen triggers inflammation. Those agents contain of DAMPs and PAMPS respectively. DAMPs bind to transmembrane NLR and DAMPs bind to TLR. NLR signal the inflammasome lead to activates caspase-1 and change cytokines to active forms such as IL-1β and IL-18. After released from the cells, those cytokines promote inflammation. Toll like receptors activate MYD88-Dependent signal transduction pathway that induces inhibitory of IκB protein phosphorylation by IKK. NF-κB

then transfer to the nucleus cells where transcription is upregulates. The transcription binding to gene of inflammatory.

CONCLUSION

Free radicals have been known to trigger the inflammatory process. Free radical molecules play an important role and contribute to the onset and progress of inflammation in the various organs. In the early stages of inflammation, proinflammatory cytokines will be formed by activation of NF-κB. In the next stage, endothelial cells will be activated because of the binding between free radicals and cytokines, will further synthesize inflammatory mediators and adhesion molecules. Ultimately, the inflammation that takes place will cause various pathological conditions, both organ and systemic scope.

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