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Inflammation and oxidative stress

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Abbreviation:

ASK1: apoptosis signal-regulating kinase 1;
ATP: adenosine triphosphate;
CDK: cyclin-dependent kinease;
cGMP: cyclic guanosine 3, 5-monophosphate;
CRP: C reactive protein;
GAPDH: glyceraldehyde-3-phosphate dehydrogenase;
iNOS: inducible nitric oxide synthase;
IP_3: inositol 1, 4, 5-triphosphate;
JNK: c-Jun-NH_2-terminal kinase;
MAP(K): mitogen-activated protein (kinase);
MPO: myeloperoxidase;
mtNOS: mitochondrial nitric oxide synthase;
NF-κB: nuclear factor-κB;
RNS: reactive nitrogen species;
ROS: reactive oxygen species;
SOD: superoxide dismutase;
SODms: superoxide dismutase mimetics;

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Abstract

Inflammation can be classified to either acute or chronic inflammation. They have distinct characteristics. Besides inflammatory interleukins, ROS and RNS play an important role in the inflammatory process. They can kill invaded microbial, but at the same time they damage the cells and tissues. Serious acute inflammatory response such as MODF is fatal. Some chronic inflammatory responses can be linked to cancer. With clearing the pathogenic triggers, anti-oxidative therapy is a helpful method to treat serious inflammation. To elucidate the mechanism that how ROS is involved in the inflammatory reaction, transgenic mice that over-expressing MnSOD can be used in both acute and chronic inflammation researches.

Introduction

Inflammation is the reaction of a tissue and its microcirculation to a pathogenic result. It is characterized by the generation of inflammatory mediators and movement of fluid and leukocytes from the blood into extravascular tissues [1].

The primary purpose of the inflammation response is to eliminate the pathogenic insult and remove injured tissue components. This process accomplishes either regeneration of the normal tissue architecture and return of physiological function or the formation of scar tissue to replace what cannot be repaired [1]. But in particular circumstances, inflammation apparently dose more harm than good. For example, when inflammation affects a joint (rheumatoid arthritis), the cartilage can be damaged by neutrophil lysosomal enzymes that enter the area. Another example is the tumors seem to arise in chronically inflamed lesions [2]. Therefore we say that inflammation is a two-edged sword.

Historically, inflammation has been referred to as either acute or chronic inflammation, depending on the persistence of the injury, clinical symptoms, and the nature of the inflammatory response [1]. The hallmarks of acute inflammation include (1) accumulation of fluid and plasma components in the affected tissue; (2) intravascular stimulation of platelets; (3) the presence of polymorphonuclear leukocytes. The hallmarks of chronic inflammation are lymphocytes, plasma cells, and macrophages.

Pathophysiology of Inflammation

The inflammation response is remarkably the same, regardless of the cause. The causes of inflammation are numerous and varied. Infection (the presence of living microorganisms within the tissue) is simply one cause of inflammation. Inflammation can easily occur under conditions of perfect sterility, such as when a portion of tissue dies because of deprivation of blood supply [3].

Acute inflammation is the immediate and early response to injury designed to deliver leukocytes to sites of injury. Once there, leukocytes clear any invading microbes and begin the process of breaking down necrotic tissues. This process has two major components: 1) vascular changes: alterations in vessel caliber resulting in increased blood flow (vasodilation) and structural changes that permit plasma protein to leave the circulation (increased vascular permeability). 2) cellular events: emigration of the leukocytes from the microcirculation and accumulation in the focus of injury (cellular recruitment and activation) [4].

Chronic inflammation arises in the following settings: viral infection, persistent microbial infections, prolonged exposure to potentially toxic agents, and autoimmune diseases. Chronic inflammation can be considered to be inflammation of prolonged duration (weeks to months to years) in which active inflammation, tissue injury, and healing proceed simultaneously [4]. Besides inflammation with mononuclear (chronic inflammatory) cells, there is also

tissue destruction, which is largely directed by the inflammatory cells, and repair, which involves new vessel proliferation (angiogenesis) and fibrosis [4].

Reactive Oxygen and Nitrogen in Inflammation

For many years, inflammation represented the only process in which reactive oxygen species could be considered to be beneficial [5]. Today, we have got much more comprehensive understanding about the role of ROS in inflammation. Rather than simply beneficial or detrimental, the eventual effect of ROS depends on a number of factors such as the integrated involvement of different cells, the chronology and the type of microvascular changes, and the interaction with other reactive species [5].

Role of ROS

Before the discovery of reactive nitrogen species (RNS), ROS was supposed to be the key factor in inflammation. In the process of acute inflammation, phagocytosis and the elaboration of degradation enzymes are two major benefits of having recruited leukocytes to the site of inflammation. Phagocytosis consists of three steps: 1) recognition and attachment of the particle to the ingesting leukocyte; 2) engulfment, with subsequent formation of a phagocytic vacuole; 3) killing and degradation of the ingested material (Figure 1) [4].

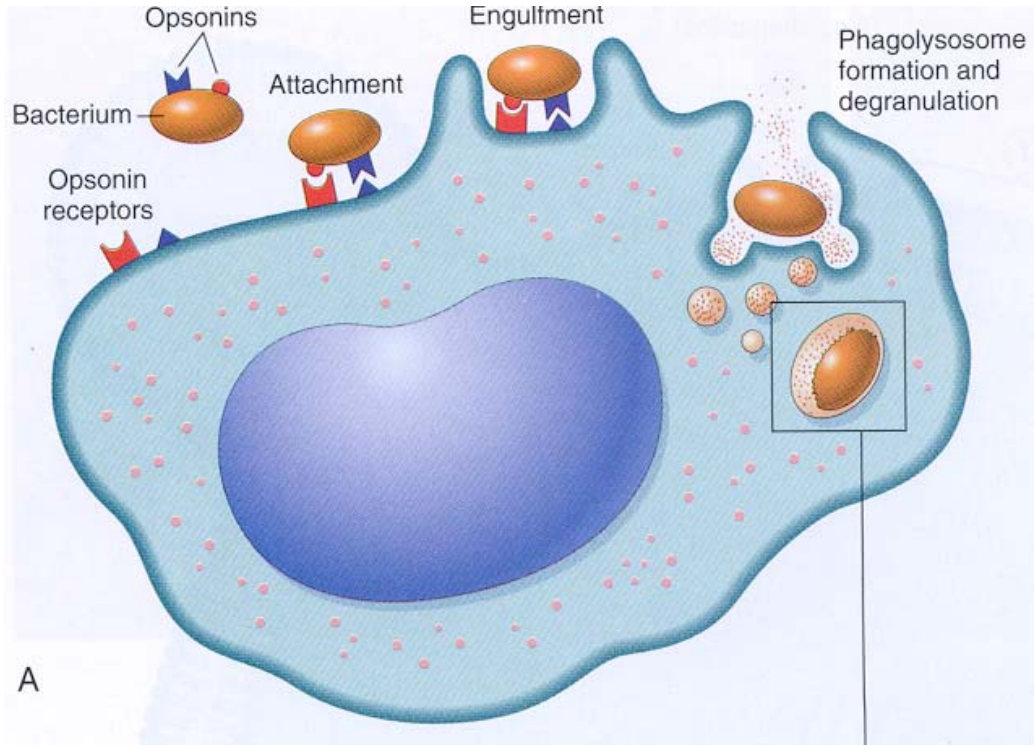

Figure 1. Three steps of phagocytosis. The square stands for the phagolysosome. (Adapted from [4]).

The final step in the phagocytosis of microbes is killing and degradation. Microbial killing is accomplished largely by reactive oxygen species. Phagocytosis stimulates an oxidative burst characterized by a sudden increase in oxygen consumption, glycogen catabolism (glycogenolysis), increased glucose oxidation, and production of reactive oxygen metabolites [4]. The generation of the oxygen metabolism is due to rapid activation of a leukocyte NADPH oxidase, which oxidizes NADPH [4]:

$$2O_2 + NADPH \xrightarrow{\text{NADPH Oxidase}} 2O_2^{\bullet -} + NADP^+ + H^+$$

Superoxide is then converted by spontaneous dismutation into H_2O_2. The quantities of H_2O_2 produced are generally insufficient to effectively kill most bacteria. However, the lysosomes of neutrophils contain myeloperoxidase

(MPO), and in the presence of a halide such as Cl^-, MPO converts H_2O_2 to $HOCl^{\bullet}$. $HOCl^{\bullet}$ is a powerful oxidant and antimicrobial agent that kill bacteria by halogenation, or by protein and lipid peroxidation (Figure 2) [4].

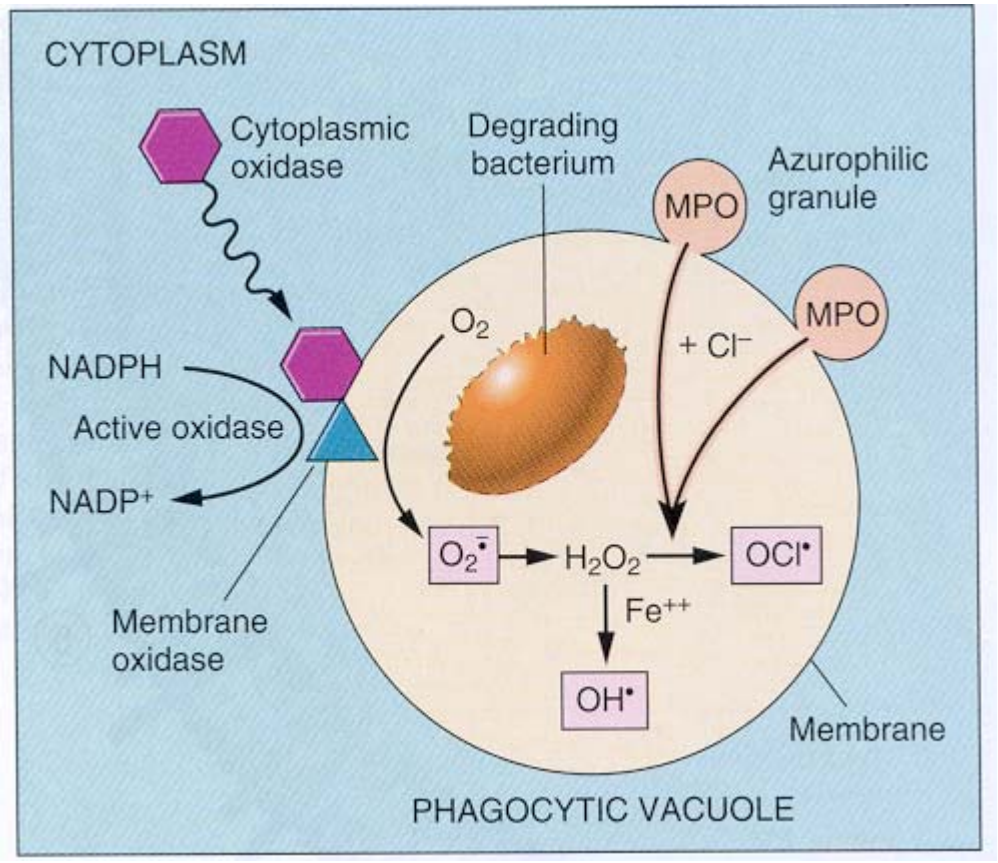

Figure 2. Killing bacteria in phagocytic vacuole. (Adapted from [4]).

NADPH oxidase is active only after translocation of its cytosolic subunit to the membrane of the phagolysosome. Thus, $HOCl^{\bullet}$ only be generated within that compartment. But, those reactive species can react with many cellular components. Figure 3 shows the pathway that reactive species generation and degradation.

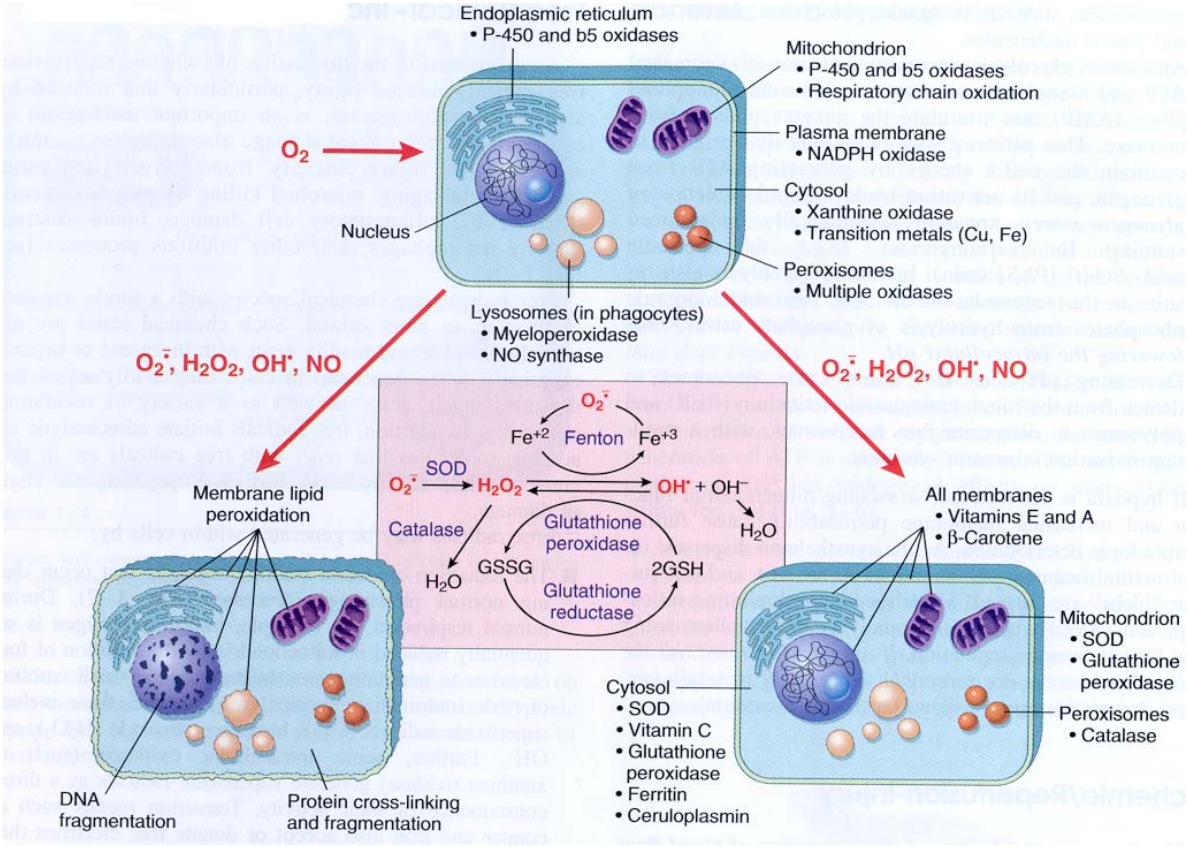

Figure 3. Generation of free radicals, the cell injury resulting from the action of unopposed free radicals, and their neutralization by cellular antioxidation mechanisms. (Adapted from [4]).

Role of RNS

Over the last few years, the overall process of the inflammation has been complicated by the potential pathogenetic contribution of RNS [6]. Many stimuli are able to up-regulate expression and synthesis of inducible nitric oxide synthase (iNOS) [5]. Now we recognize that ROS and RNS may interact with each other, resulting not only in the induction of further new reactive species, but above all, in possible changes in the concentration of these two classes of molecules [6]. Figure 4 shows the sources and effects of NO.

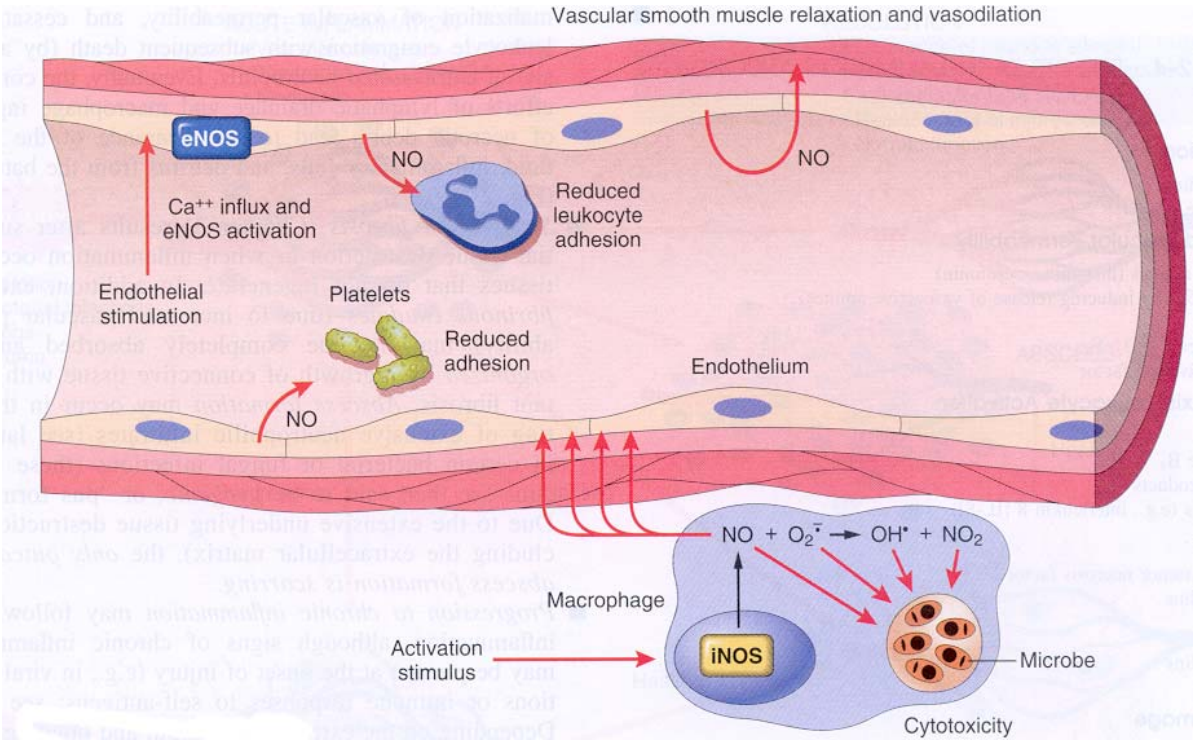

Figure 4. Resource and effects of NO. (Adapted from [4]).

The recognition of NO production by activated macrophages as part of the inflammatory process was an important milestone for assessing both the biological production of NO and the phenomenon of induction of NOS activity. This time, at the molecular level, inflammation is defined by increased concentrations of NO and of the inflammatory cytokines, mainly interleukin 1-β (IL-1) and tumor necrosis factor-α (TNF-α) in the involved biological fluids [7]. Based on this conception, the inflammation process can be described as in Figure 5.

The role of NO is different from that of ROS. NO has many actions appropriate for a pro-inflammatory agent; it is made by numerous cell types in sites of inflammation, and it increases blood flow and vascular permeability.

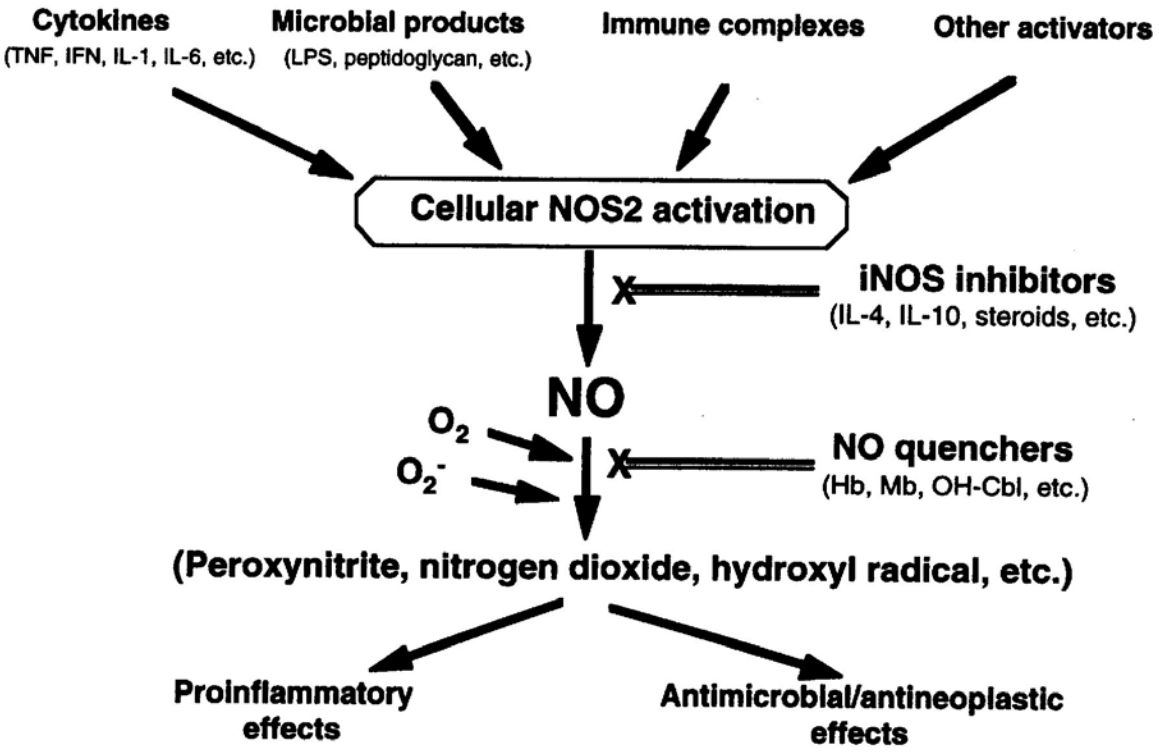

Figure 5. the role of NO in the inflammation. (Adapted from [8]).

NO has cell/tissue destructive abilities, and it can induce cyclo-oxygenase, cause pain, destroy certain protease inhibitors, and enhance production of IL-1 and TNF, and NADPH oxidase activity in myeloid cells [8]. The ability of NO to act as intracellular signal functions via second messengers. One of the primary second messengers for NO is cyclic guanosine 3, 5-monophosphate (cGMP) [9]. One intracellular target of cGMP stimulated by NO is p_{21}ras, a proto-oncogene which feeds into a signal cascade involving raf kinase and MAP kinase [9]. Besides pro-inflammatory effects, NO also has anti-inflammatory effects. Activation of cGMP may also account for some of the anti-inflammatory effects of NO [9]. By activating cGMP, NO lowers intracellular calcium levels and can attenuate platelet and neutrophil

aggregation; NO may inhibit growth factor receptor signaling by inositol 1, 4, 5-triphosphate (IP_3) by a similar mechanism; NO mobilizes intracellular calcium by stimulating cyclic ADP-ribose synthesis [9]. In addition to its effects on cGMP, NO also stimulates the ADP-ribosyltion of many intracellular proteins. For example, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), an enzyme involved in the generation of adenosine triphosphate (ATP) during glycolysis, can be inhibited by NO [9]. Thus NO contributes to cellular energy depletion.

NO may mediate inflammation and contribute to cell death by acting directly on transcriptional factors. $ONOO^-$ can either inhibit or activate NF-κB depending on the dose of $ONOO^-$ and the agent used to stimulate NF-κB [9]. Another experiment showed that NO not only induced AP-1, but also induced the phosphorylation of apoptosis signal-regulating kinase 1 (ASK1), c-Jun-NH_2-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) [10]. Thus, NO seems to involved in the apoptosis process.

Another target of NO during inflammation is mitochondria. Researchers found that during septic shock, a serious form of systemic inflammation, mitochondria isolated from skeletal muscle exhibited impaired respiration and this respiratory impairment is due to an inhibition of election transfer [7]. The inhibition of electron transfer can be understood as due to an excessive production of NO by mtNOS that leads to: (i) an irreversible effect of NO and $ONOO^-$ on NADH-ubiquinone reductase and ubiquinol-cytochrome c

reductase, and (ii) a reversible O_2-competitive inhibition of cytochrome oxidase activity (Figure 6) [7].

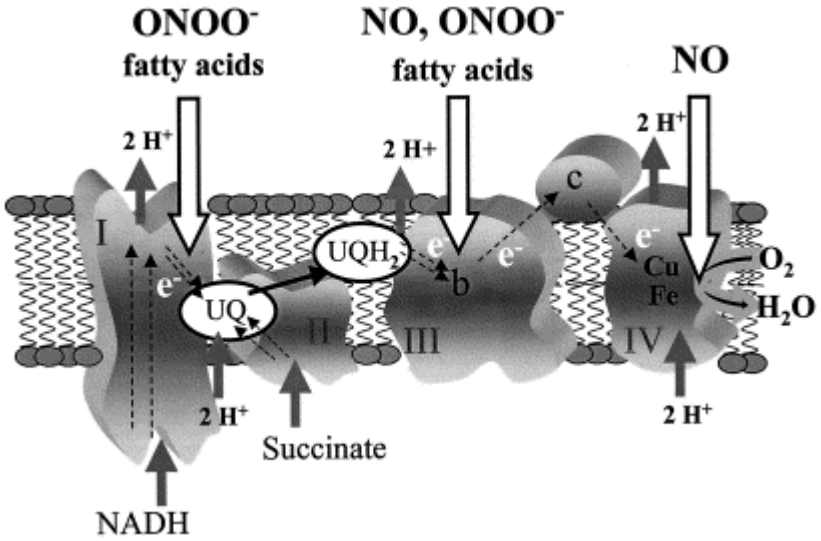

Figure 6. Scheme indicating the inhibitory effects of NO, $ONOO^-$, and fatty acids on the electron transfer of the mitochondrial respiratory chain in septic shock. The effects of NO on cytochrome oxidase, [NO] 0.5 = 0.1 M, and on complex III, [NO] 0.5 = 0.2 M, are reversible. The effects of ONOO- on Complexes I and III are irreversible. The effects of fatty acids on Complexes I and III are reversible. (Adapted from [7]).

Inflammation and Cancer

There is evidence that cancer risk occurs in tissues of the body undergoing chronic inflammation, and chronic infections contribute to about one-third of the world's cancer [11]. As mentioned previously, leukocytes and other phagocytic cells combat bacteria, parasites, and virus infected cells by destroying them with NO and superoxide, which react to form peroxynitrite, a powerful mutagenic oxidizing and nitrating agent; hypochlorite, a mutagenic chlorinating and oxidizing agent; and hydrogen peroxide, a mutagenic oxidizing agent. These oxidants protect humans from immediate death from infection but, at the same time, also cause oxidative damage to DNA, mutation

and chronic cell killing with compensatory cell division (carcinogenic process)[11]. For instance, hepatitis B and C viruses are a major cause of chronic inflammation leading to liver cancer in Asia and Africa [11]. Schistosomiasis infection is widespread in part of Asia and Egypt. In Asia, the eggs of *Schistosoma japonicum,* deposited in the colonic mucosa, cause inflammation and colon cancer; while in Egypt the eggs of *Schistosoma haematobium,* deposited in the bladder, cause inflammation and bladder cancer [11]. Asbestos can reduce O_2 to $O_2^{\bullet -}$ [12]. Exposure to asbestos cause cellular damage, leading to asbestosis, bronchogenic carcinoma, and mesothelioma in human [12].

Diagnosis of inflammation

Pathology exam is the gold standard for disease diagnosis. The pathological characteristics of acute and chronic inflammation are clear. Figure 7 and Figure 8 are respective slides for inflammation.

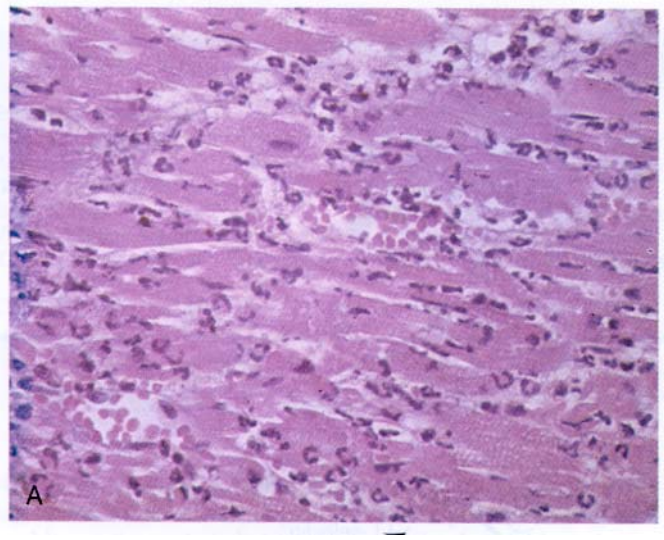

Figure 7. Acute inflammation, showing the multilobed polymorphonuclear cell infiltrate (myocardium). (Adapted from [4]).

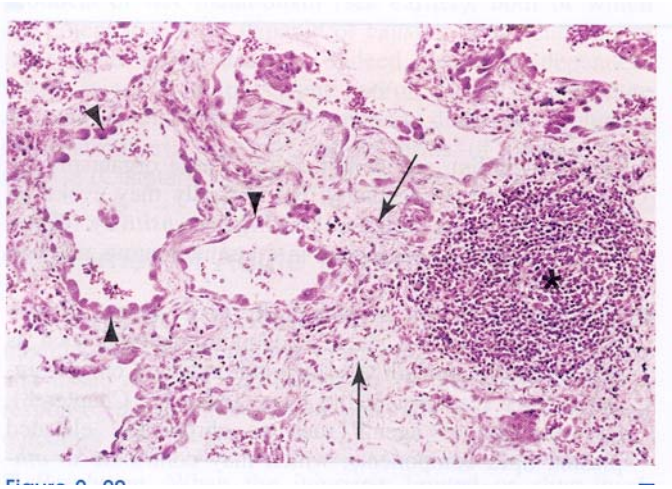

Figure 8. chronic inflammation in the lung, showing the three characteristic histological features: (1) collection of chronic inflammatory cells (*); (2) destruction of parenchyma (normal alveoli are replaced by spaces lined by cuboidal epithelium *[arrowheads]*); (3) replacement by connective tissue (fibrosis) *(arrows)*. (Adapted from [4]).

Besides the local symptoms, such as edema, pain, reddish, inflammation also has systemic symptoms. Some of them can be used as diagnosis index. Clinically, higher temperature means there is inflammatory response in patient's body. The changes in the body fluid including blood are indexes of inflammation. For example, in bacteria infection induced inflammation, the number of leukocyte increases while the percentage of neutrophils increases to more than 90%. Another index of inflammation in body fluid is C reactive protein (CRP); this protein level increases during acute inflammation. But we can see here, all the indexes mentioned above are general, and no one is pathogenically specific. So when make diagnosis of inflammation, the reason that induces the inflammatory response should be identified. Thus, the correct therapy can be delivered.

Therapy of inflammation

The primary therapy is to clear the pathogenic triggers of the inflammation, for example, using antibiotics to kill bacteria, performing an operation to

remove necrotic tissues, applying glucocorticoids to inhibit an autoimmune reaction. Since ROS and RNS play an important role in the inflammatory reaction, antioxidative therapy may be another key component of treatment for serious inflammation. Septic shock is a major cause of death following trauma and a persistent problem in surgical patients. The prevalent hypothesis regarding its mechanism is that the syndrome is caused by an excessive defensive and inflammatory response [7]. Septic shock constitutes a paradigm of acute whole body inflammation, with massive increases of NO and inflammatory cytokines in the biological fluids, with systemic damage to vascular endothelium, and with impaired tissue and whole body respiration despite adequate oxygen supply. This situation has made septic shock a tough disease to proceed therapy for many years. The elucidation of ROS and RNS involved in inflammation gives an alternative choice. Besides those traditional therapies, such as supplying body fluid, inhibiting cytokines, maintaining heart function, antioxidative therapy is an ideal method.

One strategy is applying antioxidants directly into patients. In my previous study, I applied NAC and GSH to LPS induced septic shock rabbits. These two reducing agents can significantly maintain the blood pressure after injection of LPS [13].

Another strategy is introducing the antioxidative enzymes. Directly expression of these enzymes is difficult and impractical. But superoxide dismutase mimetics may be a good choice. Native SOD2 is about 31 kDa, while synthetic

SODms are small molecules. Figure 9 shows the structure of two SODms.

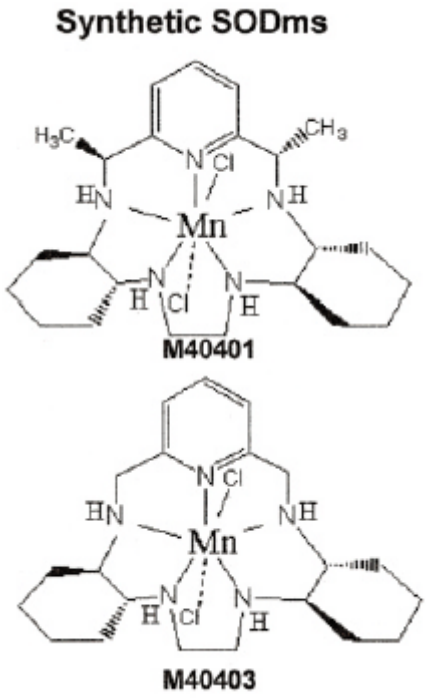

Figure 9. The structure of two SODms: M40401 and M40403. (Adapted from [14]).

These SODms have same properties: manganese containing biscyclohexylpyridine, catalytic activity equivalent if not superior to that of the native enzyme, non-peptide small molecule, non-immunogenic, penetrates cells, selective for superoxide (no interaction with biologically important molecules), stable in vivo (no Mn dissociation), not deactivated by peroxynitrite, protective in various models of acute and chronic inflammation, reperfusion injury and shock [14].

Cooling is a therapeutic method that is as old as the earliest written record. We can always see cold therapy be introduced in sports traumatology as a first aid. The mechanism of cooling-associated protection against TNF-α-induced microcirculatory dysfunction and inflammatory response is believed to be related to the HO and NOS pathways [15].

Other therapy strategies include NOS inhibitor (L-NAME), applying antioxidant supplement before development of serious inflammation stage.

Further direction for inflammation research

In my opinion, there are at least two directions that need further researches --- dealing with the serious systematical inflammatory reaction in MODF, SIRS, and septic shock; figuring out the mechanism why chronic inflammation increases the cancer risk.

Since the transgenic mice that overexpress MnSOD are available, we can use it as a tool to study the role of ROS in the inflammation process. The first experiment can be set as follow:

1), let normal control mice be group 1; MnSOD overexpressing mice group 2.

2), treat both groups with LPS.

3), record the index of blood pressure, respiration and the plasma level of TNF-α, NO, GSH, CRP.

4), pathology examination of live, kidney, lung, small intestine and blood cells. Check the protein and mRNA level for iNOS, NF-κB, JNK and MAPK.

To study the role of ROS in the relationship of chronic inflammation and cancer, we still can use the MnSOD overexpressing mice. We can set a model of *Schistosoma japonicum* in both control and MnSOD transgenic mice. That

should be a long time experiment and need a large number of animals. After successfully infect with *Schistosoma japonicum,* a serious examination should be done in a long period. We do not have any evidence in mice that *Schistosoma japonicum* infection can cause colon cancer. But by using this model, we may get some up-regulated pro-oncogenes that can lead to colon cancer in human beings.

The target genes should include both pro-oncogenes and tumor suppressor genes, such as p53, p21, c-fos, c-Jun, CDKs. The hypothesis is MnSOD overexpression may inhibit the activation of pro-oncogenes after the chronic infection of *Schistosoma japonicum*.

Summary

Inflammation is a pathophysiological process, which supposed to clear any pathogenic factors in the inflammatory area. But over –inflammatory reaction can produced much ROS, RNS and other biological active molecules that have detrimental effects. Oxidative damage involves in both acute and chronic inflammation. Chronic inflammation increases the risk of cancer. Based on the pathological changes, diagnosis can be done in a general manner. Light inflammation does not require specific therapy but serious inflammatory react sometimes is lethal and required immediate interference. Antioxidative therapy is an important component of cocktail therapy. Antioxidative therapies include applying antioxidants, SOD mimetics, cooling and NOS

inhibitors.

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