ROLE OF NITRIC OXIDE IN IMMUNE SYSTEM

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***Abstract*- Nitric oxide (NO) was discovered over twenty year ago to be a molecule that was biologically active and ever since documented as one of the most versatile components of the immune system. Nitric oxide is a readily diffusible gas that mediates cell-cell communication and thus has been branded as universal messenger. It is particularly interesting as it is part of the pathogenesis as well as the control of infectious diseases, along with autoimmunity, neurodegenerative diseases and cancer. NO can be formed from one of three NO synthases, two of which are essentially expressed in the body: endothelial NO synthases (eNOS) and neuronal NO synthases (nNOS). The third being inducible NOS where production is most commonly stimulated by proinflammatory markers.**

I. INTRODUCTION

NO was first discovered as the Endothelium Derived Relaxing Factor (EDRF) by Furchgott and Zawadki. Joseph Priestly in 1972 was the first person to term it NO, however till 1987, the biological functions of NO were not known and therefore it was merely regarded as an air pollutant or toxic gas. Only when the presence of nitrates and nitrites where detected in healthy rats and human volunteers, studies on the biological role of NO came into light which was further followed by the discovery of the tumoricidal and antimicrobial activity of NO. Later, the regulatory and protective roles of NO in cardiovascular system were described, that included blood pressure and vascular tone regulation, inhibition of leucocyte adhesion or platelet adhesion and prevention of proliferation of smooth muscles.

NO especially from iNOS (inducible Nitric Oxide synthase) Has important roles in immune regulation, inflammation and microbial invasion. NO is also implicated in the pathophysiology od various cancers viz., breast, larynx, cervix, head and neck. Tumor promoting role of NO is dependent upon the tumor type, NO concentrations and its interactions with proteins, metals or free radicals and the genetic makeup of the host cell. Lower concentration of NO is important for immune function. While NO at higher levels are shown to be immunosuppressive suggesting the dual role of this biomolecule. However, some contradictory reports also present a debating role of NO immune response. Some state NO to be a causative agent of several diseases while others argue on its protective role. This contradiction has led a conflict over whether NO serves as protector from various infections or functions as a destroyer results indifferent disorders.

II. THE IMMUNE SYSTEM

Nitric oxide plays many important roles in the immune system. It is produced in high amount from specialized cells of the immune system called macrophages. Following a bacterial infection for example, the body produces chemicals known as cytokines which activate the cells of the immune system, including macrophages and help guide them to the site of infection. The high amount of nitric oxide produced by the macrophages is actually toxic to the bacteria and plays an important role in their destruction. The production of nitric oxide in this way also help protect against other types of infection including viruses and parasites.

However, too much nitric oxide production has also been implicated in condition where the immune system is too active – diseases like arthritis and the so-called autoimmune disease.

III. HOW NO IS PRODUCE IN IMMUNE SYSTEM?

NO is synthesized from L-arginine by the enzyme NOS in the presence of two cofactors viz. NADPH and oxygen. NOS has three isoforms represented as neuronal NOS (nNOS) or NOS 1, inducible NOS (iNOS) or NOS 2, and endothelial (eNOS) or NOS 3. The gene for these isoforms 1,2 and 3 are located on the chromosomes 12, 17 and 7 respectively. All the isoforms of NOS are flavoproteins containing Tetrahydrobiopterin (THB) and heme. THB is an Important cofactor for NOS, since in its absence, NOS produces superoxide instead of NO.

Broadly, the isozymes of NOS are categorized as constitutive NOS (cNOS) and iNOS. cNOS, which is constitutively present in the cell, is calcium dependent comprises nNOS and eNOS while iNOS is calcium independent and expressed only after the stimulus is provided by cytokines. The synthesis NO by nNOS and eNOS is dependent on intracellular calcium ions and binding of these enzymes to

calmodulin. Increase in calcium level causes increased production of calmodulin that binds with eNOS and nNOS causing enhanced synthesis of NO by the respective enzymes. For the activation, nNOS in central nervous system, the glutamate first binds with NMDA (N-methyl-D-aspartate) receptors causing opening of the voltage gated ion channels and rise in calcium level. In case of eNOS, it is activated when stimulus like blood shear stress or factors like substances P, kinins or thrombin receptors etc., cause release of calcium ions from the endoplasmic reticulum. In the cells such as macrophages and monocytes, induction on iNOS by inflammatory cytokines and presence of L-arginine in sufficient amount leads to generation of NO.

IV. MECHANISM OF ACTION

It is not well understood which action of NO is responsible for induction of NO is responsible for induction of necrosis or apoptosis. The primary targets for cell death are nuclear and mtDNA, as well as mitochondrial electron transfer chain and mitochondrial electron permeability. NO interferes with heme groups of electron transfer complex IV. It can also interact directly with DNA, causing deamination. An important additional aspect is uncoupling of the electron transfer chain, which gives rise to enhanced production of oxygen free radical. These might react with NO, resulting in the formation of peroxynitrite anion, which is an extremely potent oxidant. It should be noted that different cell types differ in their resistance to the toxic effect of NO. This might be the result of varying expression of protective molecules, such as hsp70, or different pathway of NO-induced cell death such as necrosis or apoptosis.

For the immunoregulatory function of NO, several intracellular targets must be considered. These include the mitochondrial membrane permeability and the nucleus itself. It has been shown that NO disrupts Zn finger configuration by releasing Zn from thiol groups. This leads to reversible inactivation of Zn finger-containing transcription factors, and intranuclear Zn release is indeed the result of exposure of live cells to subtoxic NO concentration. In those cases where the Zn finger protein antagonize gene expression, its temporal disruption will allow for transcription to take place. Also by reacting free SH groups and forming S-NO adducts, other types of transcription factors can be inhibited, including NF-Kβ. NO also increases expression and prevent degradation of Ikβ, thereby contributing to further inhibition of NF-kβ. Again, both action of NO tend to attenuate Th1 response. By contrast, NO-mediated transcriptional activation through S-nitrosylation may also occur.

V. PHYSIOLOGICAL ROLES OF NO (CONSTITUTIVE VS INDUCIBLE NOS)

Activation of iNOS leads to the sustained production of large amount of NO while that of constitutive forms (eNOS and nNOS) cause production of low levels of NO within seconds. NO from constitutive isoforms have direct and short lasting activities. They interact with cytochrome p450, guanylate cyclase and NO itself. This leads to activation of guanylate cyclase that converts Guanosine Triphosphate (GTP) into cyclic Guanosine Monophosphate (cGMP) which in turn activates cGMP dependent protein kinase that further mediates the function of NO such as increase in vascular permeability, vasorelaxation, antioxidant roles and antiplatelet activities. In central nervous system (CNS) and peripheral nervous system (PNS), NO act as neurotransmitter and is involved in neuronal apoptosis. NO from eNOS is essential for maintaining tissue perfusion, protection against toxic lipids derived from Lipopolysaccharide (LPS) and preservation of RBC in septicemia.

The functions of NO produced by iNOS is rather different. Such NO is produced by activated macrophages and is involved in microbial killing and immune regulation. NO combines with superoxide to form peroxynitrite that further mediates toxicity of NO which includes LDL oxidation, DNA damage inhibition of TCA and mitochondrial oxidative enzymes, nitrosation.

VI. NO AND PROINFLAMMATORY CUTOKINES

Cells contain various mechanisms to regulate the signaling pathways and transcription factors. Depending upon such regulation, NO is reported to either depress or stimulate the proinflammatory cytokine expression. When the immune system is activated, there is induction of proinflammatory phase of innate immunity, a process which is termed as classical activation. Macrophages that are classically activated, also termed as M1 macrophages, results in production and release of proinflammatory cytokines.

Turpaev K et al., used DNA microarrays of macrophages cell lines in humans to identify the genes regulated by NO such as cFos, cJun and cell

cycle regulator genes. However in some cases Reactive Nitrogen species (RNS) act as regulatory molecules rather than NO.

Most of the immune pathways regulated by NO/RNS requires NF-kβ for coordinating the outcomes of innate immunity. When the calls are at resting state, NF-Kβ forms complex with the inhibitory proteins and are scattered in cytoplasm. Once the cells are activated by various stimuli such as bacterial LPS or TNF-α, there is proteosomal degradation of inhibitory proteins mediated by a cascade of phosphorylation reactions, thereby facilitating entry of NF-kβ to the nucleus where it modulates the respective gene of immune activity. Studies have shown that NO/RNS at lower concentration augment NF-Kβ mediated transcription of immune genes while higher levels inhibit the process.

VII. NO AND ANTI-INFLAMMATORY SIGNALING:

Anti-inflammatory cytokines such as TGF-β, IIL-10 and Il-4 down regulate pro-inflammatory cytokines and facilitate t tissue repair. The action of NO/RNS can be compared to that of traffic officer as they critically regulate and redirect the signaling pathways by inducing one pathway and diminishing the other. Most of the immune receptors activated by anti-inflammatory cytokines are linked with synergy between JAK that cause activation of signaling pathway via stimulation of signal transducer and activator of transcription 6(STAT6) through a cascade of phosphorylation reaction involving tyrosine residues. This finally leads to the expression of genes involved in tissue repair and restoration.

IMMUNOSUPPRESSIVE ROLES OF NO:

NO is also implicated to be involved in immunosuppressive. Various mechanisms have been postulated as follows.

- S-nitrosylation of glucose-6-phosphate dehydrogenase (G6PD) results in its nuclear translocation and degradation.
- Myeloid Suppressor Cell (MSC) mediated T cell suppression: NO produced by MSCs causes T cell immunosuppressive by orchestrating the synergetic action of iNOS and arginase since activation of both the enzymes lead to depletion of L arginine, which in turn stimulates production of superoxide by iNOs in small amount. The superoxide then reacts with NO to form ONOO- and others forms of RNS that causes T cell apoptosis.
- Impairment in signaling cascades induced by IL-2R: NO at higher concentration blocks IL-2R mediated activation of JAks (JAK 1and2) and STAT5.

VIII. TUMOR CELLS AND NO

Tumor develops due to genetic alteration that cause uncontrolled growth and proliferation of the cell. Initially the immune system act to eliminate such abnormal cells via the process known as immune-surveillance. During the first stage of immune-surveillance, the immune cell succeed to destroy the developing transformed cells and prevent the formation of tumor mass. However, if the surveillance process fails, then the emerging transformed cells transit to the second phase, also known as equilibrium phase in which immune system can control but not clear up tumor cells. During this phase the immune system constantly pressures the tumor cell in order to remove many original variants, some of which may escapes the immune-surveillance and enter the third phase known as escape phase, in which the transformed cells grow in an unrestricted manner.

Macrophages among the immune cells are the most prominent once that infiltrate deep into the hypoxic area of the tumor mass to combat and eliminate the pathogens and tumor calls. In some cases macrophages comprise about 50% of the tumor mass.

TUMOR INHIBITING BY NO: NO/RNS when present in higher concentration cause cellular death by stimulating modification of death related target protein receptors or affecting the respiratory chain via changes in the permeability of mitochondrial outer membrane causing cytochrome C release and cellular apoptosis. Besides these mechanism, another mechanism of tumor inhibition by NO/RNS includes phosphorylation of Ser15 of wild type P53 that leads to its activation and initiate the process of apoptosis. It also causes S-nitrosylation of Cys 62 of NF-κβ (an antiapoptotic factor) leading to its inhibition.

TUMOR PROMOTING ACTION OF NO: Though high levels of NO/RNS are used as a killing mechanism by phagocytes, it has also been shown that NO/RNS can cause carcinogenesis as well as support the progression of pre-existing tumor which is at escape phase from immune system.

IX. CARCINOGENIC ROLE

NO/RNS being lipophilic can easily diffuse through the cell membrane and cause oxidation or deamination of nitrogen bases, produces DNA breaks and cross links, all of which induce mutation. NO can also activate the oncogenes or cause deactivation of tumor suppression genes. S-nitrosylation or nitration of proteins involved in DNA repair by NO/RNS affects the cellular repair mechanism and genomic stability.

X. CONCLUSION

The effects of NO in humans are an area of interest for most of the researchers both at basic experimental levels or clinical studies. Remarkable studies have been conducted in the past decades to understand the role of NO in immunity. It not only act as a potent antimicrobial agent but also has a protective function against tumors. However, despite of these beneficial effects, NO has the potential to switch from the protector to destroyer, i.e., it neither protects from or leads to diseases. Over production by excess stimulation of NOS can lead to neurodegenerative disorders, cancer or inflammation. It is therefor essential to understand the role of NO in immune system which requires the discrimination between the dual natures of this biomolecule. Observation from animal models regarding the interaction between macrophages and pathogens must be correlated carefully to human studies so that the importance of NO in providing protection against emerging pathogens can be stressed out. Also NO can be used a novel therapeutic regimen in the treatment of refractory tumors which can be achieved by sensitizing the tumor cells to immunotherapy. But validation of such strategies requires further clinical trial, so that NO mediated therapies can be developed in the prevention and treatment of cancer.

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