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Review article

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NITRIC OXIDE AND SUPEROXIDE IN INFLAMMATION AND IMMUNE REGULATION.

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Nitric oxide (NO) and reactive oxygen species exert multiple modulating effects on inflammation and play a key role in the regulation of immune responses. They affect virtually every step of the development of inflammation. Low concentrations of nitric oxide produced by constitutive and neuronal nitric oxide synthases inhibit adhesion molecule expression, cytokine and chemokine synthesis and leukocyte adhesion and transmigration. Large amounts of NO, generated primarily by iNOS can be toxic and pro-inflammatory. Actions of nitric oxide are however not dependent primarily on the enzymatic source, but rather on the cellular context, NO concentration (dependent on the distance from NO source) and initial priming of immune cells. These observations may explain difficulties in determining the exact role of NO in Th1 and Th2 lymphocyte balance in normal immune responses and in allergic disease. Similarly superoxide anion produced by NAD(P)H oxidases present in all cell types participating in inflammation (leukocytes, endothelial and other vascular cells etc) may lead to toxic effects, when produced at high levels during oxidative burst, but may also modulate inflammation in a far more discrete way, when continuously produced at low levels by NOXs (non-phagocytic oxidases). The effects of both nitric oxide and superoxide in immune regulation are exerted through multiple mechanisms, which include interaction with cell signalling systems like cGMP, cAMP, G-protein, JAK/STAT or MAPK dependent signal transduction pathways. They may also lead to modification of transcription factors activity and in this way modulate the expression of multiple other mediators of inflammation. Moreover genetic polymorphisms exist within genes encoding enzymes producing both NO and superoxide. The potential role of these polymorphisms in inflammation and susceptibility to infection is discussed. Along with studies showing increasing role of NO and free radicals in mediating inflammatory responses drugs which interfere with these systems are being introduced in the treatment of inflammation. These include statins, angiotensin receptor blockers, NAD(P)H oxidase inhibitors, NO-aspirin and others. In conclusion in this mini-review we discuss the mechanisms of nitric oxide and superoxide dependent modulation of inflammatory reactions in experimental animals and humans. We also discuss potential roles of nitric oxide as a mediator of allergic inflammation.

Key words: *nitric oxide, inflammation, T helper cells, allergy*

Inflammation is a response of the organism to injury related to physical or chemical noxious stimuli or microbiological toxins, which is involved in multiple pathologies such as arthritis, asthma, multiple sclerosis, colitis, inflammatory bowel diseases and atherosclerosis. Inflammatory response is intended to inactivate or destroy invading organisms, remove irritants, and set the stage for tissue repair. The inflammatory response consists of specific immunological and non-specific immune reactions. The primary processes in inflammation include increase in vascular permeability as well as release of lipid-derived autacoids, such as eicosanoids or "platelet-activating factor" (PAF); large peptides, such as interleukin-1; small peptides, such as bradykinin; and amines, such as histamine or 5-hydroxytryptamine from injured tissues and migrating cells. These constitute the chemical network of inflammatory response and result in clinical and pathological manifestations of inflammation. Nitric oxide may play regulatory roles at virtually every stage of the development of inflammation (*Table 1*). In particular, in the regulation of pro-inflammation properties of endothelium and in the early stages of inflammatory cell transmigration into the sites of inflammation.

Nitric oxide and nitric oxide synthases

In animal tissues, nitric oxide (NO) is generated enzymatically by synthases (NOS), which oxidise L-arginine to L-citrulline (1, 2) (*Fig. 1*). There are 3 isoforms of NOS (1) NOS I or (nNOS)- the neuronal form (2) NOS II- inducible nitric oxide synthase (iNOS), present in various cell types upon inflammatory stimulation (e.g. macrophages) and (3) NOSIII (or eNOS) - constitutive enzyme primarily discovered in the endothelium (2). All three isoforms have a similar molecular structure and require multiple cofactors. Overall amino-acid sequence identity between different isoforms of NOS is 55% but is more closely homologous at important catalytic sites (*Table 2*). The neuronal and endothelial isoforms are activated by binding of the calcium regulatory protein-calmodulin

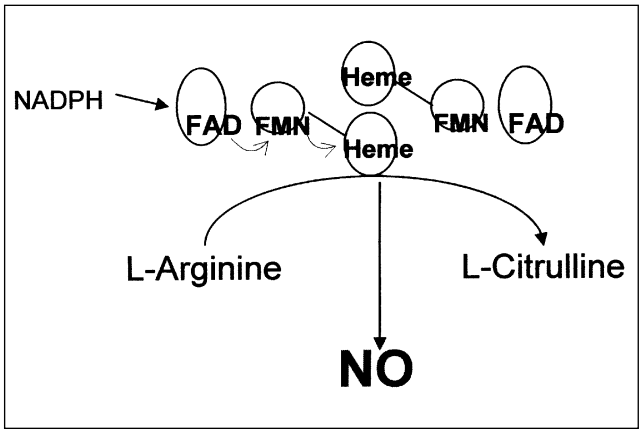

Figure 1. Production of nitric oxide from L-Arg. NOS incorporates molecular oxygen into L-Arginine in a process of five electron oxidation of the terminal guanido nitrogen of L-Arg. This process involves NADPH as co-substrate and requires other redox cofactors

Table 1. Relationships between inflammatory mediators and symptoms of inflammation. These relationships are very complex and only the most evident effects were summarised in this table.

Symptom	Mediators
Vascular permeability	Vasoactive amines Bradykinin Leukotrienes C_4, D_4, E_4 PAF Complement (C3a and C5a) Substance P Nitric oxide
Vasodilatation	Nitric oxide PGI_2, PGE_1, PGE_2, PGD_2 Hydrogen peroxide
Vasoconstriction	Tromboxane A2, Leukotrienes C_4, D_4, E_4 Superoxide
Chemotaxis and leukocyte adhesion	Chemokines LTB_4, HETE, lipoxins Complement (C5a) Bacterial antigens Superoxide
Pain	Bradykinin Prostaglandins Superoxide
Fever	IL-1; TNF; IL-6 Prostaglandins
Tissue and endothelial damage	Reactive oxygen species Nitric oxide (iNOS) Lyzosomal enzymes

and have similar NO release kinetics (3). The inducible isoforms are regulated primarily at the transcriptional level, independent of agonist stimulation and intracellular calcium levels (calmodulin is bound with very high affinity even at basal calcium levels)(4). The three NOS isoenzymes (neuronal, endothelial and inducible) are flavoproteins which contain tetrahydrobiopterin and haeme and they are homologous with cytochrome p 450 reductase. Isoenzymes of NOS act as dioxygenases using molecular oxygen and NADPH to transform L-arginine to L-citrulline and NO. Tetrahydrobiopterin (BH4) is a key cofactor for all NOS enzymes. In the absence of BH4, NOS produce superoxide, instead of nitric oxide (5, 6).

Several lines of knockout mice have been generated to distinguish the roles of each enzyme. The nNOS-deficient mice develop preserved hippocampal long

Table 2. Key features of three NOS isoforms

	eNOS	nNOS	iNOS
Originally cloned from	endothelial cells	neuronal cells	macrophages
Tissue expression	cardiac myocytes platelets, neurones	skeletal muscle, neutrophils, VSMC*	cardiac myocytes, glial cells, VSMC*, endothelium, neurones
Gene encoding and its position	*NOS3* 7q35-36	*NOS1* 12q24.2-31	*NOS2* 17q11.2-12
Major regulatory mechanism	Ca^{+2} dependent (Ca-calmodulin) Ca^{+2} independent (phosphorylation, palmitoylation)	Ca^{+2} dependent (Ca-dystrophin)	Ca^{+2} independent; transcriptional regulation e.g. by NF□B
Subcellular localisation	Golgi apparatus plasmalemmal caveolae	cytosol endoplasmic reticulum sarcollema postsynaptic densities caveolae (caveolin 3)	phagosomes

*VSMC - vascular smooth muscle cells

term potentiation, gastroparesis and muscle disorders (7); the eNOS-deficient mice develop hypertension, abnormal remodelling and increased intimal proliferation following vascular injury (8); and iNOS-deficient mice are more susceptible to inflammatory damage and tumours, but more resistant to septic shock (9). These animals teach us a valuable lesson about the importance of NO in inflammatory reactions.

NO formed by endothelial, constitutive, NOS (eNOS) is responsible for maintaining low vascular tone and preventing leukocytes and platelets from adhering to the vascular wall (2). eNOS is also found in renal mesangial cells. NO formed by neuronal constitutive NOS (nNOS) acts as a neuromodulator or neuromediator in some central neurons and in peripheral "non-adrenergic non-cholinergic" (NANC) nerve endings. NO formed by inducible NOS (iNOS) in macrophages and other cells plays multiple roles in the inflammatory response (1).

NO was discovered by Furchgott and Zawadzki as "endothelium-derived relaxing factor" (EDRF)(10). It soon became obvious that EDRF, like nitroglycerine, activates soluble guanylate cyclase in vascular smooth muscle by binding to its active haeme centre. The rise in cyclic GMP is responsible for vasodilatation and for other physiological regulatory functions of NO (2).

The activities of constitutive nNOS and eNOS are controlled by intracellular calcium/calmodulin levels. For instance, nNOS in central neurons is activated by glutamate binding to NMDA receptors with a subsequent rise in $[Ca^{2+}]_i$ due to opening of voltage calcium channels, whereas eNOS is activated by blood shear stress or stimulation of endothelial muscarinic, purinergic, kinin, substance P or

thrombin receptors. This triggers an increase in $[Ca^{2+}]_i$ at the expense of the release of Ca^{2+} from endoplasmic reticulum (11). Calcium ionophores (e.g. A23187) and polycations (e.g. poly-L-lysine) cause a rise in $[Ca^{2+}]_i$ and activate eNOS thereby bypassing the receptor mechanisms.

In contrast to the constitutive isoforms of NOS, iNOS does not require a rise in $[Ca^{2+}]_i$ to initiate its activity. In macrophages, monocytes and other cells the induction of iNOS and the presence of L-arginine are sufficient to initiate the generation of NO. Induction of iNOS can be initiated by inflammatory cytokines IFN-γ, TNF-α or IL-1(12). However, the best recognized inducer is lipopolysaccharide (LPS) or endotoxin from *Escherichia coli* which is known to be responsible for the development of Systemic Inflammatory Response Syndrome (SIRS) in the course of sepsis due to gram negative bacteria (13). Myeloid cells have a receptor for LPS on their cell membrane, m-CD14 protein. LPS, using an "LPS binding protein" (LBP), is anchored to m-CD14 and then triggers a chain of protein phosphorylation which eventually leads to the activation of NFκB transcription factor which is responsible for transcription of the iNOS mRNA (13, 14). In cells which lack m-CD14, the induction of iNOS is completed by a complex of soluble s-CD14 with LBP and LPS itself (13, 14). In a similar manner, LPS can also induce COX-2. Although NO fulfils more paracrine than autoendocrine functions, yet in the case of iNOS, large amounts of locally formed NO may inhibit iNOS itself as well as COX-2, in a negative feedback reaction. Peroxisome proliferator-activated receptor gamma activation may play an important role in the regulation of iNOS induction. It has been demonstrated that activation of PPARgamma with a specific synthetic agonist, ciglitazone, leads to a marked decrease in apoptosis, NO generation, and the expression of NOS-2 (15, 16). Glucocorticosteroids, and some cytokines, such as TGF-β, IL-4 or IL-10, inhibit the induction of iNOS.

Constitutive and inducible NOS in inflammation

Nitric oxide production kinetics by iNOS differs greatly from the production by eNOS or nNOS (*Figure 2*). Inducible NOS produces very large, toxic amounts of NO in a sustained manner, whereas constitutive NOS isoforms produce NO within seconds and its activities are direct and short acting. There are multiple intracellular mechanisms through which nitric oxide may act as a pro-inflammatory mediator (17). Low levels of NO produced by constitutive synthases primarily interact directly with positively charged metal ions of guanylate cyclase, cytochrome p450 and NOS itself (18). Activation of guanylate cyclase leads to an increase in intracellular cyclic guanosine monophosphate (cGMP), which in turn activates cGMP dependent protein kinases which mediate NO actions including vasorelaxation, increase of vascular permeability, as well as anti-proliferative, anti-platelet and anti oxidant effects of nitric oxide (19). Recent data have also indicated that NO produced by constitutive NOS enzymes may be involved in immune regulation of T helper cell proliferation and cytokine

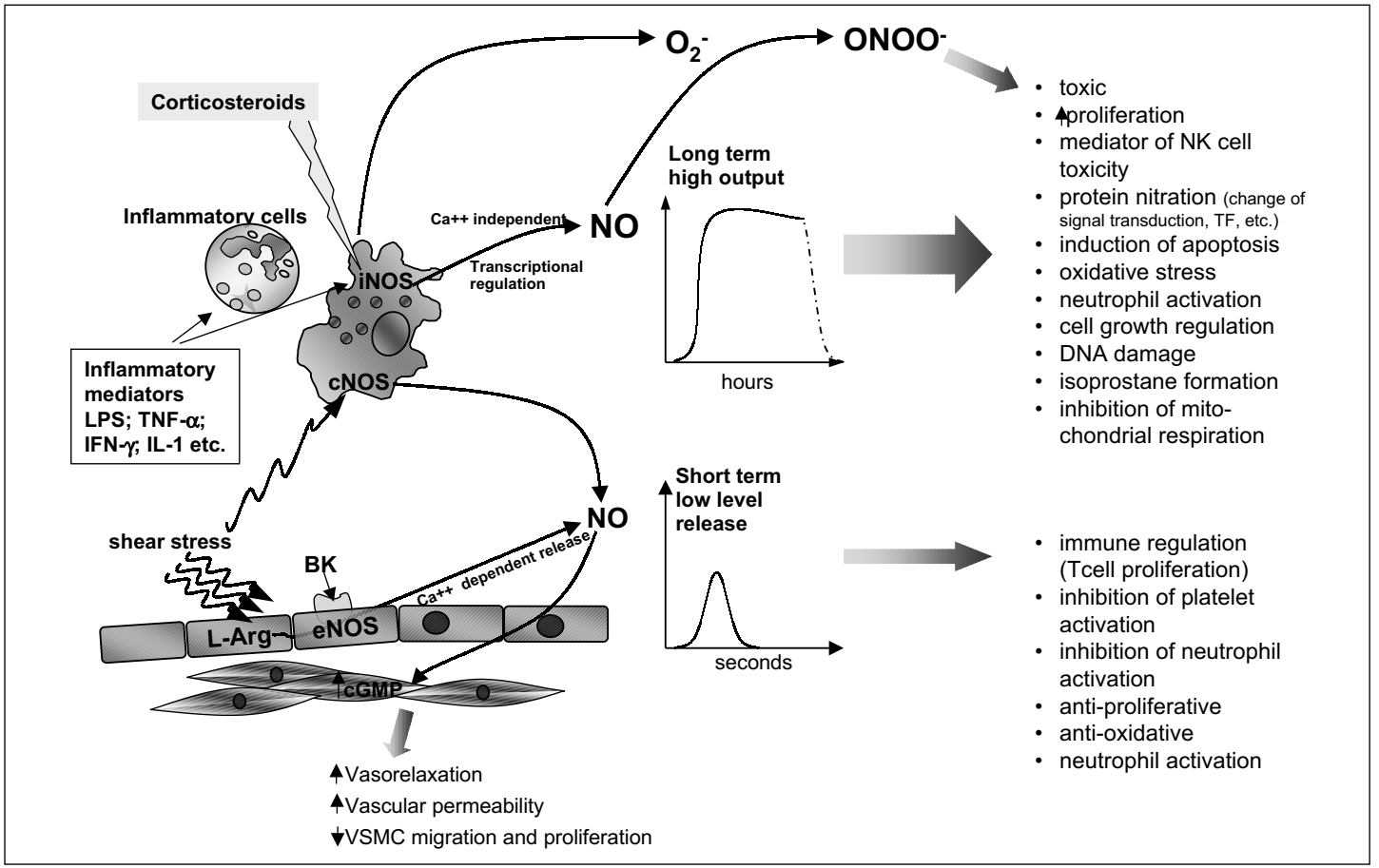

Figure 2. Kinetics and effects of nitric oxide produced by constitutive or inducible nitric oxide synthases. Importance for inflammation (see the text)

production (20). NO formed by constitutive isoforms of NOS, is stored as a nitrosothiol in albumin and may also act physiologically as N-nitrosoglutathione and N-nitrosocysteine (21, 22). NO formed by eNOS seems to be mostly cytoprotective, possibly due to its characteristic redox properties (13). Moreover, NO generated by eNOS is essential to maintain tissue perfusion with blood, to offer cytoprotection in the pulmonary and coronary circulation against toxic lipids which are released by LPS (23). NO was shown to preserve red cell deformability which becomes reduced in septicaemia (24, 25).

During the course of an inflammatory response, large amounts of NO formed by iNOS surpass the physiological amounts of NO, which are usually made by nNOS or eNOS (13). The functions of iNOS-derived NO are also different. Produced by immunologically or chemically activated macrophages, NO kills microorganisms and nitrosylates macromolecules. Eventually, within a few seconds, NO is oxidized to nitrites or nitrates. Large amounts of "inflammatory NO" from myeloid cells are usually generated side by side with large amounts of superoxide anion (O_2^-). These two can form peroxynitrite ($ONOO^-$) (26, 27) which mediates the cytotoxic effects of NO, such as DNA damage, LDL oxidation, isoprostane formation, tyrosine nitration, inhibition of aconitase and mitochondrial respiration (28). The discovery of this reaction opens a new possibilities of the therapeutic use of superoxide dismutase (SOD). Indeed superoxide dismutase mimetics have been successfully used to limit the extent of inflammation (29). Toxic properties of nitric oxide are key in the pathogenesis of septic shock (30). Over-production of NO by iNOS during septicaemia is claimed to be responsible for irreversible arterial hypotension, vasoplegia (loss of responses to noradrenaline), lactic acidosis, necrosis and apoptosis(30). However, it is important to remember that NO made by iNOS is of benefit to the host defence reaction by contributing to microbial killing. The exact role of NO in various stages of sepsis, SIRS and MODS still awaits further elucidation and evaluation.

Large amounts of NO and $ONOO^-$ may target numerous proteins and enzymes critical for cell survival and signalling. These include signalling molecules involved in cytokine signalling like JAK or STAT proteins, NKκB/IκB pathway as well as MAPK, some G proteins and transcription factors. Nitration of cysteines in these proteins may lead to their activation or inactivation (20, 31, 32).

Nitric oxide is also involved in the regulation of the release of hormones which have been shown to control inflammatory process centrally. For example, NO plays a marked inhibitory role in the CRH-induced ACTH secretion and inhibits corticosterone secretion (33).

Nitric oxide in immune regulation

In addition to its direct effector function, NO serves as a potent immunoregulatory factor. The exact role of NO in immune regulation is still ambiguous. It is mainly considered to inhibit the expression of genes involved in

cellular proliferation and growth. However, it has also been shown to have anti-apoptotic effects (34). Initial mouse studies suggested, that monocyte- and antigen presenting cell- derived NO (35) may inhibit T cell proliferation, particularly of T_{h1} subset of T helper cells (36). Further studies in iNOS knockout animals indicated a role of iNOS in the process of inhibition of T_{h1} cytokine (IFN-γ) production by T helper cells (36). iNOS knockout mice also show enhanced T_{h1} responses to *Leishmania infection* (37). Interestingly, mouse T_{h1} cells were also shown to produce NO, suggesting that the above mechanism is a part of negative feedback(38). In this way NO would inhibit T_{h1} and therefore promote T_{h2} type cytokine responses leading to humoral and allergic responses. Subsequent studies, however, indicate that both T_{h1} and T_{h2} cells produce similar amounts of NO, and both subsets respond similarly to nitric oxide (39). NO-induced changes of lymphocyte proliferation seem to be dependent more on the effects on the cell cycle proteins than due to changes in cytokine profile (40).

Nitric oxide may also regulate mast cell function. Although primarily recognised as a hallmark of allergic inflammation mast cell is now considered to be a key player in the initiation of inflammatory responses (41). IFN-γ induces nitric oxide production in peritoneal cell populations, and nitric oxide directly inhibits the IgE-mediated secretory function of mast cells (42) including histamine release from mast cells (43). Hence, activation of nitric oxide-producing cells in the tissue microenvironment may be important in the control of mast cell-dependent allergic reactions (42).

In spite of these observations it has been shown that nitric oxide may inhibit expression of numerous cytokines in lymphocytes, eosinophils, monocytes and other cells (44-47). These include cytokines critical for the development of inflammatory process like IL-1β or TNF-α expression, as well as the expression of IL-6, IFN-γ (48). These effects are exerted also by S-nitrosylation of transcription factors, including NFkB/IkB, JAK/STAT(48). All of the above transcription factors and other signal transduction pathways (e.g. MAPK etc) are critical for the signal transduction in response to cytokine and chemokine stimulation(49). Other transcription factors, like Sp1, may also be directly nitrosylated which interferes with their DNA-binding properties. In this way NO may exert effects even in the absence of changes in cytokine profile (48).

Summarising, the role of nitric oxide in immune regulation is still unclear, mainly due to the fact that it is produced by numerous cells participating at all stages of inflammation. It seems likely that its actions in immune regulation are dependent on concentrations, cellular environment and whether cells have undergone prior activation (41).

Self-protection from damaging NO effects

It is also important to note that cells which produce large amounts of toxic NO do not suffer from its toxic properties. It is mainly possible through the induction

of specific protection mechanisms. Recent studies show that GSH-GSSG anti-oxidative systems protect macrophages against iNOS generated large amounts of NO (20). Similarly endothelial cells are not the primary responder of eNOS produced NO. This is possible due to the fact that increases of intracellular calcium which mediate eNOS activation are also able to inhibit guanylate cyclase activity (50).

Pharmacological interventions in the NO-dependent mechanisms of inflammation

Studies showing importance of nitric oxide in inflammation may indicate that drugs which modulate nitric oxide production and bioavailability could be successfully used in the management of inflammatory diseases.

NO is scavenged by haemoglobin, methylene blue and pyocyanin from *Pseudomonas coereleus*. These last two are also claimed to be inhibitors of guanylate cyclase. Glucocorticoids selectively inhibit the expression of iNOS. Arginine analogues, such as L-N^G-monomethyl arginine (L-NMMA) and L-N^G-nitro-arginine methyl ester (L-NAME) inhibit inducible and constitutive NOS isoforms non-selectively. Selective iNOS inhibitors (e.g. alkylisothioureas or aminoguanidines) are being intensively investigated in the hope that selective inhibition of iNOS may prevent development of SIRS (systemic inflammatory response syndrome) or MODS (multiple organ dysfunction syndrome) (51).

Preliminary clinical experience with L-NMMA has been reasonably encouraging, as long as a low dose of the NOS inhibitor is used (52, 53). In animal models of endotoxic shock, non-selective NOS inhibitors were reported to decrease cardiac output, to increase pulmonary pressure, to decrease nutritional flow to organs, to damage gastric mucosa and to increase mortality rate (54, 55). Flavonoids are also able to inhibit the expression and activation of iNOS, and therefore could be used additionally during anti-inflammatory therapy (56).

On the other hand, inhalation of NO gas (15 ppm) in septic patients has been found to prevent the mismatch of the ventilation/perfusion ratio in their lung (57). Similarly in a model of LPS induced pancreatitis low levels of nitric oxide, along with cyclooxygenase dependent prostaglandin synthesis exerts protection from tissue damage (58, 59). Corresponding protective effects are induced by leptin, which has been shown to be mediated by anti-inflammatory properties of nitric oxide (60).

Other pharmacological therapies which act via modulation of nitric-oxide dependent pathways include statin therapy which has been shown to have pleiotropic anti-inflammatory properties partially related to their effects on nitric oxide pathway (61), but also drugs like NO donors, PPAR-gamma activators (15) or antioxidants. Finally it is important to note that non-steroidal anti

inflammatory drugs may impair protective effects of nitric oxide (62). Combination of aspirin and NO-donor could prevent these complications, however further studies are needed to evaluate this phenomenon (63, 64).

Reactive oxygen species in inflammation and immune regulation

Reactive oxygen species (ROS) production plays an important role in the modulation of inflammatory reactions. Major ROS produced within the cell are superoxide anion, hydrogen peroxide and hydroxyl radical (17). Extracellular release of large amounts of superoxide, produced as respiratory burst in leukocytes, is an important mechanism of pathogen killing and also leads to endothelial damage resulting in an increased vascular permeability as well as cell death (65).

However, vast evidence has recently implicated that intracellular ROS production plays a key role in modulation of release of other mediators of inflammation. This is related mainly to the constitutive expression of NAD(P)H oxidases (termed NOXs- non-phagocytic oxidases) in various tissues (6, 66). ROS produced by this family of enzymes can regulate adhesion molecule expression on endothelium and inflammatory cells, thus affecting cell recruitment to the sites of inflammation (67, 68). They also increase chemokine and cytokine expression (69, 70). At least part of these effects results from the ability of ROS (in particular H_2O_2) to stimulate MAP-kinases activity which leads to activation of several transcription factors. It is possible that intracellular ROS may act as second messengers in inflammatory signal transduction.

Inflammatory cytokines (like TNF-α) may in turn increase NAD(P)H oxidase activity and expression which closes vicious circle of inflammation (71). While loss of NAD(P)H oxidase activity in cells leads to diminished inflammation in the vascular wall several humoral factors may affect constitutive NAD(P)H oxidase expression in the vascular wall and therefore intracellular ROS production. These include angiotensin II, endothelins, high glucose or high cholesterol levels (6, 72). Their effects on baseline ROS production may therefore mediate modulatory effects of these factors (traditionally not considered) on inflammation.

Accordingly, attempts were undertaken to inhibit intracellular ROS production in order to limit inflammatory responses. Apocynin, an NAD(P)H oxidase activation inhibitor has been successfully used in limiting inflammation in animal model of rheumatoid arthritis (73, 74), while decoy peptide, which prevents an association of NAD(P)H oxidase subunits was shown to be effective in inflammation related to atherosclerosis (75). It is important to note that anti-oxidant properties of nitric oxide are also important in mediating anti-inflammatory properties of NO (26, 76, 77). NO inhibitory effects on NAD(P)H

oxidase can explain successful application of nitric oxide gene transfer to limit the extent of vascular inflammation (3, 78, 79).

Reactive oxygen species in immune regulation

Redox status within the cell (greatly affected by cellular superoxide production and its conversion to hydrogen peroxide, peroxynitrite and hydroxyl radicals) may regulate signal transduction pathways, therefore may affect immune regulation.

The effects of superoxide may be direct via oxidative modification of signaling molecules and transcription factors and indirect through interaction between superoxide and nitric oxide. The latter results in the loss of NO bioavailability (and its regulating properties), as well as peroxynitrite formation.

Superoxide may lead to an increase in cytosolic Ca^{2+}; however, the exact origin of Ca^{2+} is controversial. Ca^{2+} may be released from the endoplasmic reticulum, extracellular space, or mitochondria in response to oxidant-influence on Ca^{2+} pumps, channels, and transporters. Alternatively, oxidants may release Ca^{2+} from Ca^{2+} binding proteins (80). Superoxide may stimulate tyrosine as well as serine/threonine phosphorylation, and directly stimulate protein kinases as well as inhibit protein phosphatases and in this way may affect T cell proliferation (81).

Recent data indicate that H_2O_2 may be involved in lymphocyte activation, but the molecular mechanisms behind this phenomenon are not clear. Hydrogen peroxide may inhibit protein tyrosine phosphatases, and therefore act as a secondary messenger in the initiation and amplification of signaling at the antigen receptor (81). These findings explain why exposure of lymphocytes to H_2O_2 can mimic the effect of antigen (82). In addition, more recent data show that antigen receptors themselves are H_2O_2-generating enzymes and that the oxidative burst in macrophages seems to play a role not only in pathogen killing, but also in the activation of these as well as neighboring cells (83). Thus, H_2O_2 can set and influence critical thresholds for lymphocyte activation. In parallel hydrogen peroxide at higher concentrations may also be involved in the induction of apoptosis adding to its role in immune regulation (84).

Nitric oxide and superoxide in allergic inflammation

It is well recognised that allergic disorders include an important component of inflammation. Both direct cell damaging effects of NO produced in nanomolar amounts, as well as discrete immunomodulating properties may play the role in allergic inflammation. The main characteristic of allergic inflammation is a propensity to develop a sustained IgE response to common environmental antigens. T_{h2}-like TCD4+ cells are the major regulators of IgE synthesis via stimulating effects of their cytokines IL4, IL-5 and IL-12. The

CD8+ T cells as well as T_{h1} type T helper cells may suppress IgE synthesis mainly via the inhibitory effects of IFN-gamma. The role of NO in the regulation of T cell differentiation into T_{h1} or T_{h2} cells has been recently widely discussed (39). NO was found to inhibit Th1 cell proliferation and IL-2 and IFN-γ production (85). T_{h2} cells may be affected by NO to a lesser extent (36). These findings may suggest that increased amounts of NO may contribute to a preferential T_{h2} response and therefore IgE generation in allergy and asthma. Moreover, freshly isolated B lymphocytes as well as T_{h1} type cells (but not T_{h2} cells) from PBMC were found to express cNOS (21). All of these data may suggest that NO generated in sites of allergic inflammation could further enhance allergic responsiveness through its actions on IgE synthesis. However, as discussed in the previous part of this review these concepts have been challenged by some basic and clinical studies which indicate that both T_{h1} and T_{h2} cells produce similar amounts of NO, and both subsets respond similarly to nitric oxide.

Apart from apparent effects of nitric oxide on T helper cell biology it has been shown that IgE is able to increase nitric oxide synthase (iNOS) expression in human keratinocytes via IgE-CD23 interaction (86). This may further potentiate allergic reactions in urticaria patients.

Most data giving an insight into the role of NO in immune regulation are based on animal models of inflammation. Investigations in human allergic subjects are more limited. Presence of eNOS and iNOS in skin biopsies from both lesional and uninvolved areas from patients with atopic dermatitis and contact dermatitis has recently been shown by immunohistichemistry (87). Similarly NOS induction is observed in asthmatic mucosa indicating an important role for this phenomenon in this process (88).

Local inhibition of NO generation has been used to reduce erythema and oedema in both animal models and patients with atopic dermatitis and psoriasis(89). Recently, increased NOx levels have been shown in childhood AD (90). NO_x levels were increased in AD and were correlated with clinical severity (predominantly related to the area of skin lesions) or eosinophil counts. The authors concluded that local skin inflammation affects the systemic levels of serum NO metabolites in children. Indeed, the presence of increased iNOS expression in dermal vasculature and perivascular inflammatory cells was recently found in the skin lesions of AD patients during exacerbation (87). NO produced locally may without affecting systemic NO_x levels lead to the exacerbation of skin lesions, mainly via its vasodilatating actions, and through the development of oedema and erythema. In a similar study in adult patients we did not find a significant difference in NOx levels between exacerbation and remission of adult atopic dermatitis (91). We found however that NOx levels are increased in patients who show incomplete remission characterised by chronic skin inflammation. The importance of this finding remains to be elucidated.

Summarising nitric oxide may act as a double edged sword in allergic inflammation. On one hand it may act as a mediator of inflammatory responses to mast cell derived histamine, may induce Th2 dependent IgE synthesis. On the other hand NO was shown to inhibit mast cell activation, mediate inhibitory effects of IFN-γ, or inhibit vascular adhesion molecule expression thus limiting allergic inflammation. The exact mechanisms leading to predominant actions of NO in allergic inflammation remain to be described.

Genetic polymorphisms related to nitric oxide biology and inflammation

Several genetic polymorphisms have been described within eNOS and iNOS genes. Some of these may have significant functional effects. Given the importance of nitric oxide in the regulation of immune responses it is very likely that this variation could affect the inflammatory process and susceptibility to infection. Data currently available are only very sparse. eNOS gene, located on chromosome 7q35-36 contains several known polymorphisms. Only few of them however have been demonstrated to have functional consequences. Most interest has focused on Glu298Asp polymorphism within the coding sequence. Some authors have shown its functional effects (92), however data is still inconclusive (93, 94).

The second polymorphism within eNOS gene, of particular interest, is the promoter C-786T polymorphism. Several studies have shown its functional effects (95), however there are still no studies in relation to inflammation.

In the case of iNOS, genetic variation within the promoter could be most interesting from the point of its induction by LPS and cytokines. Recently a 4 bp insertion/deletion (+/-) polymorphism located 0.7 kb upstream was identified and related to coronary artery disease and the occurrence of Kawasaki disease (96, 97). Further studies are needed to determine its potential importance in the modulation of inflammation.

We would like to point out that genes encoding NAD(P)H oxidase subunits also show significant variation and could affect inflammation, immune regulation and susceptibility to infection. Indeed non-sense mutations within genes encoding NAD(P)H oxidase subunits result in the phenotype of chronic granulomatous disease. Several polymorphisms have been identified in the p22phox subunit (small membrane subunit) gene. These include coding sequence polymorphisms (C242T) and promoter G-932C polymorphisms. Both were shown to have functional significance (98, 99). Similarly to nitric oxide synthases, gene variations studies are needed to verify whether these polymorphisms have any significance for inflammation and immune regulation.

Conclusions

Nitric oxide and other reactive oxygen species play important roles in the modulation of inflammation and immune regulation. Their effects are achieved

through interactions with numerous signal transduction pathways and transcription factors. Therefore the exact effects of nitric oxide or superoxide on individual cells participating in inflammation may be ambiguous, and depend on cellular environment, NO concentration as well as other factors.

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