Role of Oxidants in Mast Cell Activation

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Abstract

Reactive oxygen species (ROS), such as superoxide, hydrogen peroxide (H₂O₂), and hydroxyl radical, have for a long time been considered as accidental by-products of respiratory energy production in mitochondria and as being useless and rather deleterious to biological systems. Contrary to such a classical view, accumulating evidence indicates that upon stimulation of divergent receptor systems, ROS are intentionally produced and even required for appropriate signal transduction and biological responses. Work by our group and that of others have shown that stimulation of mast cells through the high-affinity IgE receptor (FcεRI) induces the production of ROS such as superoxide and H₂O₂ possibly by the phagocyte NADPH oxidase homologue and that these endogenously produced oxidants have important functions in regulation of various mast cell responses, including degranulation, leukotriene secretion, and cytokine production. Subsequent studies have defined particular biochemical pathways that can be targeted by ROS and/or cellular redox balance. More recent research reveals that ROS may also play an important role in mast cell activation by divergent allergy-relevant environmental substances, for instance heavy metals and polycyclic aromatic hydrocarbons. This review summarizes current knowledge on the role of endogenous oxidants in mast cell activation.

ROS and Regulation of the Cellular Redox Balance

It is well known that respiratory energy production in mitochondria has a considerable advantage in generating energy compared to glycolysis. As the terminal electron acceptor for oxidative phosphorylation, molecular oxygen (O₂) plays an essential role in various processes. This type of oxygen use however results in the production of a variety of reactive oxygen species (ROS) such as
superoxide (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (·OH) as accidental by-products [1]. ROS readily react with cellular macromolecules, either damaging them directly, or setting in motion a chain reaction in which free radical is passed from one molecule to another, resulting in extensive damage of cellular structures such as the membrane. In addition to the respiratory energy production, various intra- and extracellular systems are involved in the production of ROS (collectively referred to as the prooxidant system). For instance, ultraviolet and ionizing radiation like γ-rays are capable of producing ROS such as H$_2$O$_2$ and ·OH. Phagocytes including neutrophils and macrophages produce large amounts of ROS, O$_2^-$ and H$_2$O$_2$, via activation of the phagocyte NADPH oxidase in response to microbial infection and soluble stimuli. The catalytic moiety of the phagocyte NADPH oxidase is the membrane-associated flavocytochrome gp91$^{phox}$ and activation of the gp91$^{phox}$ system occurs through assembly of the cytosolic regulatory proteins, p40$^{phox}$, p47$^{phox}$, and p67$^{phox}$, and small GTP binding protein Rac [for a review, see 2]. This respiratory burst however may force other types of cells to become exposed to considerable levels of ROS. Thus, ROS have been considered as being useless and rather deleterious to biological systems.

To overcome these ultimately toxic oxygen species, early forms of life needed to develop simultaneously an effective defensive system that could cope with unwanted ROS [3]. This system, called the antioxidant system, contains molecules capable of either scavenging and/or detoxifying ROS, blocking free radical chain reactions, or removing transition metals which can serve as a ready source of free electrons. Consequently, an aerobic existence is accompanied by a persistent state of oxidative siege, where the survival of a given cell is determined by the balance between ROS and antioxidants [4] (fig. 1). It is not surprising therefore that oxidative stress (a term which refers to the imbalance between the occurrence of oxidants and antioxidants) causes cell dysfunction and death.

**Generation of ROS in Non-Phagocytic Cells through the NOX/DUOX Family**

The concept of oxidative stress defines a dynamic situation in which the balance between the occurrence of oxidants and antioxidants in biological systems can indirectly influence cellular functions and phenotypes through an effect on other cellular components, particularly redox-sensitive functional groups and proteins. However, the term ‘stress’ often carries a rather negative nuance. In terms of a positive role for ‘oxidative stress’, it is proposed more accurately to use a term such as ‘oxidant-mediated regulation’. In relation to
this concept, accumulating evidence indicates that ROS act as signal intermediates in intracellular signaling to activation of mitogen-activated protein kinase (MAPK) family members, gene expression, and/or cell proliferation [5–9].

In the last decade it has been revealed that non-phagocytic cells like epithelial cells, smooth muscle cells, and endothelial cells also generate O$_2^\cdot$⁻. Our group and others have demonstrated that upon cell activation lymphocytes generate ROS, primarily O$_2^\cdot$⁻, and that ROS and/or an altered redox balance may regulate various cell functions including adhesion, proliferation, and apoptosis [6, 10–15]. Although production of ROS was frequently attributed to mitochondrial respiration, in some cases, use of inhibitors suggested that the actual source might be a flavoprotein that is similar to gp91phox. Subsequent studies revealed the occurrence of a new family of homologues of gp91phox, the
NADPH oxidase (NOX)/dual oxidase (DUOX) family in non-phagocytic cells. The NOX/DUOX family now includes NOX1 (initially referred to as Mox1, NOH-1 [9, 16]), which is predominantly expressed in colon, NOX3 (gp91–3 [17]) cloned from fetal kidney, NOX4 (Renex [18]) found in kidney cortex, NOX5 found predominantly in testis, spleen, and lymph nodes. The NOX family members have almost the same length as gp91phox (~560–580 amino acids) [19] (gp91phox is also referred to as NOX2). The DUOX family members are longer (~1,550 amino acids) because of their N-terminal extensions consisting of two EF hands (presumed binding) motifs, an additional transmembrane helix, and a peroxidase homology domain. The DUOX family includes DUOX1 (initially termed Thox1) and DUOX2 (Thox2) [20, 21]. The NOX enzymes have been proposed to function in generating ROS as mediators of signal transduction relating to growth, angiogenesis, and apoptosis [for a review, see 22].

Role of ROS in Mast Cell Activation

**FceRI Signaling in Mast Cells**

Mast cells play a critical role in allergic reactions. Mast cells express the high-affinity IgE receptor (FceRI) on the cell surface and aggregation of FceRI by IgE-antigen complexes initiates a cascade of intracellular signaling events that lead to degranulation, inflammatory mediator release, and cytokine production, contributing to allergic and inflammatory reactions [23, 24]. FceRI is a tetramer of α-, β-, and γ-chain homodimers [25], of which the α-chain binds IgE, while the β- and γ-chain mediate intracellular signaling through the receptor. Like divergent receptors found in lymphocytes, FceRI lacks intrinsic enzyme activity but the β- and γ-chains contain the immunoreceptor tyrosine-based activation motif (ITAM), which is critical for cell activation through these receptors [26, 27]. The β-chain ITAM is recognized as an important site of interaction with Lyn for signal transduction. It is now clear that the β-chain acts as a signal amplifier in mast cells and is important for augmenting allergic reactions [28–31]. In humans, it has been demonstrated that the γ-chain can act as an autonomous signaling molecule, whereas the β-chain can amplify early activation signals through the γ-chain by more than 5-fold [28]. The ITAM consensus sequence contains tyrosine residues, which are phosphorylation sites for receptor-associated tyrosine kinases and once phosphorylated, serve as a docking site for cytoplasmic proteins that contain the Src-homology-2 (SH2) domain. It is believed that aggregation of FceRI causes tyrosine phosphorylation of the β-chain ITAM by activated Lyn, and subsequent phosphorylation of Syk and the γ-chain ITAM, which in turn leads to downstream signals including Ca²⁺ mobilization and activation of protein kinase C and MAPK family
members [27, 32]. The signal transduction pathway in mast cells has been extensively reviewed elsewhere and will not be further addressed here.

Generation of ROS in Mast Cells and Basophils

Earlier studies demonstrated that rat peritoneal mast cells generated a substantial level of ROS. Both pharmacological agents (mercury and gold salts, compound 48/80, Ca^{2+} ionophores) and physiologically relevant stimuli (antigen, nerve growth factor, substance-P) stimulated the generation of intracellular ROS, although the nature of the oxidant generated was not defined [33, 34]. However, some investigators claimed that the production of ROS observed in rat peritoneal mast cells was attributed to contaminating macrophages rather than to mast cells by themselves [35]. Thus, there are conflicting data regarding whether mast cells can actually generate ROS. We have succeeded in detecting the generation of ROS in RBL-2H3 cells (rat mast cell line) and bone marrow-derived cultured mast cells [36–38]. The production of ROS is sensitive to diphenyleneiodonium, which inhibits the phagocyte NADPH oxidase and other flavoproteins, suggesting that a homologue of NADPH oxidase, possibly a NOX/DUOX family member, is the actual source of oxidants. Our previous works indicated that mast cells can produce O_2^- and H_2O_2 in response to the elevation of cytosolic Ca^{2+} concentration ([Ca^{2+}]_i). Because NOX5 and presumably DUOX1/DUOX2 can be activated in response to the rise in [Ca^{2+}], these enzymes might be responsible for the generation of ROS in mast cells.

We detected the generation of ROS especially O_2^- in human leukocytes, predominantly basophils, which were stimulated through FcεRI. Similar generation of ROS was observed when leukocytes from patients sensitized with mite allergens were challenged with the relevant allergen but not irrelevant allergen [37]. This indicates that the oxidative burst at least of basophils is allergen-specific, suggesting its relevance to allergic reactions. The fact that divergent environmental substances relevant to allergy can induce and/or enhance the generation of ROS may support the view. Mercuric chloride is well known to cause type I and IV allergic reactions. At relatively high concentrations the compound can induce the generation of ROS in rat peritoneal mast cells and at lower concentrations it augments antigen-induced ROS generation [33]. We recently revealed that heavy metals silver and gold, both of which induce severe allergic inflammation in humans, can directly activate mast cells through a ROS-dependent signaling pathway [39]. Polycyclic aromatic hydrocarbons (PAHs) are major components of diesel exhaust particles found in respirable particles of air pollutants and these consumption products of fossil fuel exacerbate allergic inflammation. PAHs such as benzo(a)pyrene and benzo(a)pyrene quinones were found to enhance antigen-induced ROS production and mediator release in human basophils [40].
Signal Transduction Pathway for ROS Generation

Studies employing Lyn- or Syk-deficient mice have demonstrated a critical role of both tyrosine kinases in FcεRI-mediated mast cell activation [41, 42]. The Tec family kinases are another class of tyrosine kinases that are implicated in mast cell activation. Btk, Itk, and Tec are expressed in primary cultured mast cells and both Btk and Itk are also activated upon FcεRI engagement, suggesting their functional roles in mast cell activation [43]. To gain further insights into roles of these signaling components in the generation of ROS, we tested the effects of divergent pharmacological inhibitors of them on the generation of ROS. As a result, Src family kinase(s), Syk kinase, and the phosphoinositide-3-kinase (PI-3K) may be necessary for the generation of ROS [38]. Lyn is a major Src family kinase in mast cells and binds to FcεRIβ loosely even in resting cells and is activated immediately upon FcεRI engagement to bind to FcεRIβ tightly and transduce downstream signals [44]. It is established that the β-chain ITAM is an important site of interaction with Lyn for signal transduction [27, 32], suggesting that the β-chain ITAM is also important for the induction of ROS production. To test this hypothesis, we generated BMMCs, in which all tyrosine residues of the β-chain ITAM are replaced by phenylalanine and compared the generation of ROS in BMMCs expressing the wild-type and the mutated type of the β-chain. The results revealed that the association between Lyn and FcεRIβ via the β-chain ITAM is necessary for the PI-3K-dependent generation of ROS [unpubl. observations]. The Btk-selective inhibitor LFM A-13 indicated that Btk may also be involved in the generation of ROS in mast cells. Activation of PI-3K results in the production of phosphatidylinositol-3,4, 5-triphosphate (PIP₃). The interaction of the pleckstrin homology (PH) domain with PIP₃ then leads to targeting of Tec family kinases to specific membrane microdomains (lipid rafts), which is a critical step for their activation upon antigen receptor stimulation. For full activation of Tec family kinases, a second step, phosphorylation of a tyrosine residue within the activation loop of the kinase domain by Src family kinases, may also be required [for a review, see 45]. Because Src family kinases and PI-3K are required for the generation of ROS, and destruction of lipid rafts abolishes the generation of ROS [unpubl. observations], the translocation of Btk to lipid rafts and phosphorylation by Src family kinases might be a critical step in the signal transduction pathway for the generation of ROS. The putative signal transduction pathway is illustrated in figure 2.

Role of Oxidants in FcεRI Signaling and Allergy

Because the generation of ROS in mast cells is sensitive to diphenyleneiodonium, the reagent is useful to investigate especially the role of $O_2^-$ in mast cell activation.
Blockade of the generation of ROS could prevent FcεRI-mediated degranulation and LT secretion in BMMCs [38]. Basically the same results were obtained with RBL-2H3 mast cells and human leukocytes, predominantly basophils. Furthermore, blocking O$_2^-$ generation also suppressed mediator secretion in allergen-challenged cells from patients with atopic dermatitis [37]. These observations imply that O$_2^-$ plays a common facilitating role in mediator release under pathophysiological conditions. The SOD mimetic MnTBaP possesses both superoxide dismutase and peroxidase activity in vitro and inhibits T-cell receptor-mediated ROS generation [14] and is expected to abolish FcεRI-mediated ROS generation. MnTBaP also suppressed FcεRI-mediated mediator release at a comparable dose. The glutathione peroxidase mimetic ebselen was employed to examine the selective role of H$_2$O$_2$ in redox regulation of mast cell responses. Interestingly, the effects of ebselen were
different from those of MnTBAp. In particular, ebselen had no significant inhibitory effect on degranulation, although it abolished cytokine production. Collectively, these observations suggest selective roles of $\text{O}_2^-$ and $\text{H}_2\text{O}_2$ in the regulation of distinct mast cell responses.

The next question is how ROS regulate chemical mediator release (degranulation) in mast cells. The observations that blockade of ROS generation and scavenging ROS reduce divergent responses suggest the common targets of ROS in the signal transduction pathways of mast cells. As described above, one of the earliest steps upon activation of mast cells is increased tyrosine phosphorylation of several proteins. Therefore, we examined whether blocking ROS generation affected the tyrosine phosphorylation of cellular proteins. In the initial studies, some proteins including the focal adhesion kinase (FAK) pp125FAK, a molecule critical for intracellular signaling to degranulation, were identified as likely targets of ROS [36]. Subsequent studies demonstrated that these molecules lie in the downstream of the activation of Ca$^{2+}$ influx. In addition, blockade of ROS generation as well as scavenging ROS also impaired Ca$^{2+}$ mobilization [38]. Thus, endogenously produced ROS and exogenously added ROS commonly affect Ca$^{2+}$ mobilization. These observations support the view that Ca$^{2+}$ mobilization is a primary target of ROS and/or altered redox status. Recent work indicated that FcεRI stimulation leads to the formation of macromolecular complex analogous to Ca$^{2+}$ signalsomes of T and B cells and contains some of the same components, including Syk, the linker for activation of T cells (LAT), Btk, Itk, SLP-76, Vav1, and PLC$\gamma$1. Assembly of this LAT-orientated complex is tyrosine phosphorylation-dependent and requires Syk and Lyn, and is essential for full activation of Ca$^{2+}$ mobilization [32, 46]. In this respect it should be noticed that tyrosine phosphorylation of PLC$\gamma$-1/2 and LAT is abolished by inhibiting ROS generation or by scavenging ROS [38], which suggests the possibility that ROS are required for the formation of and/or maintaining the Ca$^{2+}$ signalsomes (fig. 2).

**Conclusions**

Generation of ROS is a common signal transduction event in a variety of functional responses in mast cells. ROS play a critical role in the regulation of mast cell signaling including Ca$^{2+}$ mobilization, although further studies are necessary for revealing the molecular mechanisms of ROS generation and of the regulation of different cellular responses. More recent research has indicated that environmental substances that induce and/or exacerbate allergic inflammation such as heavy metals and diesel exhaust particles also stimulate and/or enhance FcεRI-mediated ROS generation and oxidant-regulated mast
cell activation. Thus, better understanding and specific targeting of the generation of ROS in mast cells might offer a promising new approach for the treatment of allergic disorders.

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