*Review*

# Microglial inflammatory reactions regulated by oxidative stress

**Yasuhiro Ishihara[1,*] and Kouichi Itoh[2]**

[1]Program of Biomedical Science, Graduate School of Integrated Sciences for Life, Hiroshima University, Hiroshima 739-8521, Japan
[2]Laboratory for Pharmacotherapy and Experimental Neurology, Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University, Kagawa 769-2193, Japan

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**Microglia are immune cells in the brain that can respond to endogenous and exogenous substrates to elicit inflammatory reactions. The transcription factor nuclear factor kappa-light-chain-enhancer of activated B induces proinflammatory gene expression in response to foreign matter via pattern recognition receptors; thus, nuclear factor kappa-light-chain-enhancer of activated B is a master regulator of inflammation. During the inflammatory process, very large amounts of reactive oxygen species are generated and promote the onset and progression of inflammation. Interestingly, nuclear factor kappa-light-chain-enhancer of activated B drives the transcription of superoxide dismutase 2 in many types of cells, including microglia. Superoxide dismutase 2 is an antioxidative enzyme that catalyzes the dismutation of superoxide anions into molecular oxygen and hydrogen peroxide. Of note, nuclear factor kappa-light-chain-enhancer of activated B can initiate inflammation to elicit proinflammatory gene expression, while its transcription product superoxide dismutase 2 can suppress inflammation. In this review, we use recent knowledge to describe the interaction between oxidative stress and nuclear factor kappa-light-chain-enhancer of activated B and discuss the complicated role of microglial superoxide dismutase 2 in inflammation.**

***Key Words*: microglia, inflammation, reactive oxygen species, NF-κB, superoxide dismutase 2**

Microglia constitute the primary immune cells of the central nervous system (CNS). Activated microglia secrete pro-inflammatory cytokines and chemokines. While release of these factors prevents further damage to CNS tissue, they can be toxic to neurons and other glial cells to exacerbate neuronal injury. Oxidative stress is also induced during inflammation and can regulate microglial activity. In this review, we describe the interaction between inflammatory reaction of microglia and oxidative stress, focusing on the function of nuclear factor kappa-light-chain-enhancer of activated B (NF-κB).

## Microglia

Microglia constitute the primary immune cells of the CNS and are activated quickly in response to external pathogens or cell debris, after which they act by releasing inflammatory factors and/or engulfing foreign bodies to mediate the inflammatory response. In addition, microglia are required for developmental synaptic remodeling. In the process of neural circuit formation, microglia are involved in the maturation of functional neural circuits by removing weakly active synapses and retaining strongly active synapses.[1,2] In 2010, lineage tracing studies strongly suggested that primitive myeloid precursors give rise to adult microglial cells in the CNS and are maintained with minimal contribution from circulating monocytes in the steady state.[3] On the other hand, PLX3397, a macrophage colony stimulating factor receptor antagonist, eradicates microglia in the murine brain, but microglia repopulate the brain again once the administration of PLX3397 has ceased.[4] Microglial repopulation is dependent on both the local self-renewal of microglia and recruitment of Ly6C$^{hi}$ monocytes in mice.[5] Therefore, there is a possibility that monocytes in the blood differentiate into microglia.

## Reactive Oxygen Species (ROS) Generated in the Microglia

Macrophages are known to produce ROS during phagocytosis and bacterial killing. M2-oriented macrophages activate NADPH oxidase to generate superoxide anions. NADPH oxidase is a multisubunit enzyme complex that transfers electrons to molecular oxygen from NADPH or, to a lesser extent, NADH. The main source of ROS in microglia is NADPH oxidase.[6] Microglia constitutively express a superoxide anion-generating Nox2 subunit (also called gp91phox) similar to macrophages.[7] When microglia detect several insults, microglial NADPH oxidase is activated to produce a large amount of ROS as a part of the defense response to protect the CNS; this also causes inflammatory damage to brain tissue.[8] Mitochondria are another source of ROS inside activated microglia. Bacterial endotoxin, lipopolysaccharide (LPS), and several chemicals, such as copper chloride, have been shown to stimulate mitochondrial superoxide production in microglia.[9,10] Naik and Dixit[11] suggested that alterations in the redox environment of the plasma membrane could cause mitochondrial ROS formation. Alternatively, when ROS generated from certain parts inside cells act on mitochondria, mitochondrial permeability transition and/or the opening of inner membrane anion channels occur, leading to intra- and inter-mitochondrial redox environment changes and subsequent further ROS generation. This regenerative cycle of mitochondrial ROS formation and release is named ROS-induced ROS release and is a process in which a small amount of ROS can be amplified.[12,13] Therefore, even if there are few ROS generated other than in mitochondria, such as in the cytosol and endoplasmic reticulum, these ROS are closely linked to mitochondrial ROS production and are able to induce oxidative stress.

ROS generated by activated microglia are thought to be involved in several CNS disorders. In Alzheimer's disease (AD), microglia in the brain strongly express gp91phox, a subunit of NADPH oxidase, suggesting that NADPH oxidase is activated in

*To whom correspondence should be addressed.
E-mail: ishiyasu@hiroshima-u.ac.jp
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microglia in the AD brain.[14] Inhibition of NADPH oxidase by apocynin or genetic deletion of gp91phox in AD model mice overexpressing mutant amyloid precursor protein (Tg2576) attenuates cerebral amyloid angiopathy.[15,16] Therefore, microglial ROS are strongly involved in AD pathology. We previously reported that microglia were activated in mice with permanent middle cerebral artery occlusion and that pretreatment with minocycline, an inhibitor of microglial activation clearly suppressed not only vasogenic edema but also infarct development,[17] suggesting that microglia are also involved in the pathogenesis of ischemic stroke. Considering that ROS and oxidative stress can contribute to the onset and/or progression of many CNS diseases,[18] additional roles of microglia in these pathologies will likely be discovered in the future.

## Dual Role of NF-κB: Proinflammation and Antioxidation

NF-κB is a master regulator of inflammation in immune cells, including microglia. NF-κB is well known to upregulate many inflammatory molecules, such as cytokines, chemokines, and cyclooxygenases.[19] NF-κB is a dimer of p50 and p65 (RelA) subunits and is sequestered in the cytosol by binding with inhibitor of κB (IκB). NF-κB is activated by 2 steps: phosphorylation of IκB and subsequent degradation of IκB, which induces the nuclear localization of NF-κB. Then, in the nucleus, p65 is phosphorylated to increase its DNA binding activity.

NF-κB is also known to increase antioxidative enzymes and antioxidative proteins to contribute to the defense against oxidative injury (Table 1). Superoxide dismutase (SOD) catalyzes the dismutation of superoxide anions to molecular oxygen and hydrogen peroxide, providing cellular defenses against ROS. SODs contain corresponding metal ions (Cu, Zn, Mn, Fe, and Ni) at their active sites. In mammalian cells, SOD1 (Cu/Zn-SOD) is present in the cytoplasm, nucleus, and peroxisomes, while SOD2 (Mn-SOD) is located in the matrix of mitochondria. SOD2 is one of the major antioxidative enzymes whose expression is driven by NF-κB. The promoter region of SOD2 has a functional NF-κB binding element,[20] and SOD2 expression is induced via the NF-κB pathway in several types of cells.[20–22] SOD1 has been reported to be upregulated by NF-κB;[23] however, it has also been reported that SOD1 downregulates NF-κB,[24] and SOD1 expression is negatively correlated with NF-κB levels.[25] Therefore, SOD1 and NF-κB might form a feedback loop to regulate the expression of each other. Catalase and glutathione peroxidase (GPx) convert hydrogen peroxide to molecular oxygen and water. GPx uses reduced glutathione as a substrate to oxidize to glutathione disulfide. Catalase and GPx are induced via the NF-κB pathway.[26,27] In addition, glutathione *S* transferase and metallothionein 3 are also upregulated in an NF-κB-dependent manner.[28,29]

NAD(P)H:quinone oxidoreductase 1 (NQO1, DT-diaphorase) is an obligate two-electron reductase for quinones. Quinones can generate very large amounts of superoxide anions via the redox cycle. Quinones receive an electron from NAD(P)H under catalysis by several enzymes, including NADPH cytochrome P450 reductase, to produce semiquinone radicals. Semiquinone radicals are unstable and thus donate an electron to molecular oxygen and turn back to quinones.[30] During this process, superoxide anions are generated. Because NQO1 can catalyze the 2-electron reduction of quinones to generate stable hydroquinones, NQO1 can suppress oxidative insults. NQO1 is reportedly upregulated under NF-κB activation.[31] NQO1 is well known to be a major target of the transcription factor nuclear factor (erythroid-derived 2)-like 2 (Nrf-2), and Nrf-2 is activated under oxidative stress conditions by Kelch-like ECH-associated protein 1 oxidation and subsequent degradation. Because inflammation is accompanied by oxidative stress, NQO1 upregulation may be due to Nrf-2 activation. This hypothesis is supported by the finding that heme oxygenase-1, another major target of Nrf-2, also increased under NF-κB activation.[32]

## NF-κB Activity and Oxidative Stress

NF-κB is widely known to be activated under oxidative stress. The major mechanism of NF-κB activation by oxidative stress is the inactivation of protein phosphatases by ROS. The phosphorylation state of NF-κB and its upstream kinase IκB are regulated by several protein tyrosine phosphatases (PTPs), such as PTP1B and PTEN. The inactivation of PTPs induces sustained phosphorylation of IκB, which enhances its degradation, followed by potentiation of NF-κB nuclear localization. PTPs have a cysteine residue at their active site; thus, PTPs are sensitive to ROS and are rapidly inactivated by ROS stimuli.[33,34] Hydrogen peroxide is known to react with the thiolate anion to initially produce sulfenic acid.[35] One of the major PTPs, PTP1B, features an $HCX_5R$ active site motif with a highly nucleophilic cysteine residue at position 215. Because of its low pKa of approximately 5.4 (where Ka is the acid dissociation constant), Cys215 is susceptible to oxidation by hydrogen peroxide.[36] Generally, irreversible oxidation of cysteine residues to sulfinic/sulfonic moieties impairs protein function. However, oxidation of the thiol groups of PTPs has been reported to be reversible, and PTP activity can be recovered by treatment with thiol-reducing agents and/or thiol reductases, including dithiothreitol, reduced glutathione, and thioredoxin/thioredoxin reductase.[37,38]

Recent reports have indicated that reactive sulfur intermediates, such as hydropersulfides (RSSH moieties) and polysulfides [$RS(S)_nH$ and $RS(S)_nSR$], have several fundamental biological properties. Importantly, a hydropersulfide moiety with the general molecular formula RSSH can be formed on specific protein cysteine residues.[39] Persulfidation (the formation of CysSSH groups) protects cysteine from irreversible oxidative loss of function by the formation of -SSOH, $-SSO_2H$ and $-SSO_3H$ derivatives that can subsequently be reduced back to native

**Table 1.** Antioxidative molecules that are upregulated via the NF-κB pathway

| Antioxidative enzyme/protein | Cell Type | Reference |
|---|---|---|
| Superoxide dismutase 1 | PC12 | Rojo *et al.*[23] |
| Superoxide dismutase 2 | PC6.3 | Kairisalo *et al.*[22] |
| Catalase | C2C12 | Zhou *et al.*[26] |
| Glutathione peroxidase 1 | U937 | Schreiber *et al.*[27] |
| Glutathione S transferase pi | HeLa | Xia *et al.*[28] |
| Metallothionein 3 | Human keratinocytes | Hinata *et al.*[29] |
| Metallothionein 3 | Human fibroblasts | Hinata *et al.*[29] |
| NAD(P)H:quinone oxidoreductase 1 | HT29 | Yao *et al.*[31] |
| Heme oxidase 1 | K562 | Lavrovsky *et al.*[32] |


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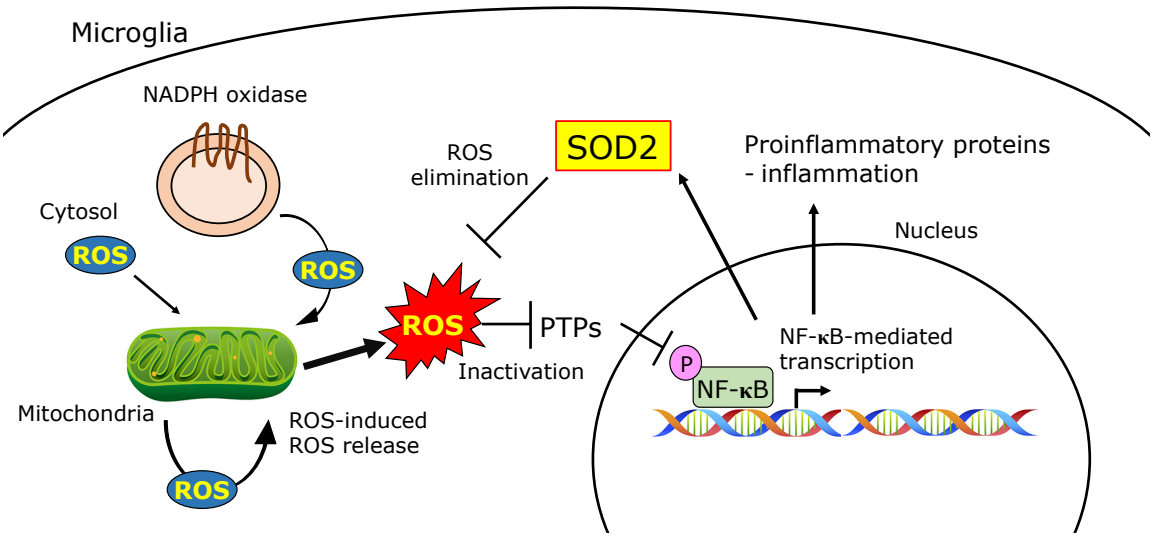


**Fig. 1.** The role of SOD2 in activated microglia. During inflammation when TLRs are stimulated, ROS are generated from NADPH oxidase and/or mitochondria, which are amplified in the mitochondria, in a process named ROS-induced ROS release. Activated NF-κB upregulates the expression of SOD2 as well as proinflammatory proteins such as cytokines and chemokines. NF-κB activity is regulated by its phosphorylation, including the phosphorylation of the Ser276 residue of the p65 subunit. ROS inactivate protein phosphatases to enhance NF-κB phosphorylation, followed by NF-κB activation. SOD2 eliminates ROS, resulting in the suppression of NF-κB activity. Collectively, transcriptionally activated SOD2 downstream of NF-κB attenuates NF-κB activity. This process might terminate the inflammatory reaction in microglia.

thiols.[40] Thus, this mechanism might protect and regulate PTPs, including PTEN and PTP1B.[41] Collectively, because ROS can suppress PTPs and persulfites can activate PTPs, this balance could affect NF-κB activity.

Direct oxidation of NF-κB by ROS is also reported to suppress NF-κB transcriptional activity.[42] Cys62 of p50 is in the Rel homology domain, and thus, its oxidation inhibits DNA binding. This oxidation is probably followed by an *S*-glutathionylation reaction since glutathionated NF-κB shows reduced transcriptional activity.[43] Cys62 is also modulated by nitric oxide to produce *S*-nitrosylated cysteine, which is catalyzed by inducible nitric oxide synthases (iNOSs).[44] Because iNOS is upregulated downstream of NF-κB, this process might form a negative feedback loop for the NF-κB transcription pathway.

Phosphorylation of Ser276 in p65 is important for DNA binding and thus for the expression of NF-κB-dependent genes.[45] Phosphorylated Ser276 is necessary for the interaction of p65 with CBP/300 and positive transcription elongation factor b.[45,46] Antioxidant treatment has been reported to suppress Ser276 phosphorylation and CBP/300 binding,[47] suggesting that oxidative stress can regulate Ser276 phosphorylation. In rat primary microglia, NF-κB p65 was localized in the nucleus but not in the cytosol under unstimulated conditions, and LPS treatment enhanced Ser276 phosphorylation and increased the expression of proinflammatory cytokines (Ishihara *et al.*, unpublished observations). NF-κB regulation can be dependent on culture conditions as well as cell type, and p65 Ser276 modulation is important, at least for the inflammatory reaction of primary microglia.

## Microglial Inflammatory Reactions Regulated by ROS

Microglia recognize the surrounding environment with pattern recognition receptors in a manner similar to macrophages and dendritic cells. Toll-like receptors (TLRs) are a family of receptors that can detect many endogenous and exogenous molecules: TLR3 can bind to RNA, TLR4 to LPS and TLR9 to CpG DNA, and NF-κB activation and subsequent proinflammatory gene upregulation occur downstream of TLR stimulation. The effects of NF-κB on antioxidation in microglia are largely unclear, although we and others reported that SOD2 expression increased in activated microglia. Sugaya *et al.*[48] reported that SOD2 was induced in the LPS-treated murine microglial cell line BV-2, which was mediated in part by nitric oxide. Treatment of rat primary microglia with interferon-gamma elicited SOD2 expression.[49] We have shown that several TLR ligands, such as poly(I:C), peptidoglycan and CpG DNA, in addition to LPS, induced SOD2 expression in rat primary microglia.[20] Kaneko *et al.*[50] reported that SOD1 and SOD2 were upregulated in murine primary microglia treated with LPS. However, SOD1 was not induced in activated microglia from rats.[20,49] Issues regarding whether there are species differences in the expression of antioxidant enzymes will be addressed in the future.

Activated microglia with increased SOD2 expression showed high tolerance to ROS, including hydrogen peroxide and superoxide anions, compared with unstimulated cells.[20,49] Furthermore, SOD2 suppressed NF-κB activity and proinflammatory cytokine expression because of ROS elimination by SOD2,[20] and thus, SOD2 has a possible role in terminating the inflammatory reaction (Fig. 1). That is, NF-κB-SOD2 signaling forms a feedback loop. ROS are generated by mitochondria in activated microglia as described above, and SOD2 can effectively eliminate superoxide anions to suppress inflammatory reactions. Collectively, NF-κB initiates an inflammatory reaction by the transcription of proinflammatory molecules and stops inflammation to abolish its antioxidative activity.

## Conclusion

Microglia can be activated by several substances, including ROS, to induce inflammatory reactions. NF-κB acts as a master regulator of inflammation by increasing the expression of cytokines/chemokines. It also upregulates antioxidative enzymes such as SOD2 during inflammation, which can terminate inflammatory reaction by eliminating ROS. Recently, the pathophysiological role of SOD2 has become more complicated. For instance, skin inflammation induces a proatherosclerotic macrophage phenotype with impaired SOD2 function, which is associated

with accelerated atherogenesis,[51] suggesting effects of SOD2 across tissues. Although there is no doubt that the major function of SOD2 is ROS elimination in the mitochondria, this seemingly simple role has a great deal of missing links that have yet to be elucidated.


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## Abbreviations

| | |
|---|---|
| AD | Alzheimer's disease |
| CNS | central nervous system |
| GPx | glutathione peroxidase |
| IκB | inhibitor of κB |
| iNOS | inducible nitric oxide synthases |
| LPS | lipopolysaccharide |
| NF-κB | nuclear factor kappa-light-chain-enhancer of activated B |
| NQO1 | NAD(P)H:quinone oxidoreductase 1 |
| Nrf-2 | nuclear factor (erythroid-derived 2)-like 2 |
| PTPs | protein tyrosine phosphatases |
| ROS | reactive oxygen species |
| SOD2 | superoxide dismutase 2 |
| TLRs | Toll-like receptors |

## Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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