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COMPREHENSIVE INVITED REVIEW

Functional Aspects of Redox Control During Neuroinflammation

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Abstract

Neuroinflammation is a CNS reaction to injury in which some severe pathologies, regardless of their origin, converge. The phenomenon emphasizes crosstalk between neurons and glia and reveals a complex interaction with oxidizing agents through redox sensors localized in enzymes, receptors, and transcription factors. When oxidizing pressures cause reversible molecular changes, such as minimal or transitory proinflammatory cytokine overproduction, redox couples provide a means of translating the presence of reactive oxygen or nitrogen species into useful signals in the cell. Additionally, thiol-based redox sensors convey information about localized changes in redox potential induced by physiologic or pathologic situations. They are susceptible to oxidative changes and become key events during neuroinflammation, altering the course of a signaling response or the behavior of specific transcription factors. When oxidative stress augments the pressure on the intracellular environment, the effective reduction potential of redox pairs diminishes, and cell signaling shifts toward proinflammatory and proapoptotic signals, creating a vicious cycle between oxidative stress and neuroinflammation. In addition, electrophilic compounds derived from the oxidative cascade react with key protein thiols and interfere with redox signaling. This article reviews the relevant functional aspects of redox control during the neuroinflammatory process. *Antioxid. Redox Signal.* 13, 193–247.

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I. Introduction

A. Definitions and scope

The term "neuroinflammation" describes an atypical inflammation because of the lack of the classic signs of inflammation: redness, pain, heat, and swelling, as observed in the periphery because of an increased vascular permeability, accompanied by a flux of cytokine-releasing phagocytic cells to the site of injury. In the central nervous system (CNS), increased glial activation, proinflammatory cytokines, and complement concentrations in response to injury occur; this is identified as "neuroinflammation" (*i.e.*, a CNS tissue reaction to injury).

Neuroinflammation involves the accumulation of astrocytes and the CNS-resident macrophages, microglia, which release factors that act on and produce responses in target cells analogous to the responses of activated immune cells in the periphery (229, 517). The term is used to describe both acute injuries and chronic CNS diseases, in which the inflammation is primarily a glia-driven process, despite the eventual leukocyte or monocyte infiltration. This is because reactive microglia may recruit peripheral immune cells apart from other glial cells to the site of injury; this implies disruption of blood–brain barrier (BBB) permeability and leukocyte/monocyte invasion. However, the primary response is a CNS-specific, microglia-driven innate immune response that, based on environment signals, may result in beneficial or harmful outcomes (483, 573). In multiple sclerosis (MS), the BBB breaks down, and autoreactive T cells cross the BBB, and MS begins as a focal inflammation that forms well-defined white-matter plaques. With chronicity comes a slow, diffuse whole-brain accumulation of inflammatory cells (276). Microglia participate in

all phases of the MS disease process, and phagocytosis by microglia/macrophages is a hallmark of the MS lesion (223).

The response can be relatively passive (glial reactivity) or active and more aggressive, known as reactive gliosis (-osis, a condition, disease or increase). In the case of glial reactivity, the occurrence of active cytologic, immunologic, morphologic, or functional responses of glial cells to CNS insults circumscribes a damaged zone and is associated with an oxidative environment and proinflammatory cytokines. Minimal and transitory stimuli produce minimal glial reactivity derived from the intercommunication between neurons and glia, with a proportional impact on redox environment, sensed by thiol-based redox sensors. Reactive gliosis, on the other side, involves astrocyte hyperplasia and hypertrophy (414) and is also accompanied by the expression of adhesion molecules, antigen-presentation molecules, cytokines, growth factors, receptors, enzymes, and protease inhibitors. It causes extensive damage, disruption of energy metabolism, and neuronal death (383).

"Neuroinflammation" is widely associated with multiple CNS pathologies. A literature survey reveals that neuroinflammation associates with chronic neurodegenerative diseases, including Alzheimer disease (AD), Lewy-body disease, and Parkinson disease (PD), as well as amyotrophic lateral sclerosis (ALS), MS (the prototype inflammatory autoimmune disorder of the CNS), Huntington disease (HD), aging, and alcohol neuropathology. In acute injuries, neuroinflammation associates with stroke, ischemia, infection, or metabolic encephalopathy. Even mental stress–related events (113, 126, 362), mental fatigue (458), or developmental disabilities such as autism (400) are associated with neuroinflammation. Thus, neuroinflammation is a widely used term for both chronic and acute disorders.

However, it does not necessarily mean neurodegeneration. Neurodegeneration is a question of dose, persistence of the stimulus, and irreversible rupture of redox homeostasis, as is described later in this review (357, 537, 563) (Fig. 1).

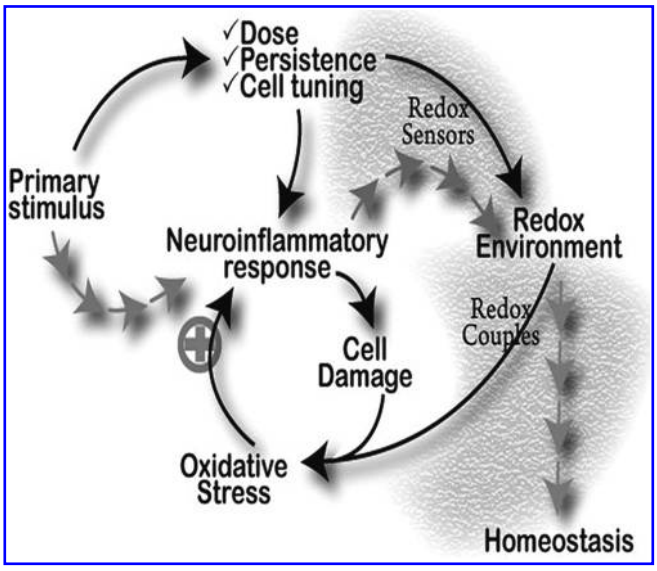

FIG. 1. A brief nonintensive primary stimulus, such as a brief ischemic episode followed by periods of reperfusion, may cause a neuroinflammatory response. However, this kind of stimulus causes minimal and reversible redox environment changes, and cells may return to homeostasis. This preconditioning pathway (*gray arrows*) not only allows the return to homeostasis, but also increases the resistance to further ischemic damage, although the mechanisms are yet to be clearly defined. A more-intense stimulus will cause a proportional impact on the redox environment, depending on its dose and persistence, as well as cell tuning. Thus, a pathologic stimulus (*black arrows*), such as protein aggregates or stroke, will not allow return to homeostasis, but will cause a persistent neuroinflammatory response and oxidative stress. The persistence of the stimulus perpetuates the cell damage and oxidative stress, and these conditions feed the neuroinflammatory response, forming a vicious cycle. Whatever its origin or the level of the stimulus may be, changes in the redox environment are sensed by redox sensors; the number and availability of thiol-based redox sensors play a key role. Meanwhile, redox couples try to maintain a reduced physiologic environment. If that is not possible, oxidative stress will be out of control and may eventually lead to disease.

II. Innate Immune Response in the Central Nervous System

A. Neuroinflammation is a team effort

The atypical inflammatory response observed in neurodegenerative diseases depends on microglial activation (517) and the release of potentially detrimental factors, such as cytokines, nitric oxide (•NO), and the superoxide anion radical ($O_2^{\bullet -}$). However, astrocytes (125) and neurons (373) modulate the activity of microglia. Neurons are not simply bystanders; they produce a complete array of well-known proinflammatory mediators, including tumor necrosis factor-α (TNF-α) (56), interleukin-1 (IL-1) (389), interleukin-6 (IL-6) (364), as well as complement (407) and cyclooxygenase-2 (COX-2) (202).

During acute neuroinflammation, microglia and astrocytes proliferate around the site of injury. The stimulus, a brief ischemic insult, as an example, induces many inflammatory genes, including cytokines, chemokines, adhesion molecules, and proinflammatory transcription factors to limit the extent of damage (164). Peripheral leukocytes do not participate, unless a BBB breakdown occurs. Glia respond to neuronal signals, which can be minimal, transitory signals, and the event finishes in a brief period. Thus, microglia provoke a controlled inflammatory reaction, and cytokines play a neuroprotector role (164, 517). However, an acute neuroinflammatory event may occur during a prolonged inflammatory response with leukocyte and monocyte infiltration in the brain, as occurs in the postischemic inflammation (271). The persistence of the stimulus determines chronicity. Neurons and glia develop a complex interaction by using cytokines, chemokines, and adhesion molecules, along with a set of damage-associated molecular patterns (DAMPs), which act as communication codes in the extracellular milieu, whereas inside the cells, a redox-dependent cascade of events attempts to maintain the cellular homeostasis (Fig. 2).

B. Microglia respond to their environment

Under normal physiologic conditions, ramified microglia are sensors of CNS homeostasis. The fine terminals of microglial processes are particularly sensitive to changes in extracellular ATP, as evaluated by time-lapse two-photon imaging

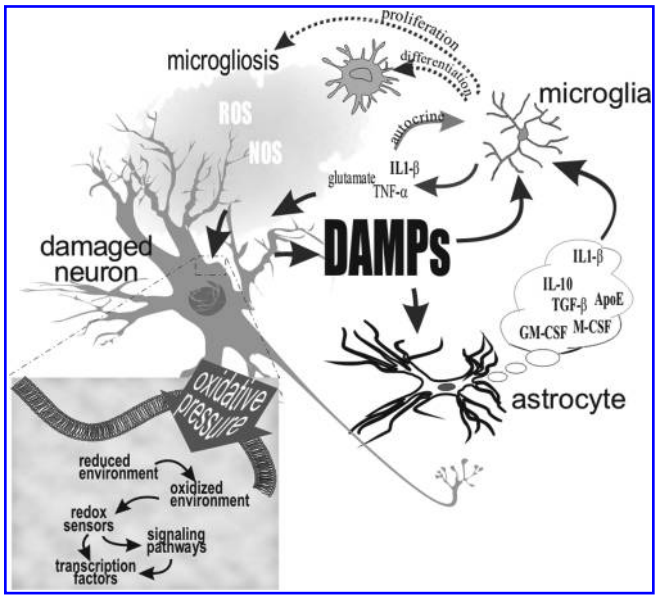

FIG. 2. Neuron–glia interactions determine and modulate microglia activity in teamwork in which cytokines, chemoattractants, and complement are key mediators. Damage-associated molecular patterns (DAMPs) are endogenous danger signals, intracellular proteins, nucleic acids, or purine metabolites, appearing in the extracellular milieu as a consequence of injury or disease, and they signal tissue damage to the innate immune system. The extracellular danger signals addressed in this review are those particularly affected by the redox status. They activate a variety of pattern-recognition receptors that initiate or prolong inflammatory responses. Inside the cells, oxidizing pressures alter the redox balance, affecting the activity of transcription factors and inducing signaling pathways. Redox couples buffer the redox-environment changes and diminish those pressures, until levels of GSH begin to decrease, for example, or the overproduced free radicals overcome the antioxidant defenses that maintained a reduced environment. IL-1β, interleukin 1-β; TNF-α, tumor necrosis factor α; IL-10, interleukin 10; ApoE, apolipoprotein E; GM-CSF, granulocyte–macrophage colony-stimulating factor; MCSF, macrophage colony-stimulating factor.

(379). Thus, ATP, delivered to the extracellular milieu, becomes a DAMP and becomes a chemotactic activator, closely related to redox variations, as described later (115, 584).

In acute neuroinflammation, microglia migrate to the injured site and behave like macrophages. However, in chronic neuroinflammation, in which the key instigating factors seem to be IL-1β and TNF-α (171, 459, 488), microglia remain in a sustained activated state, and the numerous mediators become neuroprotective or neurotoxic, depending on environmental conditions. For example, in amyloid beta (Aβ)-induced pathogenesis, overactivated microglial cells increase the efficiency of plaque phagocytosis (489), as reported in APP/PS1 mice. However, the plaque clearance *in vitro* occurs only in single-cultured microglia, and it is suppressed when astrocytes are cultured along with microglia (122), which emphasizes the importance of cell-to-cell communication and models of study. In *in vitro* experiments, it is possible to identify contradictory results regarding the neuroprotective or neurotoxic effects of cytokines. This depends on the types of cells in culture (isolated microglia, primary neurons, astrocytes, or mixed cultures), the nature of cytokines added to the culture or variations in their concentrations, or the length of stimulation as well as redox environment conditions (503).

C. Astrocytes and neurons as microglia modulators

Astrocytes, composing up 50–60% of the total cell numbers in the brain, cooperate with microglia, which provide the first wave of inflammatory cytokines. Several astrocyte-released factors, such as transforming growth factor β (TGF-β), macrophage colony-stimulating factor, granulocyte/macrophage colony-stimulating factor, IL-10 (49, 122), IL-β, and ApoE (160), modulate microglia activity (Fig. 2). Thus, inflammatory events and oxidative stress, flowing in a synchronized manner, become modulated by astrocytes (459). Astrocytes modulate the innate immune response through Ca^{2+} and ATP signaling. For example, electrical stimulation or local ATP ejection in acute brain slices elicit Ca^{2+} waves in corpus callosum glial cells, which in turn trigger responses in microglial cells (481). In addition to ATP, Ca^{2+} signals also induce the release of a variety of chemical transmitters, including glutamate and D-serine, which allow astrocytes to regulate neuronal excitability and synaptic transmission (Fig. 3). After neocortical *N*-methyl-D-aspartate (NMDA) administration in immature rat brain, the transcription factor STAT3 (signal transducer and activator of transcription), as well as the

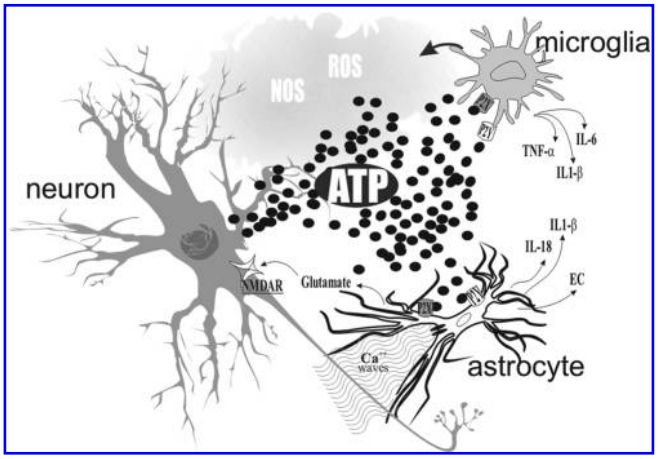

FIG. 3. Released from astrocytes in response to glutamatergic receptor stimulation or delivered by damaged neurons, extracellular adenosine 5′-triphosphate (ATP) acts as a primary extracellular messenger. Low concentrations of ATP are chemoattractants for microglia, in which the activation of P2X receptors leads to proinflammatory cytokines. Prolonged stimulation of purinergic P2X receptors opens pannexin hemichannels, which communicate between intra- and extracellular compartments and serve as a diffusion pathway for ions and small molecules modulating the range of the intercellular Ca^{2+}-wave transmission between astrocytes and neurons, which also participates in the P2X receptor–dependent inflammasome activation. ATP-elicited glial calcium waves influence neurons and microglia. Excitotoxic glutamate is released by glia, and this stimulates the *N*-methyl-D-aspartate receptor. IL-1β, interleukin 1β; TNF-α, tumor necrosis factor α; IL-18, interleukin 18; IL-6, interleukin 6; ECs, endocannabinoids.

nuclear factor-kappa B (NF-κB), becomes activated in astrocytes, leading to astrogliosis and the release of signaling molecules, with the subsequent activation of transcription factors in microglia (2, 459).

Reactive astrocytes and microglia regularly occur side by side, as suggested by imaging studies with high-resolution microautoradiography with [^{3}H]-(R)-PK11195, combined with immunohistochemical cell identification and performed on the same tissue section in MS and experimental allergic encephalomyelitis (EAE), the animal model of MS (24).

Finally, necrotic neurons activate microglia through DAMPs, delivered to the extracellular milieu and recognized by Toll-like receptors (TLR) that signal *via* the myeloid differentiation primary response protein (MyD88) to induce the expression of a proinflammatory response and the production of glutamate, which is responsible for NMDAR-mediated toxicity of neurons (395). DAMPs, particularly ATP, have many other inflammatory consequences linked to redox alterations, as we review later in this article. Interestingly, both MyD88 and NMDAR possess thiol (-SH) residues, susceptible to redox regulation (218, 306).

III. Neuroinflammation and Redox Homeostasis

Neuroinflammation is closely related to altered redox homeostasis, and this relation has physiological implications in the maintenance of the cellular homeostasis and survival (476). The extension of the oxidative stress may cause depletion of reduced glutathione (GSH), which in turn renders the cells incapable of removing ROS, leading to the irreversibility of the oxidative processes.

However, the combination of several conditions determines a different fate; some of those conditions are attributable to the primary stimulus (*e.g.*, dose and persistence), and some are cell attributes (*e.g.*, age, redox tuning, and level of activity or specialization). How the neuroinflammatory response will end is defined by the dose and persistence of the stimulus in addition to cell tuning. However, some particularities of the involved pathways, such as concurrent pathways to different transcription factors, competition for the same DNA-binding sequences, number, and availability of thiol-based redox sensors constitute the context in which those phenomena come into play (Fig. 1).

After a certain level of persistent or increasing stimulation or both from ROS, a damaging feedback mechanism develops between neuroinflammation and oxidative stress (459) (Fig. 1, black arrows). Conversely, under "normal" stress or preconditioning conditions, a balance between proinflammatory and antiinflammatory signals prevails. The endogenous antioxidant mechanisms are capable controlling the reactive species, the impact on redox environment is minimal, and cells may return to homeostasis (Fig. 1, gray arrows). Thus, neuronal signals of distress (70, 502), in addition to the dose and persistence of the damaging stimulus (151, 162), determine the final fate.

A. Endogenous danger signals in neuroinflammation and their relation to redox variations

The environment around the cell emits its own signals through molecular patterns. Microglia recognize them through pattern-recognition receptors (PRRs) or misinterpret some host-derived signals or damaged molecules as pathogen-associated molecular patterns (PAMPs), and then they are renamed DAMPs (Fig. 2). Multiple positive-feedback loops exist between DAMPs and PAMPs in addition to the overlap of receptors. Thus, bacterial LPS is considered to be the prototypical PAMP, whereas some nuclear or cytosolic proteins (even nonproteinaceous biomolecules, such as ATP, DNA, and uric acid), when released outside the cell as a result of tissue injury, could be oxidized and behave functionally as DAMPs, initiating an inflammatory response (462).

As an active monitor of the environment, microglia express a variety of PRRs, such as scavenger receptors (SRs), the receptor for advanced glycation end products (RAGEs), mannose receptor, and TLRs (48). The mannose receptor, a prototypic PRR that mediates endocytosis and phagocytosis, has been identified and characterized in microglia and astrocytes, where its expression and endocytic activity are regulated by cytokines (333). Thus, IFN-γ and IL-4 decrease or increase the mannose receptor as part of the termed alternative activation to counterbalance the proinflammatory response (613). TLRs, conversely, are implicated in neuroinflammation and neurodegenerative processes, as is described later.

Signs of damage, danger signals, or "alarmins" (40) are terms that refer to DAMPs. Their relevance to this review is attributable to their close association with oxidative pressures during oxidative stress and neuroinflammation, which is particularly true in the case of neurodegenerative diseases. However, a growing number of DAMPs have appeared, and more will likely emerge with time. Some of them, listed as "other endogenous signs of damage," are briefly described at the end of this section.

1. Extracellular ATP: immunomodulatory, NOX/H_2O_2 inducer, and possibly, a denitrosylating agent: its role in neurodegenerative pathologies. Released from neurons through secretory exocytosis, ATP directly modulates microglial function (64) and triggers an unusual NF-κB response (594) because it induces solely p65/p65 homodimers (144). These are less stable than the usual heterodimers (424) (Fig. 3).

ATP outflow increases significantly during ischemia, as demonstrated *in vivo* by intraluminal occlusion of the middle cerebral artery (341). In general, after metabolic stress, brain ischemia, or trauma, a massive extracellular release of ATP has been observed (566). However, in neurodegenerative disorders, purinergic receptors also show an important activity, mostly related to ATP release from disrupted cells. This is the case in PD, HD, and AD, as well as in ALS and MS, in which an important inflammatory component is involved.

Damaged neurons release nucleotides, including ATP, and stimulate microglia through P2 purinoceptors (217). Thus, ATP is a crucial DAMP in neuroinflammation, acting on metabotropic G protein–coupled receptors (P2Y) and ionotropic ATP-gated ion channels receptors (P2X) (62, 146). Under physiologic conditions, the ATP release from synapses occurs in the nanomolar to micromolar range (131), a dose sufficient to act as a chemoattractant for microglia (203). Conversely, ATP release associated with cell damage and astrocytic activation (Fig. 3) (562) reaches the millimolar range (1 to 5 m*M*) and activates microglial P2X ionotropic receptors (356), which initiate the inflammasome, a multimolecular structure that, through tightly controlled intracellular events, leads to the activation and secretion of IL-1β (322). IL-1β and

IL-18 are the most tightly P2X-controlled mediators (43, 421). All P2X receptors contain 10 conserved cysteines in the extracellular loop, which may form at least three disulfide bonds with known functional consequences in receptor trafficking to the cell surface (136) but potentially affecting a whole range of activities described herein. The mutation of the conserved cysteines in the ectodomain of other P2X receptors has been shown to reduce the sensitivity of the receptor to ATP (99). Moreover, P2Xs have intracellular oxidizable cysteine residues, and one of them (Cys^9) may form a disulfide bond that affects the ATP responsiveness of P2X2 (371).

Microglial P2X receptors are the primary receptors involved in H_2O_2 production, as demonstrated in the *in vitro* and *in vivo* models of neurodegenerative disorders. In the first case, primary rat microglia stimulated with ATP or its analogue 3′-*O*-(4-benzoyl)benzoyl-ATP (BzATP) produced H_2O_2 in a Ca^{2+}-dependent fashion. This phenomenon was blocked in the absence of extracellular Ca^{2+} or by using selective inhibitors of P2X7 or oxidized ATP (Fig. 3). Moreover, P2X7 receptor–deficient mice failed to exhibit neuronal cell injury in the presence of BzATP. In the brains of Tg2576 mouse, overexpressing mutant APP (K670N, M671L) that develops Aβ plaques in the aged brain, an intense staining for P2X occurs around amyloid plaques (406, 505, 511). Increased P2X7-immunoreactivity is significant in activated microglial cells/macrophages of the MS and ALS spinal cord, along with COX-2 and cannabinoid receptors (594).

P2X4R, an ATP-gated ion channel with high calcium permeability, appears enhanced in the spinal cord and causes the release of brain-derived neurotrophic factor (BDNF) during injury (547), whereas in brain, P2X4R has been characterized after traumatic brain injury or EAE (177, 605). It has been demonstrated that P2X4R upregulation results from the combined stimulation of TLR and the nucleotide-binding oligomerization domain containing 2 (NOD2) an intracellular PRR, which leads to •NO and TNF-α induction (178).

ATP-mediated ROS also are capable of activating ERK1/2 (268), a critical regulator of neuronal function, as well as PI3K and its downstream mediators (287). Additionally, ATP also participates directly in the transition from oxidative stress to excitotoxic stress, as has been demonstrated in rat hippocampus (511) and confirmed in P2X7R-knockout mice (399).

As a process or signal that is looped back to control a system within itself, the purinergic stimulation of microglia *via* P2 receptors may release ATP from microglial cells, and this feeds back onto neurons and astrocytes. Therefore, ATP released from glial cells becomes, reciprocally, a potent neuromodulator (374). Additionally, prolonged stimulation of P2X leads to the opening of a gap junction–like hemichannel pannexin (panx-1), which form nonjunctional channels that play a paracrine role by releasing ATP and, thus, modulating the range of the intercellular Ca^{2+}-wave transmission between astrocytes and neurons (Fig. 3). Panx-1 also participates in the P2X receptor–dependent inflammasome activation that, in turn, leads to IL-1β secretion as well as to activation of inflammatory caspases (63) (Fig. 3). Conversely, extracellular ATP may negatively regulate TLR, inhibiting the secretion of proinflammatory cytokines, as demonstrated in peripheral monocytes (244). These differences could be explained by the persistence of the stimulus acting on different purinergic receptors, as implied by the accumulation of P2X4 in brain cells observed as early as 6 h after traumatic brain injury (605), whereas P2X7 proinflammatory activity requires prolonged stimulation (282, 406).

ATP and purinergic receptors have a role in PD, in which microglial and NOX activation *via* P2X receptors are relevant factors. *In vitro*, ATP induces both apoptotic and necrotic features of degeneration in dissociated striatal primary cells and hippocampal organotypic cultures (7), whereas *in vivo* intrastriatal administration of ATP produces dose-dependent and P2X-receptor–mediated striatal lesions (464). Finally, P2 receptors have been mapped in the normal and damaged nigrostriatal circuit, showing that dopamine denervation in a 6-OHDA animal model of PD generates a significant rearrangement of P2-receptor proteins in these nuclei (283).

In other neurodegenerative pathologies, largely associated with neuroinflammation, such as ALS and MS, ATP derived from damaged cells may contribute, through microglial P2X7 activation, to releasing IL-1β by a mechanism that involves the activation of caspase-1 in the presence of TLR ligands. Caspase-1 is responsible for the proteolytic activation of IL-1β. This in turn initiates the upregulation of gene products to establish an inflammatory state, including matrix metalloproteinases, COX-2, interleukins, and cellular adhesion molecules. In support of this hypothesis, COX-2 and P2X7 immunoreactivity, evaluated in postmortem human spinal cord from ALS and MS patients, has been localized mainly in activated microglial cells/macrophages (594). COX-derived PGs and free radicals are usually related to inflammation at early stages, and they are found elevated in the CNS of patients with neurodegenerative processes, such as ALS (576), AD (205), dementia with Lewy bodies associated with AD pathology (471), PD (570), MS (590), and ischemia (68, 113).

Interestingly, the cleavage of IL-1β, as mentioned earlier, may be blocked with antioxidants like *N*-acetylcysteine (NAC) or a peroxynitrite scavenger, such as the metalloporphyrin FeTPPS, as demonstrated in monocytes (197). Thus, ATP/P2X-dependent NOX activation generates superoxide anions and activates caspase-1. $O_2^{\bullet -}$ rapidly forms peroxynitrite ($ONOO^-$) in the presence of •NO, as occurs in neuroinflammatory processes accompanied by oxidative stress. •NO is usually a result of NOS activity; however, it seems that the ATP-induced rapid $ONOO^-$ formation does not require NOS activity; the source of •NO in this case is protein denitrosylation. This suggests that the denitrosylating agent could be the extracellular ATP itself, although the mechanism leading to the decomposition of SNO groups after ATP stimulation remains to be resolved (197).

2. HMGB1, a redox-regulated danger signal. Redox regulation on HMGB1 connects oxidative stress to neuroinflammation, because HMGB1 triggers microglial and astrocytic activation. HMGB1 is expressed on neurons and astrocytes (316, 408), as well as on microglia, where it represents a sign of activation (8, 189). Formerly described only as a nonhistone DNA-binding protein, HMGB1 stabilizes the nucleosomal structure and facilitates gene transcription during physiologic conditions. However, during cellular damage (Fig. 4), this transcriptional coactivator, also known as amphoterin because of its characteristic bipolar structure, is passive and actively released into the extracellular milieu by damaged neurons. Once released, HMGB1 has the capacity to induce proinflammatory cytokine production *in vivo* through both TLR and RAGE, as occurs in the postischemic

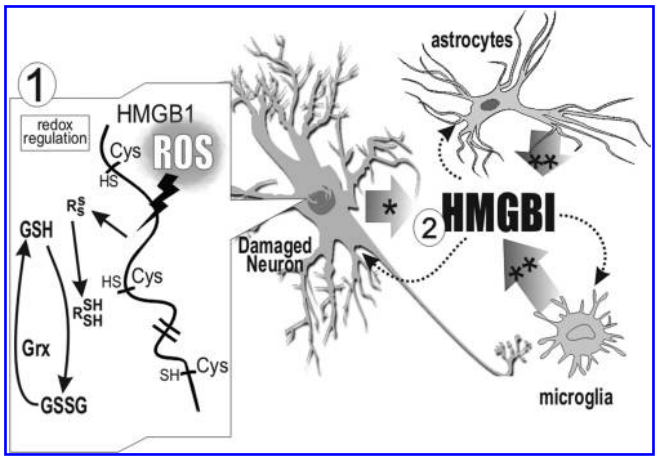

FIG. 4. (1) HMGB1 binds chromatin and acts as a transcription factor but also functions as an inflammatory cytokine. However, for this action, HMGB1 must transit from the nucleus, through the cytoplasm, to the outside of the cell. The translocation of HMGB1 during cell activation and cell death depends importantly on redox regulation because of its low pK_a cysteine residues, which can be rescued by GSH from irreversible oxidation. Mutation of cysteines in HMGB1 affects its nuclear localization. (**2**) Once released actively (**) or passively (*) toward the extracellular space, HMGB1 behaves like a cytokine, with autocrine and paracrine effects. Thus, acting through RAGE and Toll-like receptors on neurons and glial cells, HMGB1 induces an innate immune response, activating NF-κB or triggering the MAPK/ERK pathway, which leads to pleiotropic results, according to the cell target.

brain delayed neuroinflammation (254) or in active lesions of MS (8).

In microglia, HMGB1 binds to RAGE and activates Rho GTPases (360), which emits alert signals and controls p38MAPK and JNK cascades, as well as the NOX enzyme complex (180). Necrotic neurons activate a microglia-driven proinflammatory response (360, 395) in which the interaction between TLR and HMGB1 probably plays an important role through the activation of NF-κB (293). Conversely, astrocytes release HMGB1 (408) during severe tissue-damaging conditions; but astrocytes also are targets of HMGB1, as demonstrated in primary astrocytes, in which the MAPK/ERK1/2 cascade was activated as a result of the interaction between RAGE and HMGB1 (361). Thus, HMGB1 may induce COX-2, chemokines, and MMP9 in an NF-κB–independent manner to stimulate cell migration and immune surveillance in the CNS (411). However, it is possible that HMGB1 binds RAGE in neurons activating distinct downstream signaling pathways. HMGB1 may activate Rho GTPases, such as Rac and Cdc42 signaling pathways, to induce neurite outgrowth, or may lead to the activation of NF-κB through a redox-dependent pathway involving Ras-MAPK (212). In addition, HMGB1 may interact with TLR in neurons, because neurons express both TLR-2 and -4 (536). Once HMGB1 binds to TLR, it recruits MyD88, and this adaptor directly intervenes in the translocation of NF-κB to the nucleus or, through the activation of the IL-1 receptor–associated kinase (IRAK) MyD88, activates the MAPK pathway, the translocation of NF-κB to nucleus, and an inflammatory response (403, 508, 530). Hippocampal neurons from MyD88-5–deficient mice are protected from death after deprivation of oxygen and glucose (257). Moreover, by using monoclonal antibodies against HMGB1, it is possible to ameliorate brain infarction induced by transient ischemia in rats, reducing the expression of TNF-α, iNOS, and MMP-9 (309), derived from the activation of NF-κB (393).

It is worth mentioning that HMGB1, being secreted actively by inflammatory cells, such as astrocytes (408) and microglia (8) (Fig. 4), has an active role beyond oxidative stress and GSH levels. HMGB1, in response to inflammatory stimuli such as LPS, TNF, and IL-1β (568), accumulates in the cytoplasm; it is redistributed into secretory lysosomes and then released into the extracellular space. It is also susceptible to other post-translational modifications, such as methylation, acetylation, and phosphorylation. The acetylation of specific residues on HMGB1 impairs its reentry into the nuclear compartment, and this leads to its accumulation in the cytoplasm during inflammation (52). In this context, HMGB1 levels and its receptors (RAGE, TLR2, and TLR4) have been found overexpressed in active lesions of MS and experimental autoimmune encephalomyelitis (EAE), correlated with active inflammation. After the stimulation with LPS/interferon γ (IFN-γ), this phenomenon was reproduced *in vitro* and demonstrated that CNS-derived microglia were as capable as macrophages of translocating HMGB1, which provides a positive-feedback loop that amplifies the inflammatory response during MS and EAE pathogenesis (8).

Shuttled actively between the nucleus and cytoplasm, the high-mobility group protein box 1 (HMGB1) is acetylated on lysines 2 and 11 at its nuclear localization sequences in activated macrophages/monocytes. Thereafter, it is sequestrated within cytoplasmic vesicles, to be released to the extracellular milieu (52). Thus, HMGB1 becomes relevant to neurodegeneration, and it is particularly interesting because as a result of the redox-sensitive cysteine residues, it is susceptible to redox regulation (206). Indeed, HMGB1 has two cysteines on its A-domain, Cys^{23} and Cys^{45} in close proximity, which, under mild oxidative attack, form an intramolecular disulfide bridge, becoming a target for glutathione-dependent reduction by glutaredoxin. Even thioredoxin may reduce the mentioned cysteine residues, although the oxidation of HMGB1 by oxidized glutathione (GSSG) can be faster than the reduction of HMGB1 by thioredoxin when the GSSG concentration is elevated because of oxidative stress (468). A third Cys^{106} residue, this one in the B-domain, remains in the reduced form and is critical for the immunostimulatory activity of HMGB1. When Cys^{106} is oxidized during more-severe oxidative stress, it blocks the immunogenic function of HMGB1 (246), likely by allowing the nucleocytoplasmic shuttling of HMGB1 (206) for its subsequent release toward the extracellular space, where it behaves like a cytokine (Fig. 4).

3. The extracellular S100B activities rely on the oxidation of the protein cysteine residues. S-100 B is a glia-derived calcium-binding protein with physiologic relevance in the brain, where it exerts trophic effects on neurons and astrocytes. However, at relatively high extracellular concentrations, S-100B protein behaves as an endogenous alarm signal or DAMP, causes neuronal apoptosis, and activates astrocytes and microglia (42).

S100B protein is secreted via a nonclassic pathway at sites of inflammation and is considered a useful biologic marker of

acute neurologic disorders, such as ischemic or hemorrhagic stroke and traumatic head injury (518). However, S-100B also has been implicated in the pathogenesis of chronic neurodegenerative disorders, such as AD, and is associated with IL-1 overproduction. S100B induces IL-1 in microglia and neurons by means of cell type–specific transcription factors. In primary cortical neurons, Sp1 mediates IL-1β induction by S100B without evidence of a role for NF-κB, whereas in microglia, S100B stimulates NF-κB or AP-1 transcriptional activity and upregulates Cox-2, IL-1β, IL-6, and TNF-α expression in microglia through RAGE engagement (42, 295, 310). Thus, it is clear why S-100B is overexpressed in astrocytes of the cervical spinal cord of wobbler mice, an animal model of motor neuron degeneration (103).

The activity of S100B is dependent on its interaction with RAGE, and it is known to depend strictly on the redox status of the cell. S100B and its receptor RAGE have been found to be increased in AD, aging, PD, Down syndrome, as well as in tissue trauma and ischemia. Even though cysteine residues on S-100B may not be necessary for the noncovalent dimerization of S-100B, mutation of Cys^{84} to serine may alter the regulation of target protein activity (278). The oxidation of Cys^{68} and Cys^{84} on S100B induces a conformational change in the protein structure *in vitro*, unmasking a canonic casein kinase II phosphorylation site within the calcium-binding site, and it is speculated that this process is related to S100B secretion (485). At physiologic concentrations in reducing environments, S100B exists as a non-covalent homodimer; the formation of oxidized monomeric S100B, conversely, can be selectively catalyzed by $ONOO^-$; interestingly, a positive-feedback loop in glial cells can be established when extracellular S-100B protein stimulates the activity of NOS (207). The neurotrophic and mitogenic activities of the extracellular S100B protein are dependent on redox status as well as the formation of inter- or intrachain disulfide bonds (207, 278).

4. The influence of redox regulation in the crosstalk between innate and adaptive immune systems through Hsps. Released to the extracellular milieu under stress conditions, heat-shock proteins (Hsps) induce proinflammatory cytokines by interacting with convenient receptors, such as TLR, CD40, or CD14 (513). Thus, Hsps are involved in the crosstalk between innate and adaptive immune systems, and primarily mediate immune regulatory functions. Microglial activation by exogenous Hsps increases the uptake and clearance of amyloid peptide aggregates. However, Hsps also induce production of •NO, as well a set of apparently contradictory cytokines from TNF-α and IL-6, to TGF-β, IL-10, and IL-13, which could explain its role in the "conditioning" of brain tissue (556) to maintain local homoeostasis. This conditioning phenomenon is related to cell tuning, in which each microenvironment is controlled by a specific set of regulatory elements that must be finely and constantly tuned to maintain local homeostasis (32). It is important to emphasize that the NOS activator, Hsp90, may be redox regulated by *S*-nitrosylation of a Cys^{597} residue, and this modification inhibits the intrinsic ATPase activity of Hsp90 needed for its function as a chaperone protein and a coactivator of the constitutive, endothelial NOS (330).

Interestingly, intracellular Hsps are influenced by HNE to avoid cell death. During oxidative stress, HNE separates the hsp90-heat shock factor1-hsp70 complex, which represses the heat shock factor 1 (HSF-1) activity during unstressed conditions. Thus, HSF-1 translocates to the nucleus, where it promotes the expression of hsp-40 and hsp-70, which aid in the recovery of cells from thermally and chemically induced damage by promoting the stabilization of Bcl-XL. This inhibits Bax translocation to the mitochondrion and the consequent stress-induced apoptosis (225) (Fig. 5).

An interesting hypothesis that inducers of the Hsp response may be capable of triggering the oxidation of nonprotein thiols, particularly glutathione. Such oxidation should lead to formation of glutathione–protein mixed disulfides and protein–protein disulfides. Thus, thiol adduction and cross-linking would affect the structure of proteins involved, resulting in unfolding of a fraction of these proteins, causing activation of HSF. Different inducers (13 in total), as well as heat shock, may cause oxidation of glutathione and trimerization of HSF-1 (615) (Fig. 5).

5. Other endogenous signs of damage. Because of its antioxidant properties, uric acid may influence the redox status, although it is not directly involved in redox regulation. Uric acid forms hydrophobic crystals when released from injured cells, as detected by the NALP3 inflammasome (285). Reduced urate has been found in serum, cerebrospinal fluid, and postmortem substantia nigra of patients with PD when

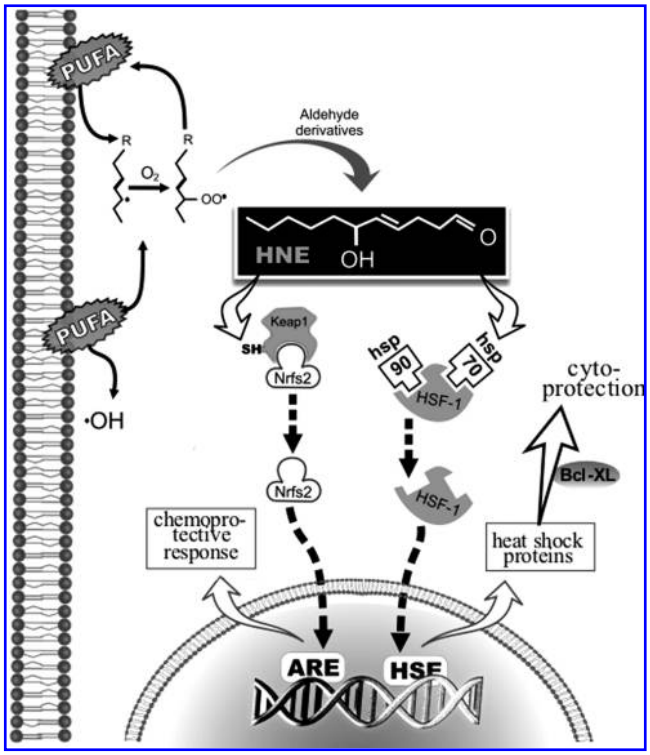

FIG. 5. Lipid peroxidation aldehyde derivative 4-hydroxynonenal (HNE) retains a complicated network of interactions. It can be beneficial by reacting with Keap-1 protein and releasing Nrf2 or allows the translocation of HSF-1 to the nucleus to promote heat-shock proteins, which in turn stabilize Bcl-Xl. This is a transmembrane molecule in the mitochondria that inhibits Bax translocation to the mitochondrion and apoptosis, a phenomenon particularly significant in brain ischemia.

compared with controls (95). Uric acid is a major endogenous antioxidant and peroxynitrite scavenger, and the infusion of uric acid after stroke may reduce free radical–mediated injury (429); however, uric acid can also mediate the production of active oxygen species by functioning as a prooxidant and, according to other reports, urate-induced oxidative stress is causative in stroke (55).

The hepatoma-derived growth factor (HDGF) is a nuclear protein homologous to the high-mobility group B protein (HMGB), evenly expressed throughout all the brain (133). It is released from damaged neurons and acts as a trophic factor in motor neurons (332), as well as in experimental autoimmune encephalomyelitis (311). To our knowledge, no a direct association occurs between HDGF and redox regulation in the brain.

Also important, but even less explored in relation to oxidative stress, redox regulation and neuroinflammation in brain are the antimicrobial cathelicidins, cysteine-rich defensins, and galectins. Galectin-1, from the β-galactoside–binding proteins, may induce astrocyte differentiation with the differentiated astrocytes, greatly enhancing their production of brain-derived neurotrophic factor (BDNF), which promotes neuronal survival, guides axonal pathfinding, and participates in activity-dependent synaptic plasticity during development (135). Thymosins, conversely, play an important role in the organization of the cytoskeleton by sequestering G-actin during development of the mammalian brain; however, after transient global ischemia in the rat brain, thymosins behave as DAMPs and are increased in microglial cells in the dentate gyrus of the hippocampal formation (256). Nucleolin, also considered a DAMP (40), is a protein with potential roles in the pathogenesis of AD, and is involved with DJ-1, a redox-dependent molecular chaperone that inhibits α-synuclein aggregate formation (128).

B. Thiol-based redox sensors

To this point, we have summarized the role of the cellular protagonists of the innate immune response in CNS and some of the mechanisms regarding how astrocytes and neurons regulate the activity of microglia during neuroinflammatory processes. We emphasized the major endogenous danger signals focused in the redox-regulation context. However, before we tackle the oxidative/nitrosative stress theme, its sources and its mechanisms of induction, the maintenance of steady-state levels, as well as the dynamic redox control in neuroinflammatory processes, we include a brief review of the instruments in redox regulation: the thiol-based redox sensors, focused on functional aspects of neuroinflammatory/neurodegenerative diseases.

Regulatory sensors monitor the redox state of the internal and external environments while maintaining redox homeostasis by converting the redox signals into regulatory outputs, usually at the level of transcription. The thiol-based redox sensors use modifications on the oxidizable -SH side-chain of cysteine (Cys-SH) to sense redox alterations.

Thiol-based redox sensors convey information about localized changes in redox potential induced by physiologic situations, such as variations in the proportion of oxidized and reduced components of redox couples or variations of •NO levels, as well as in pathologic situations with overproduction of ROS or RNS. Thus, protein cysteinyl thiols can undergo a variety of oxidative addition reactions, such as *S*-thiolation, *S*-nitrosylation, and alkylation by reactive lipid adduction. Because inflammatory events and oxidative stress occur in a synchronized manner, changing the redox environment, thiol-based redox sensors placed on signaling pathways or regulating transcription factors become checkpoints that define the course of the physiopathologic events. Furthermore, the impacts of redox variations on the cell must be considered as "effects on the neighborhood" more than effects on a singular factor. Thus, the redox environment has been defined as the summation of the reduction potentials of redox couples found in biologic fluids, organelles, cells, or tissues, multiplied by the concentration of reduced species in the redox couple (479). Recent evidence suggests that different oxidizing and reducing agents must be taken into account to measure the intracellular redox state based on thermodynamic principles, giving rise to the concept of "effective reduction potential" (331). This is important because of the multiple biases produced by very different effectors with

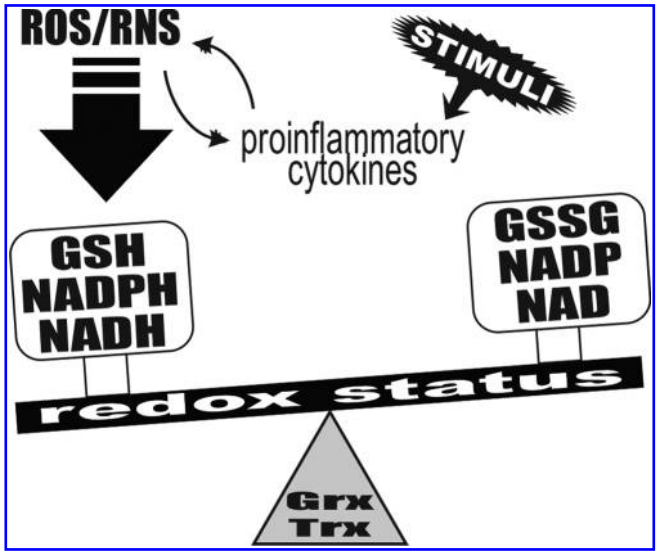

FIG. 6. The relation between neuroinflammation and redox balance is determinant in neurodegenerative diseases. Cytokines have the potential to alter the redox equilibrium, thereby affecting GSH/GSSG shuttling and recycling. For example, treatment of rat primary astrocytes with TNF-α or IL-1β leads to marked alteration in cellular redox (decrease in intracellular GSH), whereas pretreatment of astrocytes with *N*-acetylcysteine (NAC), an antioxidant and efficient thiol source for glutathione, prevents cytokine-induced decrease in GSH. Reciprocally, redox status modulates the participation of cytokines, transcription factors, and signaling pathways in the signaling process. Different oxidizing agents tip the balance between redox couples and take into account the effective redox potential, according to the thermodynamic properties. Glutaredoxin (Grx) catalyzes the reduction of disulfide bonds in proteins, converting GSH to glutathione disulfide (GSSG), whereas the enzyme glutathione reductase recycles GSSG back to GSH at the expense of NADPH. Deglutathionylation of protein thiols is also an important function for Grx. Thioredoxin (Trx) system is essential to reduce oxidized proteins by cysteine thioldisulfide exchange. By acting as an electron donor, Trx reduces peroxidases and the ribonucleotide reductase. NADPH further reduces the oxidized Trx.

available redox-sensors coupled with reversible exchange of reducing equivalents, working at the same time at any given physiological moment (Fig. 6). The concept of effective reduction potential summarizes the contribution of different oxidizing and reducing agents in the formation of an effective redox potential.

C. Redox sensors in neuroinflammation

Thiol-based redox sensors are present during all phases of neuroinflammation. The danger signals behavior, and the consequent intercellular communication may depend on the redox status, as was described earlier.

Redox sensors are determinant in neurodegenerative disorders in which *S*-nitrosylation and denitrosylation may define the functionality and fate of neurons undergoing a pathologic process. For example, modification of tyrosine residues or cysteine residues can either affect specific, disease-related substrates or affect signaling pathways, as a mechanism of redox regulation. Protein tyrosine nitration induces a shift in the pK_a of the tyrosine hydroxyl group from 10.1 to 7.2, introducing a net negative charge to the nitrated tyrosine at physiologic pH, which alters both the structure and catalytic activity of the protein (593). Accordingly, both $ONOO^-$ and nitrogen dioxide could inhibit the catalytic function of the tyrosine hydroxylase enzyme (TH) responsible for catalyzing the conversion of the amino acid L-tyrosine to dihydroxyphenylalanine (DOPA), by nitration of critical tyrosine residues (15) [identified later at positions 423, 428, and 432 (272)]. However, tyrosine nitration of TH is not sufficient to inactivate this enzyme. An extensive oxidation of cysteine residues occurs, followed by the reversible formation of disulfide adducts between TH sulfhydryls and the sulfhydryl of glutathione (*S*-thiolation) by RNS (272).

Reversibility implies regulation, in which the intracellular redox state is determinant to maintain the homeostasis. In addition to nitration of tyrosine residues, an extensive oxidation of cysteine residues by $ONOO^-$ has to occur to achieve enzyme inactivation. TH is *S*-thiolated by reactive nitrogen species in the presence of cysteine, at the expense of tyrosine nitration (466). Whatever the precise mechanism of TH regulation, *S*-nitrosylation and *S*-thiolation are reversible processes. This means that it is possible by means of *S*-nitrosylation or *S*-thiolation of its sulfhydryl residues to stop TH enzyme activity. Later, by denitrosylation or dethiolation, it is possible to reverse the phenomenon and reactivate the TH enzyme to convert the amino acid L-tyrosine to DOPA.

As we discuss later, thiol-based redox sensors play significant roles in the main neuroinflammatory pathways—in fat metabolism, for example, which in turn may define the course of neuroinflammatory events. Thiol-based redox sensors also affect directly the most important transcription factors implicated in neuroinflammation as well as phosphorylation/dephosphorylation phenomena and their concurrent pathways.

D. Redox status and proinflammatory mediators

During neuroinflammatory processes, alterations occur in the function of surrounding glial cells. These alterations are key features in the progression of neurodegeneration, in which proinflammatory cytokines and free radicals actively participate in the degenerative insults by modifying molecules. Redox status and proinflammatory mediators are highly interconnected.

The redox status, defined as the ratio of interconvertible reduced/oxidized forms of a molecule, modulates the participation of cytokines in signaling processes. Cytokines are critical mediators of oxidative stress, neuroinflammation, and neurodegeneration in the CNS. Cytokine overexpression by glia, for example, is related to the progression of neurodegeneration (171, 358), or neurodegeneration is the result of an autoreactive immune response, as occurs in MS, in which cytokines and ROS play a determinant role (251). During oxidative stress, cytokines have the potential to alter the redox equilibrium, thereby affecting GSH/GSSG shuttling and recycling (Fig. 6). For example, after H_2O_2 or amyloid-β intracerebral injection in rat brain, an association between lipid peroxidation and the levels of cytokines, in addition to a significant inverse correlation between glutathione peroxidase activity and lipid peroxidation levels, was observed (251, 459). Cytokines also use redox-sensor pathways to achieve key transcription factors, as we explain later.

The ultimate goal is to restore tissue integrity or to finish nonrecoverable defective events. However, depending on the persistence of the stimuli as well as its nature, as an amplifier system of the innate and adaptive immune responses, cytokine-induced neuroinflammation may become a harmful process affecting normal regulation of neurotransmitters, such as acetylcholine (296, 544) and excitotoxins [*e.g.*, glutamate (574) or D-serine, a co-agonist at the NMDA class of glutamate receptors (585)]. In this manner, neuroinflammation contributes to the destruction of synapses and the declines in hippocampal NMDA receptor-1 (NMDA-R1) (460).

IL-1 and TNF-α are proinflammatory cytokines expressed by and acting on neurons and glia. Both are mediators in the pathogenesis and progression of acute and chronic neurodegenerative diseases, including stroke, AD, PD, and viral infection. The activation of IL-1 and TNF-α is initiated by a set of intracellular signaling cascades, including MAPK, NF-κB (616), and JAK/STAT (599), and it runs in parallel with iNOS expression and NOX-derived ROS (325). NOX inhibitors and antioxidant enzymes suppress IFN-γ–induced expression of IL-1, IL-6, and TNF-α, as well as MAPKs (434, 524).

E. Dual role of TNF

During immune-mediated CNS inflammation, TNF activates signaling pathways and genetic transcription, particularly through NF-κB, or it directly induces apoptosis through the activation of death domain–containing proteins (326, 554). Once TNF-α binds to TNFR-1, it follows the trimerization and clustering of intracellular death domains. The first implicated is the TNFR-associated death domain (TRADD), through interactions between death domains, which recruits the Fas-associated protein with the death domain (FADD) in a redox-modulated mechanism. FADD activates caspase-8, and activated caspase-8 cleaves caspase-3 to the active form (606). GSH depletion (194) is related to the redox-modulation of caspase-3 or to the increase of p53 and Bax expression (184).

However, TNF-α activates Grx as well, and this thioltransferase catalyzes the reduction of disulfide bonds in proteins, converting GSH to GSSG (Fig. 6), and may promote the deglutathionylation of protein thiols. Thus, TNF-α–induced Grx deglutathionylates caspase-3 and modulates its activation

during TNF-α–induced apoptosis and, conversely, cysteine-to-serine mutations in caspase-3, on redox-sensitive cysteine residues, may increase the sensitivity of caspase-3 to TNF-α–induced cleavage (396).

In addition, redox alterations due to increased ROS or the consequent GSH depletion that may occur during inflammation or be caused by drug treatment (or both) may be an important dose-dependent mechanism in sensitizing cells to TNF-induced apoptosis (182, 367). Thus, logical anti–TNF-α therapies have been proposed, going from pentoxifylline [which reduces the TNF-α mRNA overexpression (487)] to p38MAPK inhibitors (363) or NF-κB antagonists, such as pyrrolidine dithiocarbamates (PDTCs), designed to prevent TNF-α transcription. Etanercept is a recombinant-DNA drug that inhibits TNF activity by competitively binding to it and preventing interactions with its cell-surface receptors; it has been successfully tested in AD patients (541). Immunomodulation therapy is also considered to increase antiinflammatory cytokines in opposition to TNF-α (105).

IV. Oxidative/Nitrosative Stress

In neuroinflammation, the overproduction of free radicals comes from three sources: (a) the microglial respiratory burst, which is a result of detection of pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) through their corresponding receptors, leading to nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase (NOX) activation; (b) lipid peroxidation; and (c) abnormal mitochondrial leakage due to dysfunctional activity of this organelle. The first mechanism responds to an insult; the second is an extension of damage; and the third is cell disease (Fig. 7).

The important ROS/RNS relevant to neuroinflammation are H_2O_2 and •NO, preceded by or in conjunction with $O_2^{\bullet -}$. These reactants mediate a variety of biologic responses, particularly through the MAPK-signaling pathways.

H_2O_2 generation is continuous in damaged tissues and lasts for extended periods. A multitude of products and damage patterns are associated with the H_2O_2-derived hydroxyl radical (•OH), which attacks lipids, proteins, and DNA sensitive bases, as well as sugars (499, 532, 535, 558). H_2O_2 directly induces c-Jun NH_2-terminal kinase (JNK)1/2, and these mediate apoptotic-like cell death in cultured rat cortical neurons [prevented by elimination of extracellular Ca^{2+} or blockage of NMDA receptors (569)]. H_2O_2 converts GSH to GSSG, which reacts with protein thiols. Proteins with low-pK_a (4.7 to 5.4) cysteine residues within the active-site motif, such as protein tyrosine phosphatases (PTP), which fine-tune the extent, duration, and subcellular location of MAPK activation, are susceptible to H_2O_2 attack. Thiol donors, such as glutathione or γ-L-glutamyl-L-cysteinylglycine (GSH), glutaredoxin (Grx), and thioredoxin (Trx) can reverse Cys oxidation, and thus, PTP activity becomes redox regulated (451).

NO, in its role as a free radical (•NO), is oxidized, reduced, or complexed with other biomolecules, depending on the reductive or oxidative microenvironment, as well as the availability of substrates (198). However, •NO itself shows a low toxicity. It does react with alkoxyl and peroxyl radical intermediates during lipid peroxidation or interferes with lipid radical chain propagation reactions. These reactions depend on the relative concentrations of individual reactive species along the route $\bullet NO + O_2^{\bullet -} \rightarrow OONO^- \rightarrow OONOH \rightarrow NO_2^{\bullet -} + \bullet HO$.

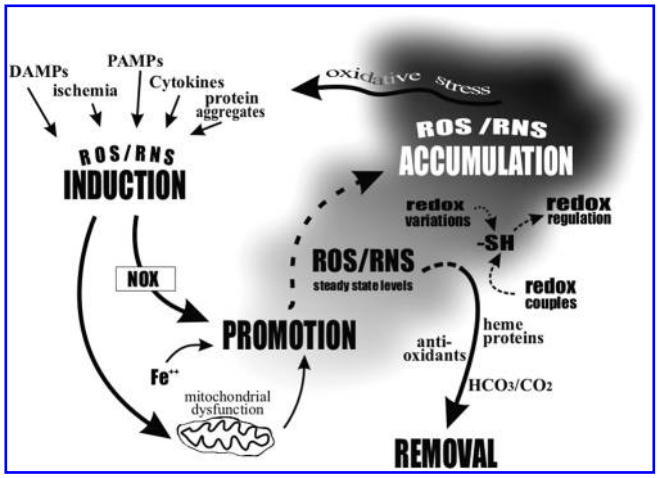

FIG. 7. Damage-associated molecular patterns (DAMPs), ischemia, pathogen-associated molecular patterns (PAMPs), cytokines, or protein aggregates induce ROS and RNS and promote them, particularly through the NADPH-oxidase (NOX) enzyme. Depending on prevalence and dose of the stimulus, along with concomitant disorders such as mitochondrial failure and leaking of free radicals, as well as the presence of metals, ROS and RNS are promoted, and they tend to accumulate. Between the promotion and the removal of those reactive species by antioxidant systems such as enzymes (SOD, Gpx, catalase), vitamins (C, E), or melatonin, in addition to heme proteins and buffer systems, a steady-state level is maintained. Thus, low levels of ROS/RNS and temporary variations in the redox state are physiologic and modulate the activity of important signal-transduction pathways. Controlled oxidizing pressures could also be part of a preconditioning system that produces protection against subsequent detrimental insults. Redox variations are sensed by thiol redox sensors in proteins, modifying the functionality by different forms of oxidation. Low-pK_a thiols are continuously attacked by redox variations and rescued by redox couples. If reactive species are overproduced or their elimination is inadequate, oxidizing pressures increase, altering the redox potential of redox-couples, which leads to irreversible oxidative changes, which in turn increase the ROS/RNS promotion, creating a vicious cycle. Oxidative/nitrosative stress feeds back to the factors that initiated and promoted ROS and RNS.

Moreover, low nitrosative stress could be part of "preconditioning," a phenomenon in which the brain protects to itself against future injuries by adapting to low-intensity noxious insults (501). However, induced as a response element of the cytotoxic cellular immune response, inducible nitric oxide synthase (iNOS) results in a 30-fold increase in •NO formation in the CNS (527). Then, •NO toxicity is linked to its ability to combine with $O_2^{\bullet -}$ to form peroxynitrite ($ONOO^-$). Thus, under proinflammatory conditions in which $O_2^{\bullet -}$ and •NO converge, $ONOO^-$ production can increase by a 1,000,000-fold (390). This unstable valence isomer of nitrate and its derivatives causes direct oxidation of lipids, DNA, and proteins, or indirectly through radical-mediated mechanisms. Both, H_2O_2 and •NO synergistically induce neuronal death through apoptosis, in a process that involves the activation of p38MAPK and caspase-3 (569). By using primary cocultures of cerebellar granule neurons and glia from rats, it was

demonstrated that the expression of iNOS or the activation of NOX separately could be relatively benign, but, when combined, these phenomena may induce neurodegeneration, particularly through $ONOO^-$ (325).

A. Steady-state levels of reactive species

The steady-state levels of the freely diffusible H_2O_2 depend on a balance between H_2O_2 production, formed by dismutation of $O_2^{\bullet -}$ or by direct reduction of O_2, against catabolizing cytosolic enzyme activities, including catalase ($H_2O_2 + H_2O_2 \rightarrow 2H_2O + O_2$), glutathione peroxidase or Gpx ($H_2O_2 + 2GSH \rightarrow 2H_2O + GSSG$), or thioredoxin peroxidase [$Trx(SH)_2 + ROOH \rightarrow TrxS_2 + ROH + H_2O$]. Thioredoxin peroxidase uses the reducing equivalents provided by Trx to reduce H_2O_2 and alkylhydroperoxides (79). The rate of removal of H_2O_2 *in vivo* as a direct function of Gpx activity×GSH has been mathematically defined (376).

The persistence of signals regulating its synthesis, the abundance and availability of •NO scavengers, as well as NOS degradation determine •NO steady-state levels. It is worth noting that autoregulation of NOS is probably another factor in determining •NO steady-state levels; this may at least be true for eNOS, which possesses a redox-reactive Cys residue susceptible of *S*-nitrosylation that disrupts the zinc tetrathiolate cluster and, thus, the active dimeric conformation of eNOS (445).

In general terms, during neuroinflammatory processes, the synthesis of •NO depends on the exacerbated activity of microglia through iNOS, which oxidizes L-Arg to L-citrulline to produce •NO by consuming O_2 and NADPH (327). The structure of this enzyme is similar to that of the cytochrome P450 reductase, containing a reductase and oxygenase domain with specific recognition sites for flavine mononucleotide (FMN), flavine-adenine dinucleotide (FAD), and for NADPH (467).

iNOS upregulation derives from diverse inflammatory signals. By inhibiting the activation of microglia with minocycline, a semisynthetic second-generation tetracycline, it is possible to attenuate •NO-mediated neuronal and axonal destruction *in vitro* in a process that involves the inhibition of the p38MAPK pathway; this pathway controls iNOS expression at the transcriptional level. Some of the inflammatory signals are viral double-stranded RNA, HIV-1 gp120 (567), bacterial endotoxin LPS (303), and cytokines like IFN-γ (258), IL-1β, TNF-α, or a combination of these (457). These signals lead to the activation of transcriptional factors, such as the interferon regulatory factor (IRF1), NF-κB, activator protein 1 (AP-1), CCAAT/enhancer-binding protein (C/EBP), Ca^{2+}/cAMP response element–binding protein (CREB), and the STAT family (175). In astrocytes, iNOS upregulation responds particularly to IL-1β and viral nucleic acids or proteins, low-density lipoprotein (LDL), and high-density lipoprotein (HDL), and, indirectly, responds to isoprenoids (392). The latter induce isoprenylation of membrane-bound small G proteins Rac and Ras, which become active and, after the extracellular signal–regulated kinase (ERK)/JNK pathways, lead to iNOS expression (324).

Endogenous scavengers of •NO are primarily heme groups, which oxidize •NO to nitrates. Additionally, GSH and bicarbonate are important •NO scavengers and play a role in redox regulation. GSH reacts with •NO to yield *S*-nitroso-L-glutathione, an important *S*-nitrosothiol •NO donor (nitrosothiols offer a mechanism with which to buffer the steady-state flux of •NO in biologic systems), whereas the coupled $HCO3^-/CO_2$ scavenges excessive •NO to produce the nitrosoperoxocarboxylate adduct ($ONOOCO_2^-$) (Fig. 7). This breaks down quickly becoming harmless. However, $ONOOCO_2^-$ adducts also react with $ONOO^-$ and enhance protein nitration (54, 348). The ease with which •NO and heme groups react with each other gives •NO an important role in cell signaling. As soon as it is produced, •NO diffuses quickly through membranes and binds to the soluble, heme-containing guanylyl cyclase (sGC) (65). It then changes its conformation and activates the enzyme for the production of the cyclic guanosine monophosphate (cGMP). Mutation of two cysteines located in the N-terminal, putative heme-binding region of the sGC β_1 subunit yields proteins insensitive to •NO (152). Paradoxically, sGC desensitization may also be caused by *S*-nitrosylation due to the overproduction of •NO (478).

•NO binds to many iron-containing proteins: *cis*-aconitase, NADH-ubiquinone oxidoreductase, lipoxygenase, and most heme proteins, possibly including a prostaglandin endoperoxide synthase, which might link •NO biosynthesis to prostanoid production (167). Through cGMP activation, •NO regulates the protein kinase B (Akt) and CREB pathways, two relevant neuroprotective pathways (452, 538). Furthermore, •NO activates CREB phosphorylation-dependent transcriptional activity through cGMP signaling, but also promotes *S*-nitrosylation of nuclear proteins that favor CREB binding to its promoters on target genes. However, high concentrations of •NO, as produced by activated microglia, lead mostly to tissue damage through the rapid formation of reactive nitrogen species (RNS) and dangerous protein interactions markedly related to neuroinflammation and neurodegenerative diseases (453).

It is worth mentioning that the activation of the phosphatidyl inositol 3-kinase (PI3K)/Akt pathway may also be mediated by H_2O_2, acting as an intracellular messenger (277, 465). Akt phosphorylates the Bcl-2–associated death promoter (BAD) on Ser136, promoting the dissociation of the Bcl-2/Bax complex, this means, Akt prevents apoptosis. HNE, conversely, dephosphorylates and inactivates Akt through a caspase/protein phosphatase 2A (PP2A)-dependent mechanism (312).

Finally, the influence of •NO steady-state levels on neuroinflammatory processes depends on iNOS degradation through the 26S proteasome. The rapid cellular rate of turnover of iNOS (half-life, 1.6 ± 0.3 h), constitutes a regulatory mechanism by itself (265).

The mechanism of iNOS degradation remains to be clarified, but it is clear that it represents a balance of forces. For example, in microglial and neuron–microglial cell culture, LPS-induced p38MAPK increases iNOS, but this action is modulated by the transforming growth factor-β1 (TGF-β1) that increases iNOS degradation (350, 565) or the peroxisome proliferator–activated receptor-gamma (PPAR-γ), which in turn decrease the expression and the activity of iNOS (253), or by both. PPAR-γ, induced by binding diverse ligands, including natural fatty acid derivatives, blocks MAPK through the PI3K/Akt pathway (174). By using pioglitazone, a PPAR-γ agonist, it was possible to inhibit *in vitro* LPS-induced iNOS expression and •NO generation, both in neurons and in

microglia, through the inhibition of p38 MAPK activity and at the expense of an increase in the PI3K/Akt activity (588). However, p38 MAPK reciprocally may phosphorylate PPAR-γ at its Ser^{82} residue, reducing its sensitivity to PPAR-γ ligands and its transcriptional activity by inhibiting PPAR-γ-DNA binding (66). Paradoxically, p38 MAPK could decrease iNOS through phosphorylated PPAR-γ through a nongenomic regulatory mechanism in which the phosphorylated PPAR-γ interacts with the NF-κB p65 subunit (83).

B. S-*nitrosylation in neurodegenerative disorders*

More than a review on nitrosative chemistry (137), herein we describe the dynamic, posttranslational regulation induced by the attachment of •NO, SNO, or several higher oxides to nucleophilic groups on proteins or particular residues on a protein (cysteine or tyrosine, for example) and their functional consequences. In this review, we use the term *S*-nitrosylation, regardless of the reaction mechanism for the following reasons. *S*-Nitrosation describes the addition of nitroso group (-NO) to form R-SNO, a third order in •NO (59), whereas "nitrosylation" refers strictly to the addition of a molecule of •NO to a metal to form nitrosyl species (228). Nonetheless, •NO is exceptionally stable and, compared with all nitric oxide species, both atomic groups are the same (329). In addition, all •NO-derived products are also highly dynamic and have short lifetimes linked to the redox state (59). "Nitrosation" implies the specific chemical addition of NO^+, which is highly unstable in water at neutral pH, immediately hydrolyzing to nitrite; therefore, the reactions are highly complex in a dynamic biologic environment. Finally, the chimera "nitros(yl)ation" has been coined to indicate the involvement of nitrosation or nitrosylation or both (59). However, when posttranslational protein modifications occur independent of the sGC/cGMP pathway, or as an alternative biochemical pathway through which •NO triggers or modulates cell signaling, the broader term "nitrosylation" is preferred, in analogy to other chemical additions containing the "yl" particle, such as in acetylation or phosphorylation. The analogy is appropriate because, like phosphorylation, *S*-nitrosylation is stimulus evoked, precisely targeted, spatiotemporally restricted, and responds to changes in the extracellular milieu (183) (Fig. 8).

In neurodegenerative diseases, such as AD, HD, or ALS, *S*-nitrosylation of cysteine residues on the endoplasmic reticulum–located protein-disulfide isomerase (PDI) contributes to the dysregulation of this enzyme. Being responsible for the correct arrangement of disulfide bonds in their fully folded state, oxidation and dysregulation of PDI lead to protein misfolding and the establishment of an unfolded protein response (UPR) pathway (94, 369). PDI contains two double-cysteine (Cys^{36}, Cys^{39}, Cys^{383}, Cys^{386}), redox-active sites, each within domains with high sequence similarity to Trx and separated by an additional two Trx-related domains lacking reactive cysteines (145).

An accumulation of protein nitrosothiols occurs in MS, in which neurofilament proteins, NMDA receptors, α/β-tubulin, β-actin, and GAPDH, as well as the neuron-specific enolase, undergo *S*-nitrosylation (45).

S-Nitrosylation also was been related to parkin and peroxiredoxin (Prx); parkin is an E3 ligase associated with a familial form of PD (551). *S*-Nitrosylation of Cys^{241} and Cys^{260} in parkin protein inhibits E3 ligase activity, becoming proapoptotic during nitrosative stress and favoring protein misfolding (94). Dopaminergic neuronal death and accumulation of insoluble α-synuclein appearing as cytoplasmic inclusions in nigral neurons have also been observed, and many of these aggregates are nitrated or oxidized. Their presence is well documented after microglial activation and oxidative stress; thus, it is possible that •NO and superoxide released by activated microglia may be mediators that link inflammation and abnormal α-synuclein in PD neurodegeneration (157). Nitrated α-synuclein is also found in brains of patients with dementia with Lewy bodies, the Lewy body variant of AD, and multiple-system atrophy brains closely related to oxidative stress (163). Conversely, Prx, which reduces intracellular peroxides by using the Trx system as the electron donor, possesses critical cysteine residues (580). C51 and C172 in Prx2 are susceptible to *S*-nitrosylation, rendering the enzyme unable to reduce peroxides, with H_2O_2, $ONOO^-$, and hydroperoxides thereby promoting oxidative stress, as demonstrated in PD brains (142). In postmortem human AD cortical tissues, Prx also is abundant and more oxidized than that in age-matched controls. PC12 cells and rat primary hippocampal neurons transfected with wild-type Prx exhibit increased Aβ resistance and higher levels of Trx and Trx reductase, two enzymes critical for maintaining Prx activity (109).

Another PD-associated protein, the redox-sensitive molecular chaperone DJ-1 (also known as PARK7: Parkinson disease autosomal recessive, early onset 7), found primarily in the cytoplasm, is an antioxidant protein that eliminates H_2O_2 by undergoing autooxidation. Within 3 h of an oxidant challenge, DJ-1 translocates to mitochondria, a phenomenon related to neuroprotection, and 12 h later, it appears in the nucleus (237). DJ-1 is required for the activity of the nuclear factor-erythroid 2–related factor 2 (Nrf2) and prevents its

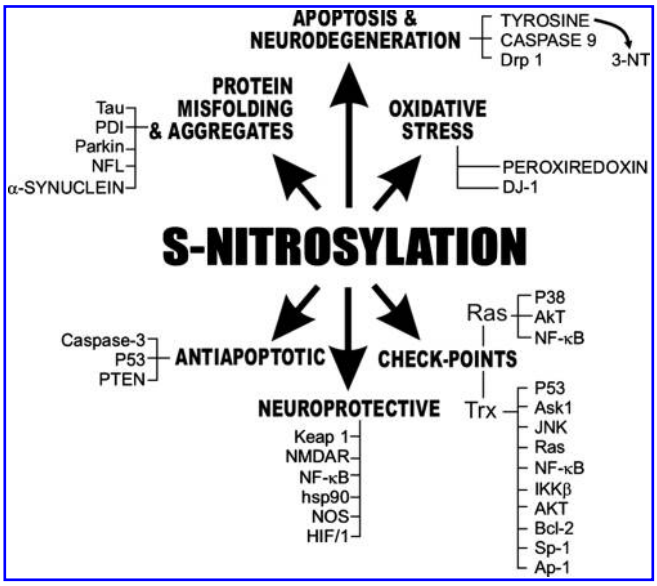

FIG. 8. Diverse functional consequences of *S*-nitrosylation reactions. *S*-Nitrosylation, as are *S*-glutathionylation or *S*-alkylation of redox-sensitive nucleophiles, are all pleiotropic events and depend greatly on dose and persistence of the stimuli, as well as the cell tuning.

association with the inhibitor protein Keap1 (97). It also modulates signaling cascades, such as the PTEN/PI3K/Akt pathway (255) and, importantly, has chaperone activity by preventing the aggregation of some proteins, such as the PD-implicated protein α-synuclein (610). DJ-1 possesses critical cysteine residues, Cys^{46} and Cys^{53}, which are prone to *S*-nitrosylation, whereas a third Cys^{106} residue is susceptible to oxidation, forming cysteine sulfinic ($-SO_2H$) or even sulfonic acid ($-SO_3H$), but not to *S*-nitrosylation (221). The neuroprotective role of DJ-1 relies on its translocation to mitochondria; this is a cysteine-sulfinic acid–driven event that depends on its Cys^{106} residue (69). However, it is speculated that *S*-nitrosylation of Cys^{46} during nitrosative stress, as occurs in PD, may disrupt the dimerization of DJ-1, which is the functional form (237). By this means, nitrosylation of DJ-1 may facilitate α-synuclein and Parkin aggregation (28, 610). Thus, under physiologic conditions or modest oxidative stress, reversible oxidation of Cys^{106} is useful to translocate and activate DJ-1. In contrast, under severe nitrosative stress, it is possible that *S*-nitrosylation of Cys^{46} inactivates DJ-1.

In contrast to the data summarized, a recent report indicates that the time frame for the translocation of DJ-1 from the cytoplasm to mitochondria and to the nucleus after oxidative stress is quite different, and that dimerized DJ-1 in mitochondria may be functional as an antioxidant not related to cysteine modification (237) (Fig. 8).

Nitrative injury is directly linked to the formation of filamentous tau inclusions as well (6). The abnormal polymerization of the tau molecule, assembled in the form of abnormal, insoluble paired helical filaments, is a seminal event in the neurodegenerative process underlying AD. Tau protein possesses five tyrosine residues on positions 18, 29, 197, 310, and 394. Tyr 18/29 nitrosylation markedly inhibits the ability of this molecule to self-associate and stabilize the microtubule lattice in a process linked to astrocyte activation (448, 450). Within an oxidative environment, both $ONOO^-$ and H_2O_2 also induce oxidation of cysteine residues in tau and microtubule-associated protein-2 (MAP2); this builds disulfide bonds that can then be reduced back to protein thiols by the glutaredoxin or the Trx reductase systems (280, 281). A strong correlation between neuronal oxidative/nitrosative damage and the progressive cognitive decline has been reported in AD, and, by extension, an increasing number of nitrosylated-proteins have been found in brains from these patients (74, 129). Tau isoforms containing both Cys^{291} and Cys^{322} may form intermolecular disulfide bonds through either Cys^{291} or Cys^{322}, producing a dimeric tau that presumably acts as a seed for initiation of tau polymerization (38). Blocking the −SH group on both Cys residues, mutating Cys for Ala, or keeping tau in a reducing environment all inhibited assembly (484). It has been postulated that elevated amounts of protein oxidation could lead directly to the formation of tau neurofibrillary tangles through a cysteine-dependent mechanism; however, the mechanism remains to be clarified (155) (Fig. 8).

The neurofilament light-chain polypeptide (NFL), which is one of the most abundant cytoskeletal component in neurons, represents intense nitrosylation and the positive correlation of this phenomenon with the severity of cognitive impairment, as demonstrated *in vivo* (6). In addition to nitrosylation, NFL undergoes hyperphosphorylation and a weak interaction with the nuclear distribution element-like (NUDEL), an essential protein in maintaining the stability of the neurofilament (6) (Fig. 8).

The nitrosylation of the phenolic ring of tyrosine yields 3-nitrotyrosine (3-NT), whose concentration as well as the 3-NT/tyrosine ratio are considered common markers of nitrosative stress. Both of them, as evaluated in the CSF, significantly increase with advancing age; however, the increase is more than sixfold greater in patients with AD compared with controls of a similar age (543). In addition, beyond its role as marker of nitrosative stress, 3-NT by itself causes apoptosis and neurodegeneration (46).

Recently, a dystrophin-related protein 1 (Drp1) that intervenes in mitochondrial division, fission, and fusion processes (506), has been discovered to be sensitive to *S*-nitrosylation. This phenomenon could explain the mitochondrial fission, synaptic loss, and the neuronal damage observed in AD, alterations that could be abrogated by preventing nitrosylation of Drp1 by cysteine mutation (89) (Fig. 8).

Whereas multiple nonenzymatic mechanisms are known to produce nitrated tyrosine residues, most tyrosine nitration events involve catalysis by metalloproteins, such as myeloperoxidase, eosinophil peroxidase, myoglobin, the cytochrome P-450s, SOD, and prostacyclin synthase. A cysteine residue (Cys^{99}) and three histidine residues coordinate the propeptide domain and the Zn^{2+} ion at the matrix metalloproteinase catalytic site. Cys^{99} is susceptible to nitrosative and oxidative stress (173, 514), as documented in cerebral ischemia models. *S*-Nitrosylation or sulfonation of Cys^{99} culminates in the release of the propeptide's cysteine sulfur "blockage" of the active site and promotes MMP activation (413). Such a redox regulation in MMP-9, along with its upregulation by the proinflammatory cytokines IL-1β and TNF-α, through NF-κB (138), could aid in explaining a potential extracellular proteolysis pathway to neuronal cell death, and the involvement of matrix metalloproteins in the pathogenesis of other neuroinflammatory/degenerative diseases, such as AD, PD, MS, and ALS (170). Once activated, MMP also causes opening of the BBB, immune cell invasion of neural tissue, and apoptosis (173).

Extensive nitrosylation is a protagonist in the pathogenesis of ALS, in which oxidative stress and neuroinflammation lead to progressive motor neuron degeneration in brain and spinal cord. In spinal cord protein extracts of a transgenic mouse model of familial ALS (FALS), 32 proteins were found *S*-nitrosylated *in vivo* under physiologic or pathologic conditions (73). Several important proteins, such as α- and γ-enolase, ATP synthase β chain, and heat-shock cognate 71-kDa protein, as well as actin, were found Tyr-overnitrated before clinical manifestations.

The effector protease caspase-3 associates with damage progression and neuronal death in AD. Even more, caspase-3 is involved in the proteolytic cleavage of Alzheimer amyloid precursor protein (APP) and constitutes an alternative, non-secretase processing of Aβ during apoptosis (161, 415). Caspase-3 also is related to neuronal death in traumatic brain injury (96), ischemia (113, 386), and seizures (193), besides a variety of neurodegenerative disorders (336). Caspase-3 possesses cysteine redox-sensitive residues susceptible to *S*-nitrosylation, as demonstrated in a human promyelocytic leukemia cell line U937 (353), as well as in HeLa cells by using *S*-nitrosogluthathione (GSNO)-sepharose that mimics site-specific *S*-gluthathionylation, a tool to identify GSNO-

induced mixed-disulfide formation (261). Thus, nitrosative stress, paradoxically, may reduce apoptosis by causing caspase-3 *S*-nitrosylation with the consequent lower caspase-3 activity (292, 353) (Fig. 8). Additionally, it is likely that glutathionylation and subsequent deglutathionylation of Cys^{184} and Cys^{220} regulate the TNF-α–induced caspase-3 cleavage and apoptosis (396).

In ischemic neuronal death, the activation of caspase-3 along with JNK activation and the consequent mitochondrial alterations are key events. Thus, the inhibition of caspase-3 by *S*-nitrosylation could explain the beneficial antiapoptotic influence of •NO on multiple cell lines, as well as in lymphocytes, endothelial cells, eosinophils, splenocytes, ovarian follicles, and hepatocytes (292). However, *S*-nitrosylation of caspase-3 is not sufficient to rescue neurons from death, as evaluated *in vitro* by using cortical neurons in culture treated with staurosporine and camptothecin, broad-specificity kinase inhibitors and inducers of apoptosis; thus, it could be possible that inhibition of caspase by •NO unmasks a caspase-independent form of cell death (609). Caspase-3 denitrosylation, conversely, may lead to enzyme activation either by relieving its interaction with an inhibitory protein or by unblocking the cysteine at the active site (351).

Caspases are key enzymes in neurodegenerative processes. Apart from caspase-3, caspase-1, a highly regulated cysteine-dependent protease that plays an essential role in host defense and the execution of the innate immune response, which is activated within the inflammasome, may be redox regulated as well. Caspase-1 also has at least two potential levels of regulation by *S*-nitrosylation [*i.e.*, modulation of protein–protein interaction within the inflammasome or *S*-nitrosylation of Cys^{285} at the active site of the enzyme (579)]. By adding exogenous NO, it is possible to inhibit the activity of caspase-1 and subsequent IL-1β processing in monocytes and macrophages (259).

V. Role of GSH Depletion and Its Replenishment

The intracellular redox environment influences DNA synthesis, enzyme activation, selective gene expression, cell-cycle progression, proliferation, differentiation, and apoptosis. A reduced redox potential of the cytosol is vital for proper cell function. In the CNS, in both chronic neuroinflammatory responses and acute injuries, as long as GSH can be replenished and a reducing intracellular environment prevails, microglia may promote, repair, and remodel the injured brain tissue, thereby restricting toxic substrates released by the damaged cells (87, 379, 547). A reduced intracellular environment depends on a balance between ROS production and GSH demands (Fig. 6). The activation of the transmembrane glutamate/cystine-antiporter system in conjunction with excitatory amino-acid transporters maintains the GSH levels.

An interesting connection exists between neuroinflammation/neurodegenerative factors and a redox environment (26, 289). The transport of cystine into cells is rate limiting for glutathione synthesis, and the antiporter system Xc is one of the main transporters responsible for cystine transport in the CNS where it determines the redox homeostasis (61). Cysteine, working in a sodium-independent manner, takes up cystine by 1:1 exchange with glutamate across the cell membrane (571). Thus, thanks to the antiporter system Xc, cysteine and its disulfide cystine (Cys/CySS) are the principal thiol/disulfide control system and maintain a redox potential (Eh) around −80 mV (234), whereas in subjects with disease, this redox state becomes oxidized to between −62 and −20 mV (233). When oxidized, the Eh Cys/CySS induces ROS and IL-1β, as observed in monocytes, suggesting that strategies to preserve Eh Cys/CySS may represent a means to control IL-1β in inflammatory disease states (222), as is the case in neuroinflammatory diseases.

When NADPH is activated by reactive microglia and orchestrates the $O_2^{\bullet -}$ production, the superoxide dismutase (SOD) enzyme catalyzes the dismutation of the $O_2^{\bullet -}$ to O_2 and H_2O_2 (346). Gpx in turn removes H_2O_2 through the oxidation/reduction cycle of the active center, by using GSH as the reductant (376). During oxidative stress, lipid-peroxidation by-products, such as the diffusible electrophile HNE, are extensively produced and react easily with cellular nucleophiles while the enzyme glutathione *S*-transferase (GST) conjugates GSH through the sulfhydryl group to the HNE electrophilic center (225). By this means, HNE also produces nerve-terminal toxicity by forming adducts with sulfhydryl thiolates on proteins involved in neurotransmission. Thus, the treatment of cells with HNE results in depletion of intracellular GSH. Moreover, GSH also is depleted by excessive demand (19). By using *in vivo* fibrillar Aβ (fAβ)-injected rat brains, we found an inverse correlation between the GSH-peroxidase activity and lipid peroxidation levels (459); 84 h after intracerebral fAβ injection, the enzyme was almost depleted, whereas lipid peroxidation by-products reached their higher concentrations. Once oxidative stress, lipid peroxidation, and high extracellular glutamate deplete GSH, the glutamate/cystine-antiporter system Xc takes up cystine to regenerate GSH, essential at high doses for effective organic hydroperoxide detoxification (302); however, the increased extracellular glutamate deplete cells of cystine by blocking the gradient-driven glutamate/cystine antiporter system.

A cooperative action of glutamate transporters and cysteine/glutamate antiporter system Xc exists to protect from oxidative glutamate toxicity (289). The excitatory amino-acid transporters (EAATs) serve as support of the Xc system by reducing glutamate concentrations in the extracellular space, preventing glutathione depletion caused by high extracellular glutamate. For example, EAAT1-5, Na^+-dependent glutamate transporters, present in neurons and glia (30), play an important role in glutamate uptake and are a support system to prevent GSH depletion. Important neurodegenerative disorders implicate EAAT dysfunction. In ALS, reduced levels of the mainly astroglial glutamate transporter EAAT2, as well as multiple abnormal EAAT2 mRNA species, have been found in motor cortex and spinal cord and in ALS brain tissue (224). In epilepsy, particularly in temporal lobe epilepsy patients, alternative splicing of glutamate transporter EAAT2 RNA in neocortex and hippocampus of the temporal lobe have been described (204). EAAT abnormalities also have been implicated in cerebral stroke, epilepsy, AD, HIV-associated dementia, and malignant glioma (30, 496).

The glutamate-overridden glutamate/cystine-antiporter system may lead to an excessive influx of cations, particularly Ca^{2+}, which activates a number of enzymes, including phospholipases, endonucleases, and proteases, such as the calcium-dependent cysteine-protease calpain (120). This process

is defined as excitotoxicity. In addition, the depletion of GSH activates neuronal 12-lipoxygenase, which contributes directly to a greater increase in ROS and intracellular Ca^{2+}, whereas its metabolites activate sGC, which may mediate apoptosis during GSH depletion by the sGC/cGMP pathway (297). An interesting hypothesis postulates that neurotoxicity exhibited by microglia is not so much due to ROS, but to excitotoxic stress through the liberation of a neurotransmitter, sufficiently stable and diffusible to affect neurons over intermediate distances (26). The redox-sensitive transcription factor Nrf2, responsible for activating transcription in response to oxidative stress, has an important neuroprotector role in this circumstance because neither NMDA nor glutamate seems to be able to affect Nrf2 translocation to the nucleus. Thus, Nrf2 transactivates the expression of detoxifying enzymes, antioxidant enzymes, reducing molecules, and Nrf2 itself. Neurons lacking Nrf2 are more susceptible to excitotoxic damage, as demonstrated in primary murine cortical cultures (270).

When oxidative stress is out of control and GSH demands exceed the available substrate for GSH replenishment, GSH is depleted, and microglial overreaction becomes dangerous. In AD, for example, microglial activity is necessary for the plaque burden in transgenic mice. However, as long as GSH levels continue decreasing, the microglia-guided inflammatory response becomes more severe (34). It has been demonstrated *in vitro* that Aβ, added to rat cortical neurons, causes the depletion of GSH, which seems to be related to a reduction in Gpx and GR activities (342). GSH depletion induces cognitive impairment in rats and mice, as demonstrated by the disruption of the short-term spatial memory in the Y-maze (119). Intracellular levels of GSH have been measured in lymphoblast lines carrying mutations in genes associated with AD, with GSH levels being significantly decreased with respect to controls (76). Melatonin, conversely, reduces Aβ-induced ROS, allowing GSH replenishment *in vivo* (459), which correlates with a significant reduction of the proinflammatory response (176).

In PD, oxidative stress with low levels of GSH is related to the progression of the disease, which is particularly important in mitochondria (344) and is associated with the buildup of defective proteins. This leads to cell death of dopaminergic neurons by impairing the ubiquitin-proteasome pathway of protein degradation (37). It has been demonstrated that, after GSH depletion, the role of low •NO concentrations as a neurotrophic agent on dopaminergic cells switches to neurotoxicity due to the persistent activation of the ERK-1/2 signaling pathway in glial cells (67).

The reduction in GSH levels may cause NSC34 motor neuron–like cells to increase the production of ROS, accompanied by a significant proinflammatory response in addition to mitochondrial dysfunction, translocation of the apoptotic-inducing factor (AIF), cytochrome *c* release, and caspase 3 activation. These changes probably imply that GSH depletion has a key role in the motor neuron degeneration observed in ALS (87).

Protection against GSH depletion in cultured cortical neurons under glutamate neurotoxicity by using low concentrations of 15-deoxy-Δ12,14-prostaglandin J2 (15Δ-PGJ_2) or HNE, induces both the intracellular GSH and gene expression of glutamate-cysteine ligase (469). The latter is the rate-limiting enzyme in GSH synthesis through the induction of an adaptive response, primarily through the upregulation of the intracellular GSH synthesis.

VI. Dynamic Redox Control in Neuroinflammatory Processes: Glutathionylation/Deglutathionylation

Posttranslational modification of proteins is directly linked to redox status. ROS and RNS induce modifications on –SH residues from cysteine modifying the structure or activity of the involved proteins and the GSH system, composed of NADPH, glutathione reductase, GSH, and glutaredoxin, plays a crucial role by protecting protein cysteines from irreversible oxidation (200, 376).

S-Glutathionylation is a physiologic, reversible protein modification of cysteine residues with glutathione in response to mild oxidative stress. Thanks to reversibility, *S*-glutathionylation has modulatory effects on protein function and occurs during physiologically and pathologically relevant situations. Thus, the formation of mixed disulfides between glutathione and low-pK_a cysteinyl residues, coupled to the reduction of protein GSH-mixed disulfides (deglutathionylation), maintains a GSH/GSSG ratio with a cytoplasmatic redox potential of about −240 mV (479). Under physiologic conditions, this is a high ratio, in which the GSSG concentration is less than 10% of the GSH concentration. In brief, GSH may take one electron from a free radical being converted into the glutathionil radical, and two glutathionil radicals yield a GSSG molecule. In the glutathione redox cycle, GSH is oxidized to GSSG by free radicals and ROS, with the intervention of the Gpx enzyme. Eventually, GSSG may be reduced back again by using NADPH reducing equivalents. Oxidized glutathione may form a covalent S-S bond with another oxidized glutathione, or may glutathionylate an oxidized Cys residue in a protein. Then, through thioredoxin or the glutathione reductase (GR) enzyme in the presence of NADPH, oxidized glutathione can be rescued to its reduced GSH form (200) (Fig. 6).

The glutathionylation/deglutathionylation process prevents irreversible oxidation of protein thiols, which also acts at the same time as a switch that turns off and on the protein function, according to redox variations in its environment. Once the oxidative pressures (ROS/RNS overproduction) reach thiol groups (−SH) on susceptible cysteine residues (low pK_a), they may undergo progressive oxidation, going from –SH to –SOH (sulfenic acid), then –SO_2H (sulfinic acid), and then –SO_3H (sulfonic acid). The Cys–SOH moiety readily reacts with GS^- by forming intermolecular disulfides and resulting in glutathionylated proteins (P-SSG); this occurs not only under pathologic but also under physiologic conditions. Thus, in addition to its reversibility, these properties confer to *S*-glutathionylation a significant role during growth, differentiation, cell-cycle progression, metabolism, and transcriptional activity. The removal of the GSH moiety from those protein mixed disulfides (*i.e.*, deglutathionylation), depends first on the concentrations of GSSG and the levels of reduced thiols in the cellular environment. However, deglutathionylation is not necessarily a random process but an enzymatic controlled process in which three enzymes play a critical role: glutaredoxin, sulfiredoxin, and thioredoxin (111, 112, 347).

Glutaredoxin, localized essentially in the neuronal cells (23), reduces the GSH-mixed disulfide of inactive oxidized nuclear factor-I and thus restores its DNA-binding activity (25). During MPTP-induced neurotoxicity in rats, mitochon-

drial complex I dysfunction due to protein thiol oxidation at its active site is reversed by glutaredoxin. This implies the redox regulation of the enzyme through a glutathionylation/deglutathionylation mechanism. When antisense oligonucleotides to glutaredoxin were administered, the recovery of mitochondrial complex I was prevented (249). These results are consistent with the low levels of GSH typically observed in the substantia nigra of PD patients, which may reveal a failure to maintain a reduced thiol status of mitochondrial complex I (417). It is hypothesized that mitochondrial dysfunction could induce α-synuclein oligomerization through ATP depletion–driven microtubule depolymerization and through ROS increase–driven protein oxidation (139). During the neuroinflammatory response, Grx-dependent reversal of *S*-glutathionylation of IKK-β constitutes a protective mechanism that modulates the extent and timing of activation of NF-κB in response to redox changes by protecting IKK-β from irreversible inactivation (449).

Sulfiredoxin is a small ATP-dependent oxidoreductase first characterized for its regulation of Prx(s) through reduction of the conserved cysteine from sulfinic to sulfenic acid, which is involved in the regulation of downstream transcription factors and kinase signaling pathways (44). Induction of sulfiredoxin expression in both neurons and glia is mediated by Nrf2, acting through a cis-acting ARE in its promoter, which contains an embedded AP-1 site. This directs the induction of sulfaredoxin by synaptic activity (509). By this means, Prx-$SO_2/^3H$ can be reduced to the catalytically active thiol form, and Prx is restored to the thioredoxin cycle, preventing permanent oxidative inactivation of Prxs by strong oxidative insults (580). Sulfiredoxin possesses its own critical cysteine residue located at the COOH terminus, which participates in the catalytic reversal of •NO-induced protein glutathionylation (44). With this mechanism, sulfiredoxin has been identified as a regulator of PTP activity (149) because glutathionylation of PTP inhibits, whereas deglutathionylation restores its phosphatase activity (27). In this manner, phosphorylation/dephosphorylation and glutathionylation/deglutathionylation, two posttranslational mechanisms of control and adaptation, become linked. In neuroinflammation, this has significant effects on mitochondrial enzymes in which the glutathionylation/deglutathionylation mechanism of switching to regulate enzymatic activity could be beneficial (148). For example, mitochondrial $NADP^+$-dependent isocitrate dehydrogenase (IDPm), which supplies NADPH in the mitochondria needed for the regeneration of mitochondrial GSH, is inactivated by glutathionylation in brains of MPTP-treated mice (252); as a result, sulfiredoxins may have a therapeutic role in PD. The number of glutathionylated proteins in different neurodegenerative pathologies is growing, such as the proapoptotic protein p53 (123), GAPDH, which catalyzes the conversion of glyceraldehyde-3-phosphate to 1,3-phosphoglycerate in the first oxidation–reduction reaction of the glycolytic pathway (375), or the endoplasmic reticulum–located protein-disulfide isomerase, which is involved in protein misfolding (368, 546). Importantly, 12-lipoxygenase, which inserts molecular oxygen into free and esterified polyunsaturated fatty acids, because of its multiple cysteine residues, may be glutathionylated in glutamate-challenged neural cells, leading to cell death (67, 402). Thus, the mechanisms of deglutathionylation may protect against glutamate-induced neurotoxicity.

The thioredoxin system comprises the selenoenzyme thioredoxin reductase (TrxR), Trx, and NADPH. TrxR is an NADPH-dependent, FAD-containing disulfide reductase and catalyzes the electron transfer from NADPH to thioredoxin. TrxR possesses two vicinal cysteines ($Cys^{497}/SeCys^{498}$) in the active site, which are susceptible to alkylation at pH 6.5, and Trx has a pair of redox-active Cys residues (Cys^{32}/Cys^{35}) sensitive to various alkylating reagents (Fig. 9). In its reduced form (−SH2), Trx donates electrons to protein substrates [for example, ribonucleotide reductases, peroxiredoxins, and methionine sulfoxide reductases], becoming oxidized (−S2) to form an intramolecular disulfide bond. Then, the NADPH-coupled TrxR reduces oxidized Trx back to its reduced state (93, 201).

During oxidative stress and lipid peroxidation, as occurs in neuroinflammatory diseases, the brain levels of HNE show a significant increase. HNE is a potent hydroxyalkenal derivative agent that reacts with nucleophilic sites in DNA, alkylates proteins, and modifies redox residues both in TrxR and in Trx, blocking the entire Trx system (141). Considering that the TrxR-Trx system regulates redox-sensitive processes essential for cell growth, differentiation, and genomic integrity, with an important influence on the activation of NF-κB and AP-1 (190, 200), the inhibition of Trx by HNE may explain in part the pathophysiologic role of HNE in neurodegenerative disorders (Fig. 9). In addition, HNE is a potential inducer of signaling pathways that involve JNK and p38 activation, both c-Jun inducers, with the consequent activation of AP-1 (200, 550) (Fig. 10).

Trx modulates the function of protein substrates through the redox state of thiol groups. Several important targets in nonneuronal cells are controlled by *S*-nitrosylation, *S*-alkylation, or direct oxidation of Trx cysteine residues (Fig. 9), including Ask1, JNK, Ras, NF-κB, IKKβ, Akt, FLICE inhibitory protein, Bcl-2 (196), AP-1, Sp1, and p53. Trx reduces one

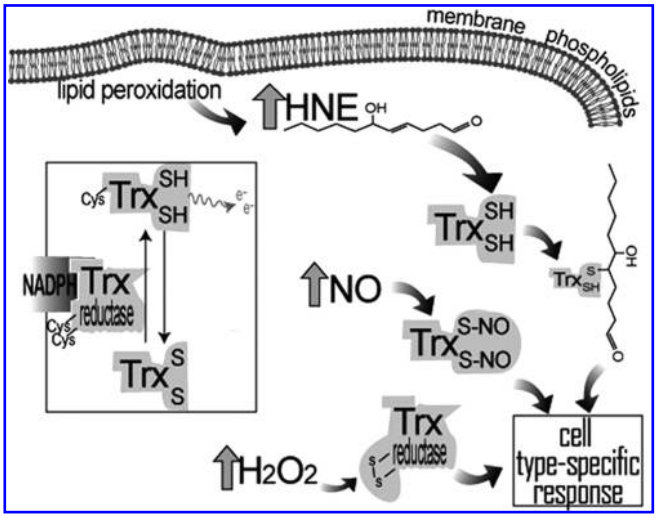

FIG. 9. The thioredoxin system is responsible for maintaining a reduced redox environment, and its presence is determinant for many physiologic processes. Under excessive oxidative stress conditions, the Trx system may be inhibited by ROS, RNS, or lipid-peroxidation derivatives, acting on Trx or on Trx reductase. The response is cell-type specific. Furthermore, it varies depending on the cell compartment in which these phenomena occur.

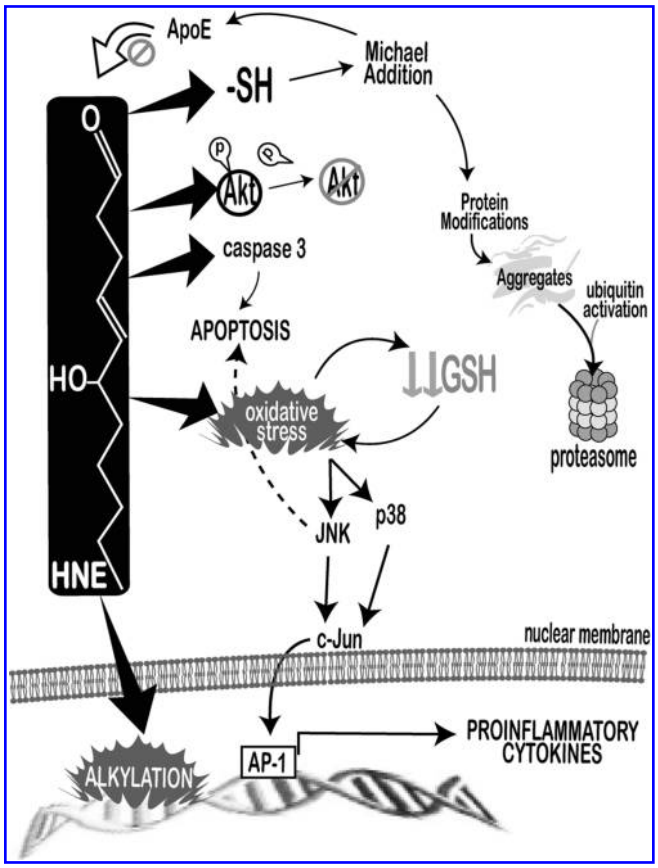

FIG. 10. HNE increases in brain tissue and cerebrospinal fluid of AD patients, the same as in the spinal cord of ALS patients. HNE directly scavenges GSH, with the consequent increase in oxidative stress. Conversely, HNE activates caspase-3, JNK, and p38 proinflammatory pathways, and it facilitates protein misfolding and Akt dephosphorylation. Haplotypes 2 and 3 of ApoE react with HNE through cysteine residues and hamper those damaging interactions. Apoe4, a known risk factor in AD, does not contain Cys residues and does not stop HNE.

of the essential Cys residues on NF-κB by forming a covalent bond between Cys^{32} on Trx and Cys^{62} on p50 NF-κB (436). Moreover, by reducing the N-terminal domain of Ref-1, Trx allows the activation of p53 and its binding to DNA (552). Conversely, in control of oxidative stress, Trx removes H_2O_2 directly or activates Gpx and the Trx-dependent peroxidase or peroxiredoxin (78). Thus, the thioredoxin system functions both as a protein disulfide reductase and as a hydrogen donor. Overexpression of Trx is sufficient to halt the apoptotic current surge in neurons (16). Transgenic mice overexpressing Trx1, the Trx cytoplasmic isoform, show an extended life span and are relatively resistant to ischemia-mediated brain damage, whereas intravenous administration of Trx decreases brain damage after transient focal cerebral ischemia in mice (188).

Uncoupling the dimer Trx/Ask1 represents a crosstalk between redox and phosphorylative processes (Fig. 11). The activity on NOX plus mitochondrial leakage (Fig. 7), an important feature in neurodegenerative processes associated with neuroinflammation, leads to ROS overproduction (58), which oxidizes Trx and activates Ask1 by uncoupling the dimer Trx/Ask1 (470). This is important in AD pathology, in which it has been found that Aβ cause deregulation of Grx and Trx antioxidant systems, as well as nuclear export of Daxx, an adaptor protein that binds to the N-terminal noncatalytic domain of Ask1, and thereby activates JNK downstream of Ask-1 (5). Ask1 dimerizes and activates SEK1 (MKK4), which in turn releases the JNK and p38 MAP kinase pathways required for TNF-α–induced apoptosis (542). The activation of JNK and P38 pathways may be strengthened by lipid peroxidation derivatives, such as HNE, commonly implicated in neuroinflammatory diseases (550). A marked activation of the Ask-1–JNK pathway over the Ask-1–p38 pathway leads to apoptosis, whereas the overactivation of the Ask-1–p38 pathway leads to differentiation of neuronal cells (531). JNK acts on an SCFβ-TrCP-ubiquitin ligase complex as well, promoting ubiquitination of the phosphorylated IκBα molecule.

Trx expression, as well as its translocation from the cytoplasm to nucleus, is induced by PPAR-α and, conversely, Trx suppresses PPAR-α transcriptional activity as well as its binding to the PPAR-response element (308). PPAR-α is a member of the nuclear-receptor superfamily that protects the brain from excessive oxidative stress and inflammation in traumatic brain injury and stroke (143). The use of PPAR-α agonists results in neuroprotection against Aβ, probably activating the Wnt signaling pathway (215).

VII. Redox Control of Enzymes Involved in Neuroinflammation

A. NADPH-oxidase is controlled by redox variations

The exposure of microglia to inflammatory stimuli or to certain molecular patterns (PAMPs or DAMPs), initiates the activation of multiple signaling cascades. The cytosolic Rac protein of the Rho family of small G proteins, anchored by the interaction of its C-terminal prenyl moiety with the GDP dissociation inhibitor (RhoGDI) (611), passes from an inactive guanosine diphosphate (GDP)-bound state to an active guanosine triphosphate (GTP)-bound state. The transition is mediated by guanine-nucleotide exchange factors (GEFs), although the precise mechanism remains to be established (444). Additionally, another cytosolic protein complex formed by $p47^{phoxPHOX}$, $p67^{phox}$, and p40, which remained stable through Src homology 3 (SH3) domain-peptide interactions (118), separates when $p47^{phoxPHOX}$ and $p67^{phox}$ become phosphorylated in a process that involves a diversity of kinases according to cell type and function. For example, ERKs are particularly relevant for the phosphorylation of $p47^{phox}$ in microglial cells (433); however, protein kinase C-delta (PKC-δ) is the involved kinase in human monocyte/macrophages (36) for the same target. $p47^{phox}$, extensively phosphorylated when the oxidase is activated, possesses four cysteine residues (116, 131, 228, 443), and at least three of them are susceptible to redox regulation (214). This suggests that the activity of NOX could be controlled by redox variations (20). Additionally, ATP stimulation induces the assembly of NADPH oxidase through P2X receptors (197), a redox-related phenomenon, as we saw earlier on extracellular danger signals.

Once activated, water-soluble cytosolic proteins Rac-GTP, phosphorylated $p47^{phox}$, phosphorylated $p67^{phox}$, and p40 translocate to the membrane, where they form a complex with cytochrome b_{558}, which will be the catalytic core of a new

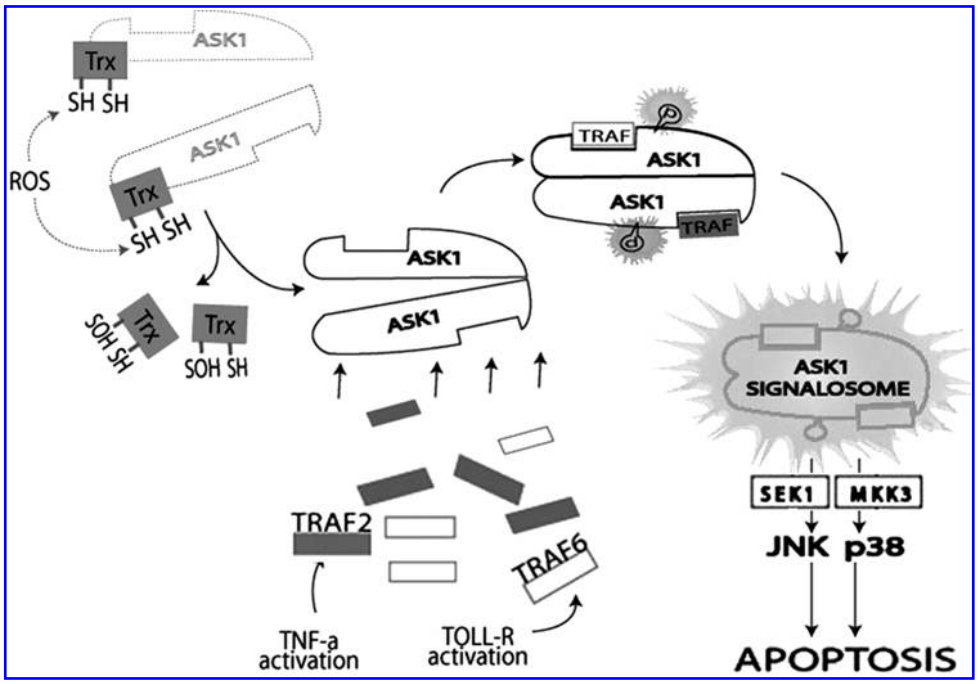

FIG. 11. Oxidation of cysteine residues on Trx allows the integration of ASK1 with TRAF components (coming from the activation of TNF receptors or Toll receptors). ASK1 is phosphorylated, and the ASK1 signalosome becomes activated, proceeding with the activation of the JNK and p38 signaling pathways. This represents an important redox-regulated pathway.

multimeric NOX used to transfer electrons from NADPH to molecular oxygen, generating $O_2^{\bullet -}$ (232, 486). Cytochrome b_{558} consists of a large glycoprotein gp91^{phox} or Nox-2 and a small protein p22 (405). Gp91^{phox} contains binding sites for flavin adenine dinucleotide [FAD; two hemes for each FAD- (20) and NADPH (523)]. Thus, NOX catalyzes the reaction: $2\ O_2 + \text{NADPH} \rightarrow 2\ O_2^{\bullet -} + \text{NADP}^+ + \text{H}^+$.

B. Microglial overactivated NOX in neuroinflammatory diseases

The importance of NOX induction in microglia is underlined in experiments in which microglia isolated from mice deficient in the catalytic component of the oxidase, gp91^{phox}, failed to induce oligodendrocyte death when LPS was added to microglia/oligodendrocyte cocultures (291). These experiments may help explain the role of the innate immune response in white-matter disorders, such as periventricular leukomalacia and MS (314).

A well-known correlation exists between neurodegenerative diseases and overactivated microglial NOX. fAβ, the major pathogenic component of amyloid plaques in AD, causes microglial proliferation mediated by NOX, an event that correlates with a marked translocation of the cytosolic factors p47^{phox} and p67^{phox} to the membrane in brains of AD patients, as well as the overproduction of both TNF-α and IL-1β (231, 498). In rat primary culture of microglial cells and human neutrophils and monocytes, fAβ activates NOX, an effect potentiated by interferon-γ or TNF-α, but blocked by tyrosine kinase inhibitors (39). Tyrosine kinases and small G proteins (Gα1 and Gα0) are involved in the activation of ERK by ROS. Moreover, in the interaction between fAβ and microglia, a specific Vav GEF has been identified, facilitating the exchange of GDP for GTP and increasing the amount of active Rac, a crucial component of NOX (577). Finally, a novel oxidative–degradative mechanism has been found, by which oligomeric Aβ induces ROS production mediated by NOX, while it stimulates the arachidonic acid release, possibly through NMDA receptors (491).

Neuroinflammation and NOX activity play key roles in PD. A major component of Lewy bodies responsible for neurodegeneration, the α-synuclein, forms aggregates (regardless of etiology of nigral neuronal damage) and activates microglia and microglial NOX, as evaluated in primary rat and mouse midbrain cultures (604). By this means, microglia enhance 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic neurodegeneration. The astroglial MPTP metabolite, 1-methyl-4-phenylpyridinium (MPP+), is taken into dopaminergic neurons by a dopamine transporter; it blocks mitochondrial respiration. NOX complex integration and activation has also been demonstrated in rodent models of PD in which inflammation-induced dopaminergic neurodegeneration [due to different substances, in addition to MPTP (512), including supranigral administration of LPS (219), rotenone (156), or paraquat (586)], is achieved. Moreover, NOX inhibition by drugs such as squamosamide derivative FLZ (602), sinomenine (a natural dextrorotatory morphinan analogue) (434), or dextromethorphan (315) protect dopaminergic neurons from neuroinflammation and neurodegeneration. Finally, *in vitro*, in LPS-induced dopaminergic neurotoxicity, the antiinflammatory cytokine IL-10 inhibits NADPH activation by preventing the translocation of the cytosolic subunit p47^{phox} to the membrane *via* a Janus kinase 1 (JAK1)-dependent mechanism (431).

It is important to note that ROS also are generated in response to hormones, growth factors, and cytokines, which

emphasize their varied physiologic roles in addition to the innate immune response. The latter occurs in nonphagocytic cells in which ROS are involved in cell signaling and metabolism and are being generated at low levels by NOX, where the $gp91^{phox}$component is represented by different homologues, such as Nox-1, Nox-3, Nox-4, Nox-5, Duox-1, and Duox-2 (245). Neurons also express the phagocyte, functional Nox-2 (533); this is also true for astrocytes when they are challenged by fAβ (1). In addition, cultured rat astrocytes express mRNAs encoding for NOX1, 2, and 4, and the dual oxidases 1 and 2 (447). During PC12 cell differentiation, after nerve growth factor (NGF)-induced neurite outgrowth, Nox1 increased to almost 10 times higher than the phagocyte type subunit, Nox2, and the consequential increase in $O_2^{\bullet -}$ production suppressed excessive neurite outgrowth (611). The activity of Nox-2 in microglia during brain ischemia, for example, coincides with the activity of Nox4 in neurons. Rather than being a part of the innate immune response, Nox4 probably has a role in angiogenesis (611).

NOX activity in microglia significantly influences the outcome. Thus, in SOD1G93A transgenic mice, a model of ALS in which a strong redox stress prevails, the levels of $O_2^{\bullet -}$ and the $gp91^{phox}$ component from NOX in spinal cord microglia are high, particularly Nox1 and Nox2. It is possible to delay the progression of motor neuron disease in SOD1G93A transgenic mice by disrupting these X-linked Nox1 or Nox2 genes (327). Nox2-KO mice present a marked decrease in the number of activated microglia after LPS treatment, avoiding the loss of nigral dopaminergic neurons *in vivo* (438). Conversely, experimental evidence for a role of Nox2 in AD has emerged (601). Thus, NOX has become a therapeutic target with a number of potential NOX inhibitors, including dextromethorphan, naloxone, morphine, diphenyliodonium, minocycline, IL-10, and dopamine, among many others (47, 229). All of them reduce microglia-mediated neurotoxicity in both mice and cortical neuron glia cocultures by inhibiting NOX (91, 290, 307, 320, 430, 432, 437); however, although promising, most of these compounds are incompletely characterized or show numerous other pharmacologic properties or even lack desirable druglike qualities (229). Thus, they are not ready for clinical use.

C. An oxidizable antioxidant, SOD

Among the enzymes involved in neuroinflammation, Cu-Zn superoxide dismutase-1 (SOD1) plays a key role. SOD1 is a cytosolic enzyme involved in dismutation of $O_2^{\bullet -}$ to H_2O_2. One of the most plausible theories about how nerve cells in the brain and spinal cord that control muscle movement are particularly sensitive to SOD1 mutations relates to the accumulation of harmful $O_2^{\bullet -}$. SOD1 regulates NOX-dependent $O_2^{\bullet -}$ production by binding Rac1 and inhibiting its GTPase activity, as shown in the *in vitro* experiments using SH-SY and M059J cell lines (186). The SOD1/Rac1 binding is redox dependent, and H_2O_2 levels seem to be the switch that connects and disconnects SOD1 from Rac1. Elevated H_2O_2 oxidizes Rac1 and SOD1, and they separate from each other, allowing the hydrolysis of Rac-GTP and the subsequent inactivation of the Nox2 complex. According to Harraz *et al*. (186), SOD1 mutants, such as those related to the familial form of ALS, are insensitive to local concentrations of ROS at sites of Rac/Nox2 complex activation and cannot dissociate from Rac1. Thus, Nox2 continues producing $O_2^{\bullet -}$. SODs, especially SOD1, have an important role in other neuroinflammatory diseases. For example, a decreased SOD1 activity was found in the CSF of patients with AD, PD, HD, and ALS, in which an imbalance exists between •NO overproduction and decreased SOD defense (51). Transplanted neurons showed a better survival in the presence of SOD1 in a rat model of PD (370).

The SOD1 gene is encoded in chromosome 21 (*21q22.1*). Thus, SOD is constitutively overexpressed in Down syndrome (DS). In this case, SOD overexpression does not coincide necessarily with a concomitant increase in Gpx and catalase, but is a result of the trisomy (11).

SOD1 is an antioxidant enzyme that catalyzes the conversion of single electron–reduced species of molecular oxygen to H_2O_2 and O_2; paradoxically, SOD1 is susceptible to oxidative stress by itself. SOD1 possesses several cysteine residues, which are important for protein appropriate folding (250) or are directly involved in catalysis (418). SOD1, composing 90% of the total SOD, has a Cys residue at position 146 in the C-terminal region, which is susceptible to irreversible sulfonation, as revealed by mass spectrometry in AD and PD brains (90). Thus, SOD1 may undergo oxidative damage by its own reaction product, H_2O_2, and forms proteinaceous aggregates, which appear associated with amyloid senile plaques and neurofibrillary tangles in AD brains (90, 154) and possibly with Lewy bodies in PD brains (381).

VIII. Main Redox-Related Pathways in Neuroinflammation

A. The central innate immune sensors: Toll-like receptors

Toll-like receptors may sense oxidative stress or can use oxidants as ligands, two features that make them particularly important in neuroinflammatory diseases. Structurally, TLRs are type I transmembrane receptors that possess varying numbers of extracellular N-terminal leucine-rich repeat (LRR) motifs, followed by a cysteine-rich region (important target for oxidative regulation in many proteins), a transmembrane domain, and an intracellular Toll/IL-1R (TIR) motif, which is common to the IL-1 R/TLR superfamily (338). TIR uses four adaptor proteins: MyD88, TIR domain–containing adaptor inducing IFN-β (TICAM-1), MyD88 adaptor–like/TIR-associated protein (MAL/TIRAP), and the TRIF-related adaptor molecule (TRAM). It is known that at least one of them, MyD88, is regulated by *S*-nitrosylation, which causes a retardation of TLR signal transduction and initiation of acute-phase immune responses *in vivo* (218) (Fig. 12).

MyD88 is an adaptor that promotes the initiation and amplification of inflammatory responses, at least in part, through induced expression of multiple proinflammatory gene products and, through MyD88, several inflammatory pathways become regulated by redox variations. Necrotic neurons, for example, enhance microglial neurotoxicity by inducing the glutamate-producer enzyme, glutaminase, through an MyD88-dependent pathway, which increases significantly the IL-6, TNF-α, and COX-2 levels (395). Acute brain injury also triggers MyD88-dependent, TLR2/4-independent inflammatory responses (262). Nonetheless, MyD88 is susceptible to *S*-nitrosylation at Cys^{216} (Fig. 12). When this occurs, MyD88 interactions are impeded, but can be reversed by denitrosylation by using antioxidants and oxidoreductases (218).

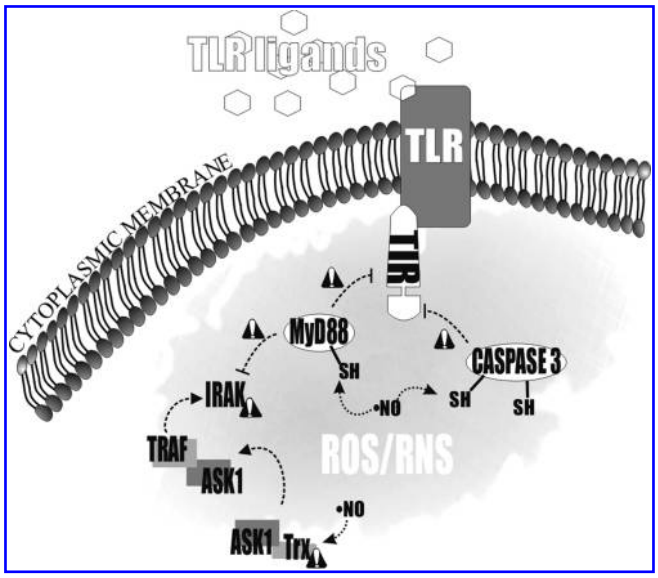

FIG. 12. The integration of the active Toll-like receptor, a crucial factor in neuroinflammation, depends on redox-regulated events. Key components of TLR signaling, such as MyD88 or the IL-1R–associated kinases (IRAKs), as well as the recruitment of TRAF family proteins to the ASK1 signalosome, essential for oxidative stress–induced cell death, may depend on redox regulatory events determined by ROS/RNS levels, as represented by the *gray shading* wrapping the different signaling components. ⚠ = direct redox regulation through redox sensors.

Acute stimuli, such as ischemia or bacterial infection, involve TLR4 signaling to microglia and astrocytes (57, 305). Other endogenous TLR4 ligands include the monosodium urate, heat-shock proteins (Hsp-60, Hsp-70), heparan sulfate, fibrinogen, and hyaluronan or HMGB1, which can be released from the cell surface as endogenous danger signals (4, 515). HMGB1 or Hsps, released from the cell surface and linked to the redox-regulated caspase-1 activation and IL-1β processing (515), differentially uses TLR4 signaling (which initiates both MyD88-dependent and -independent signaling cascades) or TLR2 signaling that is fully dependent on the intracellular adaptor protein MyD88 (262). TLR4 activates NF-κB, and by this, it is connected with a variety of physiopathologic situations in a stimulus-dependent fashion (18, 241): from innate adaptive immunity to neurodegeneration (273) and from neuroprotection to apoptosis (340), all of them situations in which multiple factors intervene, especially the redox status, as explained later. Other TLRs, such as TLR3, TLR7, TLR8, and TLR9, are expressed in the CNS and represent further candidate receptors for the initiation of the innate immune response after acute brain injury.

It also is possible that Toll-like receptors, specifically TLR-2, use oxidants as ligands, as was documented in studies using knockout mice (TLR2$^{-/-}$ or TLR4$^{-/-}$) treated with smoke extract (409). Thus, oxidant stress is sensed by TLR2-dependent pathways, and endogenous TLR2 signaling strongly affects early glial cytokine and chemokine responses, which exacerbate postischemic damages in the brain (612). TLR2-like TLR4 receptors are expressed both in glia and neurons and under ischemic conditions probably induced by DAMPs, Hsp70, and hyaluronan. Both TLR2 and TLR4 activate proinflammatory pathways: TLR2 activates the JNK-AP-1 pathway (536), whereas TLR4 activates NF-κB signaling pathways (18, 72).

Aβ, central to AD pathogenesis, induces oxidative stress with the consequent protein oxidation, lipid peroxidation, DNA oxidation, and 3-nitrotyrosine derivatives. In this context, TLRs become important as Aβ innate immune receptors. Antisense knockdown of TLR2 or functional blocking antibodies against TLR2 may suppress Aβ-induced expression of proinflammatory molecules and integrin markers in microglia; thus, fAβ peptides activate microglia through TLR2 (227). TLR2$^{-/-}$ mice show increased levels of Aβ accompanied by memory impairments, whereas restoring TLR2 clears Aβ and delays the cognitive decline (454). TLR4 could be also involved in clearance of Aβ deposits, as demonstrated in mouse models homozygous for a destructive mutation of TLR4, which showed significant increases in diffuse and fibrillar Aβ deposits (528). Conversely, if Toll-like receptors act as natural innate immune receptors to clear Aβ, they serve also as Aβ inflammatory mediators. For example, Aβ oligopeptides behave as foreign particles capable of initiating an inflammatory response through TLR signaling, which is responsible for the synaptic impairment observed in AD pathology (472).

Important receptors, such as the class B scavenger receptor CD36 and the LPS-binding molecule CD14, signal through TLR2. CD36 recognizes a variety of ischemic by-products acting as ligands, including oxidized low-density lipoprotein (LDL), long-chain fatty acids, thrombospondin-1, and fAβ; it is also a mediator in free radical overproduction after ischemic events in the brain (274) and the activation of proinflammatory pathways through NF-κB. CD14, expressed by microglia in ischemic brain injury signals by using TLR2 or TLR4 and the strong association between TLR2 and CD14 in experimental autoimmune encephalomyelitis, is important, given that those regions with more chronic expression of TLR2 and CD14 showed a larger activation of NF-κB (600).

Conversely, from studies on neutrophils, the oxidant-dependent signaling events downstream of TLR4 under stimulation with LPS, it was also demonstrated that oxidant events promote TLR4 signaling before the activation of IL-1 receptor–associated kinases (IRAK-1 and IRAK-4) (18). IRAKs are serine/threonine kinases associated with IL-1 receptor (IL1R) stimulation and become necessary for the IL-1–induced activation of NF-κB and c-Jun N-terminal kinase (435). IRAK/IRAK4, as well as other NF-κB–upstream associated kinases such as MAPKs and TRAF-associated NF-κB activator–binding kinase 1 (TBK1), are targets for ROS modification (18, 264) (Fig. 12). IL-1 binding to the receptor complex recruits the adaptor molecule MyD88 and IRAK (508). IRAK becomes autophosphorylated and then interacts with the TNF-receptor (TNFR)-associated factor 6 (TRAF6), which transduces the IL-1 signal downstream of NF-κB and JNK activation in a process in which H_2O_2 increases the strength of this interaction, as does inhibition of phosphatase activity (294). Because IRAK possesses thiol groups, thiol-modifying agents may block the recruitment of IRAK to IL-1R, an event that it is possible to reverse by adding GSH. Thus, it has been postulated that the recruitment of IRAK to the IL-1R is redox regulated by the glutathione system and that a reduced status is a prerequisite for an appropriate IL-1 response (50) (Fig. 12).

B. A redox-regulated pathway and a target to reduce neurodegeneration and neuroinflammation: Nrf2

Nrf2 coordinates the expression of genes required for free radical scavenging, detoxification of xenobiotics, and maintenance of redox potential, playing an important role in modulating ischemic injury *in vivo* (497, 539). It is constitutively produced, and its transcription does not change in response to antioxidants or xenobiotics; its regulation is a posttranslational event (216).

A diversity of sources, such as low-density lipoproteins, HNE, ROS, and proinflammatory cytokines, activate an electrophile response element also known as antioxidant response element (ARE)-dependent cytoprotective response. This response begins with the release of Nrf2 from the cytosol, attached to the oxidative-stress sensor Kelch-like ECH-associated protein (Keap1), which in turn is anchored to the actin cytoskeleton. Once released, Nrf2 translocates into the nucleus, where it binds to ARE and determines its transactivation (561). ARE is a cis-acting regulatory sequence present within the 5′-regulatory region of diverse phase II detoxifying enzyme genes, and its presence results in a coordinated induction of the protective enzymes in response to prooxidants or electrophiles (Fig. 5).

Keap1 has a broad complex–tramtrack–bric-a-brac (BTB) (317) with a cysteine residue at the 151 position, which inhibits the Keap1-dependent degradation of Nrf2 by oxidative stress or by electrophilic compounds, such as sulforaphane, that directly interacts with Keap1 by covalent binding to its thiol groups (603). Keap1 also has an intervening region (IVR) sequence, outside the BTB domain, responsible for the association between Keap1 and the Cullin-based E3-ligase (Cul3), a subunit of the ubiquitin E3 ligase complex, and transfers ubiquitin to Nrf2 protein (153). This IVR sequence has two reactive cysteine residues, Cys^{273} and Cys^{288}, critical for repressor activity (108). The ability of Keap1 to repress Nrf2 can be experimentally impeded by site-directed mutagenesis of Cys^{273} and Cys^{288} (317), by disease-associated mutation (380), or by thiol-reactive oxidants and electrophiles (603). *S*-Nitrosylation of Keap1 thiols also has been demonstrated by using HEK293 and HEK293 cell lines, which overexpress hemagglutinin-tagged Keap1, treated with nitric oxide and *S*-nitrosocysteine (60); thus, nitrosylated Keap1 accumulates in the nucleus with a time course similar to that of Nrf2.

1. Electrophiles attack Keap1 and provide neuroprotection. Through the inactivation of Keap1, the half-life of Nrf2 increases about fivefold and accumulates in the nucleus, where it forms heterodimers with small Maf proteins (musculoaponeurotic-fibrosarcoma virus) before it binds to ARE. By binding to Keap1, inactivation of Nrf2 is avoided; Nrf2 may translocate then to the nucleus, where it induces ARE-regulated phase 2 enzymes (glutathione transferases; NADPH:quinone reductase; glucuronosyltransferases) in parallel with HO-1 (the Keap1/Nrf2/HO-1 pathway), ferritin, γ-glutamylcysteine ligase, glutathione reductase, aldehyde dehydrogenase, dihydrodiol dehydrogenase, leukotriene B_4 dehydrogenase and glutathione *S*-conjugate efflux pumps (191, 284). By nullifying Nrf2, both constitutive and inducible gene expression through ARE become impeded; thus, Nrf2 has been shown to be essential in ARE transactivation and neuroprotection.

ROS-derived lipoperoxides, particularly those containing an electrophilic carbon center, such as isoprostanes (366), oxidized low-density lipoproteins and HNE (220); these endogenous, highly reactive electrophiles act on the Keap1/Nrf2 pathway. HNE activates the Nrf2/ARE pathway by attacking –SH groups on the oxidative-stress sensor Keap 1 (Fig. 5). Resveratrol, a phytoalexin that increases MnSOD activity and induces glutathione synthesis by activation of Nrf2, protects against HNE-induced apoptosis by blocking JNK and c-Jun/AP-1 signaling (275). Natural electrophilic compounds, such as sulforaphane (603), as well as proposed therapeutic electrophilic compounds, such as neurite outgrowth–promoting prostaglandin (NEPP), *tert*-butylhydroquinone (t-BHQ), or carnosic acid, a catechol-type electrophilic compound (475, 476, 477), also induce neuroprotection through release of Nrf2 from Keap1. Exogenous t-BHQ, for example, blocks neuronal cell death induced by glutamate-induced oxidative damage in primary neurons *in vitro* (490). t-BHQ activates ARE in astrocytes (270), whereas NEPP compounds (Δ7-prostaglandin A1 analogues) accumulate preferentially in neurons (477). It is possible to activate the Keap1/Nrf2 transcriptional pathway and provide neuroprotection against middle cerebral artery ischemia/reperfusion by using catechol ring–containing compounds such as carnosic acid, which becomes electrophilic quinone on oxidation and binds to specific Keap1 cysteine residues (476).

Preclinical experiments have demonstrated that BG12, an immunomodulatory dimethyl fumarate (DMF), can activate the Nrf2-ARE pathway and significantly reduce brain lesions in MS (243, 377). The electrophilic compound DMF-induced expression of ARE-mediated gene expression appears to be largely restricted to astrocytes (365).

By using low concentrations of 15Δ-PGJ_2 or HNE in cultured cortical neurons under glutamate neurotoxicity, it is possible to maintain intracellular GSH levels as well as gene expression of glutamate-cysteine ligase, the rate-limiting enzyme of GSH synthesis, through the induction of an adaptive response, primarily through the upregulation of the intracellular GSH synthesis (469).

Neurons lacking of Nrf2 are particularly susceptible to oxidative stress, but they can be rescued by inducing Nrf2 overexpression. For example, $Nrf2^{-/-}$ mice show a significantly greater neurologic deficit after 90-min occlusion of the middle cerebral artery, followed by 24-h reperfusion, than do their counterpart wild-type mice. However, in the presence of Nrf2, ARE becomes active, and a correlation between expression of ARE-driven genes and reduction in infarct size is evidenced (284, 490).

In spite of these theoretic considerations and *in vitro* experiments, when Nrf2 was investigated in brains from patients with chronic neurodegenerative disorders and neuroinflammation, such as AD and PD, despite the prevalence of oxidative stress in both diseases, this transcription factor did not behave as expected. For example, a significant decrease in nuclear Nrf2 levels in AD brains in both neurons and astrocytes was observed, whereas in PD nigral neurons, Nrf2 was strongly nuclear (442). Another condition is aging, in which brain oxidative stress prevails because of a reduced capacity of cellular homeostatic mechanisms that protect the body against a variety of oxidative insults (495). Thus, Nrf2 would be expected to be active in the nucleus. However, a 50% decline in nuclear Nrf2 levels was revealed, correlated to

a significant loss of glutathione synthesis (520). Discrepancies could be explained because potential alterations in the redox-sensing capacity of Keap1 or the Nrf2 regulation may be related to other control pathways; such as protein phosphorylation–mediated pathways, connecting both neuroinflammatory and oxidative events (442). PI3-kinase could be directly implicated as an Nrf2 regulator by controlling the nuclear translocation of Nrf2 in response to oxidative stress, in a process that involves rearrangement of actin microfilaments (242).

However, another hypothesis claimed that phosphorylation of Nrf2 at Ser-40, through a PKC-based mechanism, plays a critical role in the dissociation of Nrf2 from Keap1 (420). Several other protein kinases have been also implicated in Nrf2 regulation, including MAPK (596), PKC (209), or even the ER-localized pancreatic endoplasmic reticulum kinase (PERK), after the oxidative stress–derived endoplasmic reticulum stress (107).

Control of Nrf2 release is not exclusive to Keap1 oxidation-sensitive shuttling. Four serine residues (Ser-40, Ser-378, Ser-439, and Ser-589) and three threonine residues (Thr-417, Thr-418, and Thr-594) are all susceptible to phosphorylation (209, 596). Whether Keap1 redox-shuttling and ser/thr phosphorylations on Nrf2 are just complementary to each other or alternative pathways remains unclear.

The close relation between redox-sensitive pathways and Nrf2 is obvious during experimentally induced oxidative stress with GSH depletion *in vivo*. A generalized alarm response, including ERK2 activation and nuclear accumulation of Nrf2 in brain, is accompanied by enhanced transcription of Nrf2, the cystine/glutamate exchange transporter, γ-glutamylcysteine synthetase (γGCSr), and Trx1. Conversely, MAPK inhibitors or the addition of exogenous thiol *N*-acetylcysteine, abrogates the activation of ERK2, which is accompanied by nuclear Nrf2 accumulation (304).

C. The redox-regulated hypoxia-inducible transcription factor-1: its role beyond ischemia

Besides the Nrf2/keap1/ARE pathway, the hypoxia-inducible transcription factor-1 (HIF-1) and the cAMP-responsive element–binding protein (CREB) are significant in detoxifying ROS, playing an important role in acute neuroinflammation and oxidative stress regulation. Both HIF-1 and CREB are in turn regulated by redox-sensitive transcription factors, NF-κB and AP-1.

Depending on time and severity of the oxygen deprivation and aided by oxygen-sensing signal cascades, such as the prolyl-4-hydroxylase domain, HIF-1 determines the cellular fate during hypoxic events. HIF-1 is a heterodimeric protein consisting of two subunits, the O_2-regulated HIF-1α and HIF-1β not regulated by O_2; it is induced in areas in which blood flow is persistently decreased, and oxygen delivery is impaired. HIF-1 activates a variety of hypoxia-inducible genes, such as erythropoietin, tyrosine hydroxylase, iNOS, vascular endothelial growth factor (VEGF), glucose transporter-1 (GLUT-1), HO-1, all glycolytic enzymes including phosphofructokinase and lactate dehydrogenase, as well as the metal transcription factor-1 that mediates metal-response element responses to hypoxia in metallothionein genes (81). When hypoxia is prolonged, the HIF-1 inducible α-subunit is stabilized, preventing posttranslational and oxygen-dependent hydroxylation by EGL-nine homologue (EGLN) enzymes and subsequent degradation through the proteasome (279). Hypoxia is linked to free radical overexpression, and $O_2^{\bullet -}$ could be the stabilizer of HIF-1α, as evaluated in MCF-7 cells. HIF-1α has a cysteine residue at the position 800, which is a potential target for *S*-nitrosylation and recruitment of the p300 coactivator that is necessary for transcriptional activity of the HIF-1 complex (591) (Fig. 13).

Antioxidant enzymes like MnSOD may be the final HIF-1 regulator (239). If this be the case, $O_2^{\bullet -}$-generating systems, such as NOX, are key HIF-1 regulators as well. Once again, the link between oxidative stress, redox regulation, and inflammation-related pathways is manifest. NOX activates HIF-1α and apoptosis signal-regulating kinase 1 (Ask-1, of the JNK and p38MAPK pathways) through the overproduction of $O_2^{\bullet -}$ and, based on studies in THP-1 human myeloid monocytic leukemia cells, LPS-induced TLR4 signaling triggers crosstalk of Ask-1 and HIF-1α (522). Both pathways are activated through redox-dependent mechanisms, associated with the activation of PKCα/β, mediated in turn by tyrosine kinase/phospholipase C-1γ, which are known to activate NOX. Ask-1, through p38 MAPK, may also relate to the stabilization of HIF-1α. Thus, protein phosphatases, controlling phosphorylation pathways, such as the protein phosphatase 5 (PP5), assume a significant role in hypoxia (607) (Fig. 13). •NO, conversely, is able to mimic a hypoxic response and

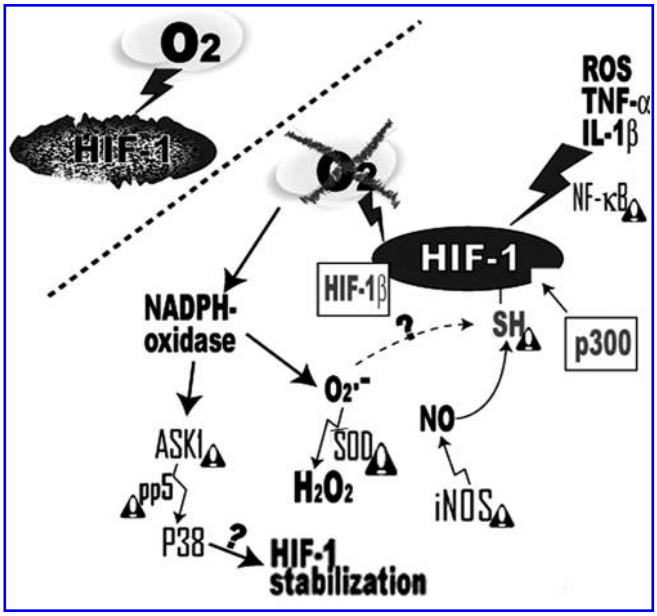

FIG. 13. Under hypoxic conditions, HIF-1 becomes active and stable, preventing posttranslational hydroxylation and subsequent degradation through the proteasome. A Cys residue on the C-terminal activation domain of HIF-1 can be nitrosylated or alkylated, facilitating the recruitment of p300, as shown, although the underlying molecular mechanisms have not yet been well established. Several mechanisms take a role in the activation of HIF-1, including NADPH-oxidase, the ASK1/p38 pathway, iNOS, SOD, and NF-κB, all of them redox-sensitive pathways. This phenomenon may explain why HIF-1 responds to many stimuli under normoxic conditions, including lipopolysaccharide and cytokines such as TNF-α. ⚠ = direct redox regulation through redox-sensors.

stabilizes HIF-1α (608), which might have implications in pathophysiologic processes. Studies in HEK-293 cell cultures stably expressing human iNOS revealed that concentrations of •NO less than 400 n*M* prevented the accumulation of HIF-1α in hypoxia, but •NO concentrations greater than 1 μ*M* always resulted in HIF-1α stabilization, under both hypoxic and nonhypoxic conditions, as well as independent of the mitochondrial respiratory chain (334).

1. HIF-1α a very active protagonist in neuroinflammation. HIF is a key mediator in the cellular adaptation to hypoxia, but also responds to nonhypoxic stimuli. In brain, under nonhypoxic conditions, the accumulation of Aβ, a well-known inducer of oxidative and nitrosative stress, is able to induce the accumulation and nuclear translocation of HIF-1α, and this mediates a neuroprotective response, presumably by regulating glucose metabolism (510).

The connection between hypoxia, HIF, and neuroinflammation involves a spreading depression phenomenon that induces, at some distance from the infarct, the immediate early gene, c-fos (382). Thus, the AP-1 transcription factor becomes activated, and an extended, proinflammatory response begins (127). However, the crosslink with neuroinflammatory, redox-regulated transcription factors goes well beyond this. The HIF-1α gene promoter possesses several consensus sites for NF-κB proteins and responds to many stimuli [the same in normoxic conditions as in hypoxic conditions], involving NF-κB in the regulation of hypoxia, in addition to its well-known role in neuroinflammation and oxidative stress control (169) (Fig. 13). Thus, ROS as well as cytokines may induce HIF-1 by a nonhypoxic mechanism. Under normoxic conditions, all NF-κB subunits may bind the HIF-1α promoter and activate HIF-1α, but none of the NF-κB subunits may activate a truncated version of the HIF-1α promoter construct without the NF-κB consensus site (557). Both HIF-1α mRNA and HIF-1α protein may be induced by TNF-α in a NF-κB–dependent manner. Additionally, under hypoxic conditions, the induction of HIF-1α could be prevented by blocking the NF-κB activation pathway (557). Moreover, a certain basal level of NF-κB activity is necessary for HIF-1α accumulation during brain hypoxia, in a process in which IKK-β becomes essential, because mice lacking IKK-β showed a defective induction of HIF-1α target genes (455).

Inflammation uses several factors to induce HIF-1α: low levels of oxygen and glucose, high levels of cytokines and ROS/RNS, plus their metabolites. In this context, the crosslink between the HIF-1α and the NF-κB pathways becomes relevant (Fig. 13).

D. Redox-regulated NMDAR

Glutamate is an excitatory amino acid that activates different types of ion channels, forming receptors (ionotropic) and G protein–coupled receptors (metabotropic) to develop its essential role in the functional activity of the brain. Ionotropic receptors are subdivided into AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors, kainate receptors, and NMDA (*N*-methyl-D-aspartic acid) receptors, named for their selective agonists. NMDA receptors (NMDARs) seem to be related to synaptic plasticity mediated by calcium entry through the NMDAR-associated channel (120, 460) (Fig. 3). When NMDARs trigger an excessive entry of Ca^{2+}, initiate a series of cytoplasmic and nuclear processes, such as the proteolytic calpains or the calmodulin kinase II that promote neuronal cell death by promoting a disparated phosphorylation of a number of enzymes (369).

NMDA receptors are particularly important in neuroinflammation because they convert excitotoxic stress into oxidative stress. ROS overproduction may come from dysfunctional mitochondria because of a massive influx of Ca^{2+} or by the activation of calcium-dependent enzymes, including xanthine oxidase and the enzyme phospholipase A2 (PLA2). This in turn initiates the mobilization of fatty acids from membranes. Thereafter, as explained later, COX-2 mediates the necessary catabolic processes leading to the formation of $O_2^{\bullet -}$ (29). Furthermore, calcium activates NOS, increasing •NO levels. Excessive Ca^{2+} influx–derived •NO activates guanylylcyclases, reacts with $O_2^{\bullet -}$ to form $ONOO^-$, and leads to *S*-nitrosylation (369). These events change the redox status and alter many physiologic processes or lead to pathologic situations, as we summarized elsewhere in this review (Fig. 3).

During neuroinflammation, microgliosis and astrocytosis occur, accompanied by an important increase in IL-1 and TNF-α, and operate through NMDA receptors, an event reproduced by infusing LPS intracerebrally from 28 to 37 days in rats (578).

Excessive NMDA current can be modulated by *S*-nitrosylation of Cys residues on NMDARs (306). Thus, it is possible to inhibit NMDAR activity through *S*-nitrosylation of a cysteine thiol at the position 399 of its NR2A subunit, a probable eventuality only under hypoxic conditions or stroke, when free thiol groups in the molecule are more readily available to react with •NO to form *S*-nitrosothiol. This implies the existence of an "•NO-reactive oxygen sensor motif," which includes two other Cys residues (744 and 798) that sensitize the NMDAR to inhibition by •NO under hypoxic conditions (529). It is probable also that Cys residues on NMDARs may be susceptible to redox regulation through glutathionylation/deglutathionylation (519).

Considering this, a potential exists for pharmacologic interventions in various glutamate-mediated excitotoxicities, such as occurs in neuropathic pain, drug withdrawal and dependency, epilepsy, PD, AD, AIDS, HD, or ALS. For example, redox-active compounds, such as the oxidant 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) and the redox cofactor pyrroloquinoline quinone (PQQ), may have therapeutic use in epilepsy by reversing NMDAR-mediated pathophysiology without blocking physiologic NMDAR function (473). By inhibiting the excessive NMDAR activity with memantine, a low-affinity voltage-dependent uncompetitive antagonist at glutamatergic NMDARs, it is possible to ameliorate excessive production of •NO, protein misfolding, and neurodegeneration. (368). Dimebon, an antihistamine compound with demonstrated efficacy in phase II AD and HD clinical trials, may stabilize glutamate-induced Ca^{2+} signals. It functions as an inhibitor of NMDARs and voltage-gated Ca^{2+} channels in a cellular model of HD (583).

IX. Fats and Alternative Mechanisms of Redox Regulation

The control of the neuroinflammatory response depends importantly on mobilization of membrane lipids and metabolism of oxidized fatty acids, two redox-related processes (Figs. 5 and 9).

Most lipids contain readily oxidizable polyunsaturated fatty acids (PUFAs) yielding a huge number of oxidatively modified lipid molecules. Liberated from membrane phospholipids by phospholipase A2 (PLA2), arachidonic acid is converted by COX-2 (PG-endoperoxide H synthase) to prostaglandin H2 (PGH2) (130, 553), which in turn is subsequently reduced to PGF2α by a PGF2α synthase or is isomerized to other prostanoids of the 2 series, including PGE_2, $PGF_{2\alpha}$, PGD_2, PGJ_2, PG endoperoxides (PGG_2, PGH_2), thromboxane A_2 (TXA_2), and prostacyclin (PGI_2) (172, 507) (Fig. 14). It is worth mentioning that chronic neuroinflammation induces neurodegeneration by itself, being capable of reproducing some AD neuropathologic components (125) in a process that involves prostanoid synthesis (349).

Both PLA_2 and COX-2 are coupled to oxidative pathways (524), and NF-κB has binding sites on PLA_2 and COX-2 promoters (13, 443, 549), related to intracellular redox-mediated modification of proteins. NOX- or PLA_2-induced ROS activates NF-κB, which in turn induces gene expression of secretory PLA_2, COX-2, lipoxygenases (LOX), SOD, NOX, iNOS, and proinflammatory cytokines, in addition to adhesion molecules and matrix metalloproteinases (443) (Fig. 14). Under stress conditions, ERK and p38MAPK phosphorylate cytosolic PLA2 and, by this means, enhance ROS production (491, 492).

COX-2 is abundant in activated microglia, in which it is usually activated by a number of proinflammatory conditions such as the presence of neuronal IL-1β (29, 354), or the Ca^{2+}-modulated protein S100B, acting through the RAGE (41).

Oxidative metabolism of endocannabinoids by COX-2 is another pathway to neuroinflammation. Endocannabinoid-derived prostanoids, such as prostaglandin glycerol esters [PGE2G, ester derivatives from 2-arachidonylglycerol (208)] and ethanolamides [PG-EA, amide derivatives from arachidonylethanolamide (anandamide), such as prostaglandin E2 (269)] serve as intercellular lipid messengers acting through cannabinoid receptors (116, 391). Their role in cellular pathophysiology is under scrutiny. High doses of PGE2-G increase the activation of NF-κB, and low doses decreased NF-κB, as evaluated in a macrophage cell line (116, 269). Moreover, apparently through diverse pathways, other than the cannabinoid receptor 1, such as ERK, p38MAPK, IP3, and NF-κB, PGE2-G enhances the hippocampal glutamatergic synaptic transmission and neuronal damage (474). Thus, PGE2-G becomes a common factor in neuroinflammation, and the inhibition of its synthesis could prevent neuronal damage, as proposed in ALS patients (213). Anandamide, conversely, protects neurons from inflammatory damage by cannabinoid receptor–mediated induction of mitogen-activated protein kinase phosphatase-1 (MKP-1) in microglial cells, switching off MAPK signal transduction activated by stimulation of PRR (134). Amines that inhibit the anandamide transporter inhibit its uptake or degradation or both, which is considered for the treatment of such diverse pathologies as HD or MS (21). Anandamide may act also through the ERK1/2 and JNK pathways to downregulate IL-12p70 and IL-23, two functionally related cytokines that play a crucial role in MS (102).

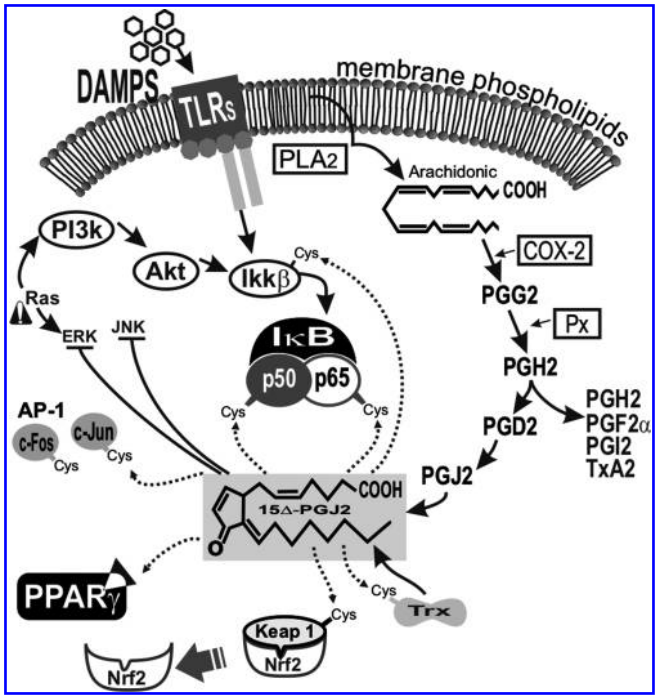

FIG. 14. The NF-κB activation, wherever it comes from [Akt neuroprotective pathways or Toll-like receptors, to respond to an aggression] can be blocked by the cyclopentenone prostaglandin 15Δ-PGJ_2. The α,β-unsaturated carbonyl group in the cyclopentenone ring may react with sulfhydryl groups of cysteine residues of proteins by Michael addition. By this mechanism, 15Δ-PGJ_2 acts on several other thiol residues and modulates important pathways such as ERK, JNK, or Nrf2, apart from its direct effect on AP-1 and the activation of PPAR-γ. These antiinflammatory actions point to 15Δ-PGJ_2 as a therapeutic agent in neuroinflammatory disorders; however, the blockade of the NF-κB activity may also facilitate proapoptotic events in neurons. ⚠ = direct redox regulation through redox sensors.

A. Oxidation of N-6 PUFAs

PUFA oxidation is involved in energy metabolism. A lipid-radical theory considers the role of α-tocopherol and cytochrome *b*5, two membrane factors involved in the functioning of lipid-radical cycles in mitochondria as well as the participation of lipid-radical reactions in key membrane processes (124). The reduction of peroxyl radical (LOO•), located in the inner membrane ($cyt.b5red + LOO\bullet \rightarrow cyt.b5ox + LOO^-$), secures, within a reduced environment, an interaction of α-TOH with another intermediary LOO^- (384). However, excessive oxidation of PUFA and LOOH formation (LOO• → LOOH) would cause a shortage of lipid-radical cycles in membranes with a defective function of adenine nucleotide translocator and ATP synthesis in mitochondria (124). Thus, activity of key enzymes in mitochondrial membranes depends on the redox reactions involving lipid radicals.

Oxidation of N-6 PUFAs has a critical role in the induction of neuronal apoptosis during oxidative processes. The 4-oxo-2-nonenal (ONE), a by-product of the peroxidation of N-6 PUFAs, is a potential inducer of p53 phosphorylation, as evaluated in human neuroblastoma SH-SY5Y cells, and also a ONE-2′-deoxyguanosine adduct has been found in spinal cord motor neurons of patients with sporadic ALS (494). 15Δ-PGJ_2 and HNE, as well as a variety of isoprostane derivatives, are electrophilic lipid compounds originated from PUFA peroxidation and may react with nucleophilic amino acids

such as cysteine (-SH group), histidine (imidazole group), and lysine (ε-amino group) by Michael addition reactions. By these means, alternative mechanisms of regulation emerge. Thus, the addition of these electrophiles to cysteine thiols (*S*-alkylation) is another form of "redox" signaling, analogous to *S*-thiolation. 15Δ-PGJ_2 is the dehydration end product of PGD_2 and an endogenous ligand for the intranuclear peroxisome proliferator–activated receptor γ (PPAR-γ) (158) (Fig. 14). It possesses only one electrophilic carbon, whereas HNE possess both an aldehydic function and an electrophilic carbon; both may modify nucleophilic protein residues or form secondary ROS from the mitochondrion (266). Because of its aldehydic function, HNE may compete with or interfere with other pathways that feature thiol/redox-dependent signaling (Figs. 6, 8, and 9) (19, 141, 220, 313). Redox regulation of HNE may have functional consequences in neurodegenerative disorders, as shown later.

B. ApoE in detoxifying HNE, an example of functional aspects of redox regulation

The apolipoprotein E4 isoform (apoE4) has been related to increased risk, earlier onset, or negative prognosis in some other pathologies, such as PD (299), ALS (300), traumatic brain injury (372), and HIV-encephalitis (101). ApoE plays a specific role in brain innate immunity as well. It is expressed in astrocytes and apparently modulates its activity (526), as demonstrated in bacterial lipopolysaccharide (LPS)-stimulated astrocyte cultures from targeted replacement APOE mice, in which human APOE genomic fragments replace mouse apoE through homologous recombination (321). The alternative activation of p38 mitogen-activated protein kinase (p38MAPK) or NF-κB signaling in microglia and astrocytes has been revealed as a mechanism for this modulatory role of ApoE. APOE4 targeted replacement causes the greatest innate immune activation and paracrine damage to neurons through the activation of p38MAPK (321). Microglia derived from APOE4/4 targeted replacement mice exhibit a proinflammatory phenotype that includes altered cell morphology, increased •NO production, and higher proinflammatory cytokine production (TNF-α, IL-6, IL-12p40) compared with microglia derived from APOE3/3 targeted replacement mice (564). Finally, animals expressing the APOE4 allele have significantly greater systemic and brain elevations of the proinflammatory cytokines TNF-α and IL-6, as compared with their APOE3 counterparts, suggesting an isoform-specific effect of the immunomodulatory properties of apoE (319).

The three ApoE isoforms (E2, E3, and E4) differ by single amino acid interchange at residues 112 and 158: E3 (Cys^{112}-Arg^{158}), E4 (Arg^{112}-Arg^{158}), and E2 (Cys^{112}-Cys^{158}) (572). Redox-sensitive cysteine residues in E3 and E2 are susceptible to electrophilic attack, attaching free radicals (335) or being regulated by this mechanism, which is not possible on E4 because of the lack of cysteine residues on those positions. By studying the interactions between ApoE and the lipid peroxidation by-product, 4-hydroxynonenal (HNE), it was shown that ApoE plays a major role in detoxifying HNE. However, this action depends on the cysteine content of ApoE, which differs among the ApoE isoforms. Thus, ApoE2 with two cysteine residues and ApoE3 with one residue are capable of interacting with HNE and protect against HNE-induced apoptosis (Fig. 10), as evaluated in cultures of mouse spinal cord motor neurons, as well as in cultures of rat hippocampal neurons challenged with Aβ peptide. The ApoE4 isoform, a known risk factor in AD, has no cysteine residues, does not bind to HNE, and thus, it is unable to reduce HNE toxicity (410).

C. 15Δ-PGJ_2, a controversy around electrophilic compounds

15Δ-PGJ_2 is highly reactive with thiol-containing intracellular components like glutathione or thiol-containing proteins through Michael addition, and thus, it may decrease oxidative/nitrosative mediators in the brain after acute stress in rats, and it is considered an antiinflammatory prostaglandin (158, 480). However, 15Δ-PGJ_2 might also induce apoptosis, as demonstrated *in vitro* in SH-SY5Y human neuroblastoma cells, in which 15Δ-PGJ_2 induced the accumulation of p53 and the consequent activation of a death-inducing caspase cascade mediated by Fas and the Fas ligand (266). By interfering with the NF-κB transcriptional activity in the nucleus, 15Δ-PGJ_2 is a microglia downregulator; it suppresses iNOS promoter activity as well as iNOS mRNA and enzyme levels (419). Some other mechanisms could be related to 15Δ-PGJ_2 downregulation of microglia, including the induction of HO-1 or Hsp-70 (263). Hsp-70 inhibits the nuclear translocation of NF-κB and the degradation of IκBα induced by TNF-α (318).

15Δ-PGJ_2 modulates NF-κB through *S*-alkylation. Added to macrophages, 15Δ-PGJ_2 alkylates nucleophilic cysteine residues, Cys^{38} of the p65 subunit, and Cys^{62} of the p50 subunit of NF-κB (516). Even more, 15Δ-PGJ_2 binds to Cys^{179} on IKKβ as well, attenuating the NF-κB–dependent proinflammatory gene expression (132) (Fig. 14).

However, the blockade of neuronal NF-κB activity is a double-edged sword. It could be antiinflammatory (480), but it is neurotoxic by enhancing COX-2 expression in neuronal cells in a PPAR-γ–independent manner, through the p38MAPK pathway (301).

15Δ-PGJ_2 *S*-alkylates the SH group of Cys residues in several other key proteins, such as Keap1 (Cys^{151}), the serine/threonine kinase (Cys^{210}), AP-1 (Cys^{269}), H-ras (Cys^{184}), thioredoxin (Cys^{35} and Cys^{69}), and PPAR-γ (Cys^{285}) (476) (Fig. 14). Experiments *in vitro* using SH-SY5Y human neuroblastoma cells revealed that 15Δ-PGJ_2 induced apoptotic cell death by the accumulation and phosphorylation of p53, which was accompanied by a preferential redistribution of the p53 protein in the nuclei of the cells and by a time-dependent increase in p53 DNA-binding activity (266).

Oleic and linoleic acids are susceptible to nitration by nitric oxide–derived species, yielding nitroalkene derivatives, which react with GSH and cysteine residues also through the Michael addition reaction, but at rates significantly greater than those for the GSH reaction with H_2O_2 and non-nitrated electrophilic fatty acids, such as 15Δ-PGJ_2 (85). The nitrated fatty acid species play an important role as pluripotent adaptive signaling mediators, inducing posttranslational and reversible modifications on proteins (22). For example, nitroalkene derivatives of oleic acid (OA-NO_2) and linoleic acid (LNO_2) are capable of covalently nitroalkylating the p65 subunit of NF-κB and influencing the inflammatory processes (106); or they can be recognized at nanomolar concentrations

by PPAR-γ, which represents a convergence of lipid and nitric oxide–mediated signaling (298).

D. The immunoregulatory and redox-regulated PPARs

The most significant inflammatory signaling systems, such as NF-κB, STAT, AP-1, or NFAT, may be affected by PPAR-mediated transrepression.

PPAR is a metabolic nuclear receptor governed by binding of small lipophilic ligands, mainly fatty acids, and derived from nutritional or metabolic pathways, which interferes with important neuroinflammatory pathways, such as NF-κB, AP-1, phosphatase 2A, ERK, and JNK (394, 404). It particularly suppresses the p38 MAPK phosphorylation while increasing the activity of the PI3K/Akt pathway, inhibiting iNOS expression and •NO generation (588) (Fig. 15). The activation of PPAR-γ in cerebellar granule cells by using NSAIDs or endogenous 15Δ-PGJ_2 downregulates cytokine levels and iNOS expression, reducing LPS-induced cell death and proinflammatory cytokines (192). Conversely, PPAR-α agonists significantly inhibit the radiation-induced microglial proinflammatory response by decreasing the nuclear translocation of the NF-κB p65 subunit and phosphorylation of the c-jun subunit of AP-1 in the nucleus (441), while it induces IκBα (75, 158). Thus, the activation of PPAR-α can also modulate the microglial proinflammatory response. A physical association between the p65 subunit of NF-κB and PPAR-γ—facilitated by the phosphorylation of PPAR-γ through ERK1/2—has been demonstrated (614). In AD, PPAR-γ agonists inhibit the expression of TNF-α, IL-6, iNOS, and COX-2, as well as glial activation (35) (Fig. 15). Both α and γ PPARs, when activated, modulate the microglial proinflammatory response and reduce the expression of NOX while increasing SOD, because the gene of SOD has PPAR-response elements. For example, by using the PPAR-γ agonist rosiglitazone in epileptic rats, it is possible to suppress the ROS and enhance the antioxidative activity of SOD and GSH, together with decreased expression of HO-1 in the hippocampus (597).

During inflammation, PPAR-γ also regulates stress-activated protein kinases and the PI3K/Akt pathway. Acting on PPAR-γ receptors, 15Δ-PGJ_2 downregulates the PI3K/Akt pathway and inhibits the inflammatory response, as evaluated in primary rat astrocytes (14). This cyclopentenone isoprostane also alkylates cysteine residues in binding domains of NF-kB, IKKβ, and AP-1 (75, 77, 129, 416), as well as the proper PPAR ligand-binding pocket through a Michael addition in a cysteine residue. However, 15Δ-PGJ_2 could not be the only eicosanoid metabolite able to bind PPAR; other oxidized eicosanoids containing an α, β-unsaturated ketone can covalently bind to a cysteine residue in the PPAR-γ ligand-binding domain. In this manner, PPAR-γ may sense oxidized fatty acids as signaling molecules and assume a role as an inflammation modulator (500) (Fig. 15).

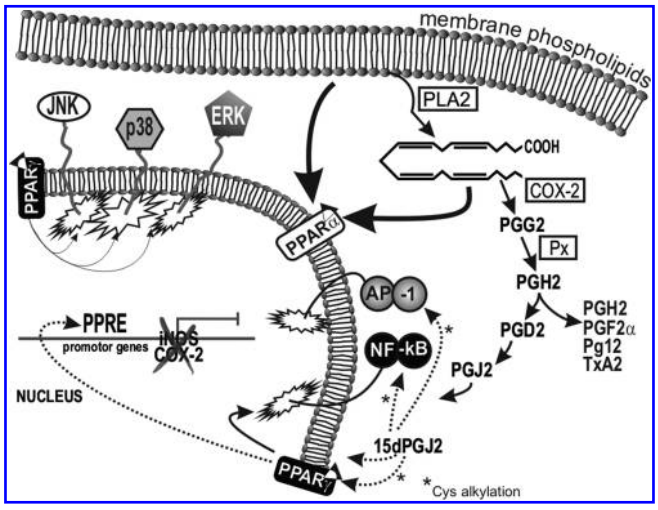

FIG. 15. PPAR-α and PPAR-γ activation induces antiinflammatory and antioxidant effects. Both are expressed mainly in tissues with high fatty acid catabolism, such as the central nervous system, and are linked to its metabolism. Thus, endogenous ligands for PPARs comprehend long-chain fatty acids, polyunsaturated fatty acids such as linoleic and arachidonic acid, the same as saturated fatty acids and eicosanoids (including eicosanoid metabolites, such as 15Δ-PGJ_2). Once activated, PPARs may block genes from oxidative and inflammatory factors in addition to stress-related pathways (JNK, ERK, p38) and proinflammatory transcription factors, such as NF-κB and AP-1. (PPRE = PPAR response element; iNOS = inducible nitric oxide synthase; Cox-2 = cyclooxygenase-2).

X. Redox-Regulated Transcription Factors

A. NF-κB: a pluripotential key regulator of neuroinflammation

A well-known correlation exists between oxidative stress, the neuroinflammatory response, and redox regulation of transcription factors, particularly NF-κB. NF-κB regulates major histocompatibility proteins, adhesion molecules, and the cytokines IL-2, IL-6, interferon-β, transforming growth factor-β, and TNF-α. For example, in the nuclei of dopaminergic cells of patients with PD, a 70-fold increase in NF-κB immunoreactivity has been found (211); meanwhile, in nuclear extracts from dopamine-treated PC12 cells, NF-κB–p65 proteins appeared bound to the consensus DNA sequence only 30 min after dopamine exposure (397). In AD, the main constituent of senile plaques, Aβ, induces microglial NF-κB activation, a feature that is at the center of the amyloid hypothesis (84). Both fibrillar and nonfibrillar Aβ activate NF-κB signaling in microglia; the fibrillar Aβ interacts with scavenger receptors, RAGE, or even TNFR1, whereas it is possible that nonfibrillar Aβ binds α_v-containing integrins on glia and neurons, and this triggers either TNF-α release or Aβ internalization, both effects resulting in TNF-R1 activation (461). Conversely, NF-κB target genes (iNOS, cathepsin B, proinflammatory cytokines) create a feedback loop that increases Aβ aggregation, oxidative stress, and neuroinflammation. In MS as well as in ALS, NF-κB plays a key role in conjunction with AP-1 (53, 348), so much so that NF-κB has been considered a potential therapeutic target for the treatment of MS (589).

NF-κB is a transcription factor consisting of homo- or heterodimers of the Rel protein family, with a pivotal role in inflammation, cell survival, and proliferation. In unstimulated cells, the inhibitory protein IκBα binds to NF-κB. Phosphorylation of IκBα by the IκB kinase (IKK) complex (IKKα + IKKβ + NF-κB essential modulator or NEMO) leads to its dissociation from NF-κB and its proteasomal degradation. Once released, NF-κB translocates to the nucleus and is the major transcriptional factor in iNOS transcriptional

induction occurring in glial cells, but it is a pluripotential transcription factor that participates in the control of transcription of more than 150 target genes (393), including cytokines, chemokines and their modulators, immunoreceptors, cell-adhesion molecules, acute-phase proteins, stress-response genes, regulators of apoptosis, growth factors, early-response genes, and transcription factors, among others (113, 337). NF-κB–inducing kinase (NIK) is a further upstream kinase that phosphorylates IKK, and evidence indicates that both NIK and IKK are targets of redox regulation (82).

1. Redox regulation of NF-κB. Nitrosylation of the cysteine residue in the DNA-binding domain of the NF-kB p50 subunit suppresses DNA binding and the transcriptional activity of NF-kB. The NF-κB p65 subunit, which possesses the transactivation domain, also is targeted by •NO, as demonstrated after cytokine stimulation of respiratory epithelial cells and macrophages; a conserved cysteine within the Rel homology domain susceptible to *S*-nitrosylation has been identified (247). *S*-nitrosylation of p65 inhibits NF-κB–dependent gene transcription. Conversely, specific inhibitors of thioredoxin reductases impair the phosphorylation of the c-Rel protein, suggesting that the redox regulation of the protein controls its phosphorylation (165) (Fig. 16).

The relation of ROS to NF-κB is less clear. By using a steady-state titration in which cells (MCF-7 and HeLa cells) are exposed to constant, low, and known concentrations of H_2O_2, maintaining at the same time the redox homeostasis, it was demonstrated that H_2O_2 actually has a weak capacity to activate NF-κB by itself. However, in the presence of TNF-α [as in a well-established inflammatory response], H_2O_2 shows a significant synergism in the activation of NF-κB (117). In this context, it is important to make clear that ROS are not always necessarily mediators in NF-κB activation despite a strong correlation with it. Interpretation of comparative experiments by using antioxidants requires caution because antioxidants disturb the cellular redox status conditions essential for the activation of NF-κB. However, in 1996, it was demonstrated by using electrophoretic mobility-shift titration experiments and incorporating different ratios of GSH/GSSG, that changes in GSH and GSSG concentrations, corresponding to redox potential differences of as little as ± 15 mV, enabled or abolished binding of NF-κB and AP1 to their cognate DNA sites (98). More recently, the native forms of NF-κB subunit p50, with a Cys^{62} residue as well as a p50 mutant (Cys^{62S}), were exposed to changes in the redox pair GSH/GSSG ratio. When the range was between 1 and 0.1, a significant 40 to 70% inhibition of the DNA binding of p50 wild-type was found (422). The specific *S*-glutathionylation of p50 in the Cys^{62} residue was demonstrated in this study, but also the oxidation of Cys^{62} and sulfenic acid formation was found, suggesting that more than one modification of Cys^{62} is feasible.

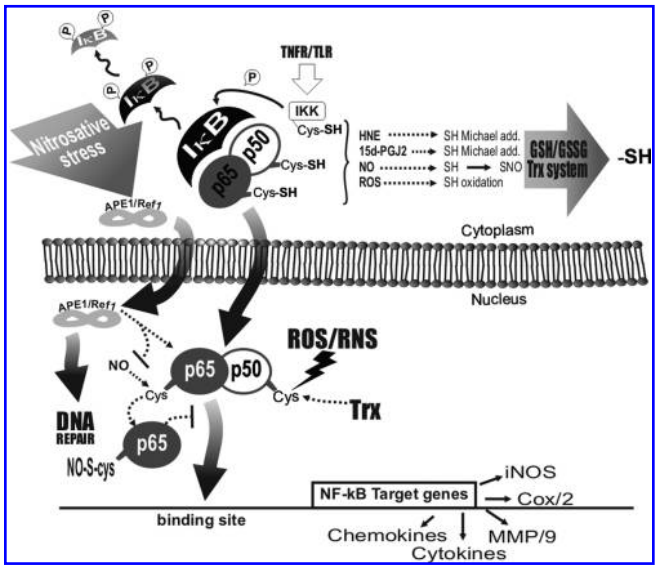

FIG. 16. NF-κB plays a pivotal role in inducing genes involved in the neuroinflammatory response, and it is induced by oxidative stress. Both NF-κB subunits have redox-reactive cysteine residues, the same as the IκB kinase, necessary for NF-κB activation. These residues are susceptible to a diversity of modifications induced by HNE, cyclopentenone prostaglandins, NO, and ROS. The durations of those interactions are under control of the major redox systems, such as the GSH/GSSG couple and the Trx system. Even after the translocation to the nucleus, NF-κB is under oxidative attack. Thioredoxin regulates the DNA-binding activity of NF-κB by reduction of a disulfide bond in the p50 subunit, which is susceptible to attack by ROS or RNS. p65 can be inactivated by *S*-nitrosylation, but it is possible that the APE1/Ref1 complex reduces those redox-sensitive cysteines or facilitates their reduction by GSH or Trx.

Trx is able to interact with NF-κB by reducing the mentioned disulfide bond involving Cys^{62} of the p50 subunit and thus regulating NF-κB activity (436). However, Trx has a controversial role in regulating NF-κB activity (449). Apparently it depends on Trx concentration. In the cytoplasm, Trx interferes with the signals to IκB kinases and blocks the degradation of IκB, which implies restriction of NF-κB activity (114). However, if NF-κB becomes active, Trx translocates from the cytoplasm to the nucleus and associates directly with the NF-κB p50 DNA-binding domain through its Cys^{32} residue in the catalytic domain. In this way, Trx potentiates NF-κB–dependent transcription (Fig. 16).

H_2O_2 oxidizes a conserved cysteine 179 (Cys^{179}) in the kinase domain of IKKβ, and by this reversible mechanism, H_2O_2 may inhibit IKK, the gatekeeper for NF-κB activation (449). Furthermore, IKKβ is susceptible to *S*-glutathionylation with the formation of a sulfenic acid intermediate, and such S-glutathionylation was responsible for the reversible inhibition of IKK in H_2O_2-exposed cells. Even more *S*-glutathionylation, as an important regulatory switch, allowed the rapid regeneration of IKK enzymatic activity through GRX-dependent catalysis (449).

2. Redox-regulated pathways involved in NF-κB activation. IL-1 induces NF-κB (114, 508) in a well-known process involving the IL-1 receptor with its cytoplasmic Toll/IL-1R domain that requires the interaction with MyD88 and the recruitment of IRAK and TRAF-6 (82) (Figs. 12 and 16). However, the small GTPase Rac1, a key regulator of the intracellular redox state, is required for the IL-1β–stimulated activation of NF-κB and the subsequent NIK-TRAF6 complex formation (230). The affinity of the H_2O_2-modulated NIK (294) for TRAF6 may be enhanced directly by a disulfide bond–mediated conformational change in the protein, or it may be indirectly stimulated by reducing the activity of a regulatory phosphatase; by inhibiting Rac1 and therefore the

consequent Rac-dependent NOX as H_2O_2 source, the NIK-TRAF6 complex is inhibited. In this context, some other, more-complex mechanisms of NF-κB regulation by redox sensors also were proposed. This includes the regulation of TRAF by the redox-regulated Ask1 (352) and the inflammatory cytokine-activated PI3-kinase/Akt, which directly phosphorylates IKK, leading to the activation of NF-κB, independent of MEKK-1 and NIK (456, 504) (Fig. 14). Reciprocally, the overexpression of p65 leads to Akt phosphorylation in the absence of extracellular stimulatory factors, whereas overexpression of IκBα reduces Akt phosphorylation (343).

Akt is activated by several cytokines, growth factors, and neurotransmitters, or it can be mediated by PI3-kinase. It is positively regulated by Hsps, whereas the tensin homologue deleted on chromosome 10 (PTEN) is a known negative Akt regulator. PTEN dephosphorylates membrane inositol phospholipids at the 3′-OH group of the inositol ring, preventing PI3K-dependent activation of Akt (80). However, Akt also is vulnerable to *S*-nitrosylation because of a redox-vulnerable cysteine residue in the catalytic domain, which makes this pathway prone to redox regulation (595). Similarly, *S*-nitrosothiols oxidatively modify PTEN, leading to reversible inhibition of its phosphatase activity.

Another IKK-independent mechanism for redox regulation of NF-κB could involve the c-Src–dependent tyrosine phosphorylation of IκBα and the subsequent activation of NF-κB, an event controlled by intracellular H_2O_2, as demonstrated in HeLa cells after hypoxia/reoxygenation injury (140).

NF-κB and AP-1 become convenient targets for the apurinic/apyrimidinic endonuclease 1/redox factor 1 (APE1/Ref1 complex), which either directly reduces critical redox-sensitive cysteine residues, thereby facilitating its DNA-binding and transcriptional activities, or facilitates NF-κB and AP-1 reduction by other reducing molecules, such as glutathione or Trx (181). Thus, in addition to its well-known role in DNA repair (559), APE1/Ref1 is a "redox chaperone" stimulating the DNA-binding activity of Fos-Jun heterodimers, Jun-Jun homodimers, and Hela cell AP-1 proteins, as well as that of several other transcription factors, including NF-κB, Myb, and members of the ATF/CREB family. Functioning as a chaperone, APE1/Ref1 does not require more than physiologic concentrations in the cell nucleus (more than 1 μM) or additional levels of GSH (1 mM) or Trx (1 μM) (9). Conversely, •NO induces *S*-nitrosylation of Cys^{93} and Cys^{310} in the APE1/Ref1 complex; thus, the nuclear-cytoplasmic translocation of APE1/Ref1 is linked to nitrosative stress, a mechanism that could explain how the repression of the APE1 endonuclease activity enhances DNA damage by nitrosative stress (439) (Fig. 16). Evidence also suggests that APE1/Ref-1 is dependent on ROS generated after purinergic-receptor stimulation (P2Y receptors) by ATP, which correlates with an increase of its endonuclease and redox activities; these effects are associated with the simultaneous increase of two important second messengers, Ca^{2+} ions and 1,2 diacylglycerol, a physiologic activator of PKC (423).

B. DNA-binding properties of AP-1 depend on the redox state of its cysteine residues

AP-1 is crucial for cell adaptation to many environmental changes and controls neuroinflammation and cell survival. It is important in this review on the functional aspects of redox control during neuroinflammation to make clear how AP-1 becomes redox regulated. The induction of AP-1 appears to be part of a general mechanism of regulation of gene expression by oxidative stress.

AP-1, downstream of MAPK signaling pathways, is a ubiquitous regulatory protein complex that interacts with AP-1 binding sites of target genes and regulates the neuronal and glial response to injury. It is a dimer composed of Jun or Jun and Fos proteins, with regulatory sites in a number of promoter genes, which are necessary in neuroinflammation, such as matrix metalloproteinases, proinflammatory cytokines, chemokines, and nitric oxide (267, 359, 582).

The activation of AP-1 is determined by H_2O_2 levels (425), whereas the DNA-binding properties of AP-1 depend on the redox state of its cysteine residues (424). A correlation is found between electrophilic (quinone)-mediated production of •OH, a decrease in GSH levels, and the induction of AP-1 binding activity, in addition to GST gene expression (33). Conversely, in a well-established inflammation–oxidative stress vicious cycle, HNE activates stress-signaling pathways that lead to overexpression of c-Jun and the subsequent AP-1 activation, whereas it increases H_2O_2 strongly and promotes GSH depletion (550) (Fig. 10).

Several cysteine residues have been described for Jun-Jun or Jun-Fos AP-1 dimers, and cysteine-to-serine mutants show that the inhibition of AP-1 activity after •NO treatment is dependent on the presence of Cys^{7272} and Cys^{154} in the DNA-binding domain of Jun and Fos, respectively (378). The associated Ref-1 and Trx also interact directly with Cys^{252} of Jun and become essential to AP-1 activation by redox modification (199). It is well known that changes in the ratio of reduced to oxidized glutathione provide the potential to oxidize c-Jun sulfhydryls, thereby producing either protein disulfide formation or *S*-glutathiolation (260). Then glutaredoxin or thioredoxin may catalyze the reduction of the glutathionylated protein (200). Thus, c-Jun DNA-binding activity may depend on glutathionylation/deglutathionylation reactions.

C. Reactive cysteines contribute to a negative regulation for p53

p53 plays a pathogenic role in chronic neuroinflammation, in which it is linked to nitrosative stress. This redox-active transcription factor triggers apoptosis in response to cellular stress, while it induces the transcription of redox-related genes, encoding proteins that in turn lead to more oxidative stress and promote the oxidative degradation of mitochondrial components (426). Dependent on the rate and timing of •NO production, as well as the concurrent $O_2^{\bullet -}$ overproduction, amino acid modification in p53 through *S*-nitrosylation may impair its function. p53 possesses three critical cysteine residues at positions 173, 235, and 239, which, when replaced by serine, markedly reduce *in vitro* DNA binding, completely block transcriptional activation, and lead to a striking enhancement rather than a suppression of transformation by p53 (440). Thus, p53 is subject to modulation through oxidation–reduction of cysteines at or near the p53-DNA interface. At the same time, p53 is modulated in macrophages by the macrophage migration inhibitory factor (MIF) through a direct, physical interaction by using p53 cysteine residues at the

81, 242, and 238 positions within its central DNA-binding domain (236).

A mixed disulfide glutathione adduct on the Cys^{182} residue of p53 could account for the observed stoichiometry of oxidized thiols and structural changes. The glutathione adduct may prevent proper helix–helix interaction within the DNA-binding domain and contribute to tetramer dissociation (525). In addition, p53 can be activated in response to $ONOO^-$ stimulation by an indirect route (240), in which the ras/ERK pathway plays a significant role, as evaluated in murine primary neural cells. Nonetheless, the activation of p53, leading to apoptosis in neuroinflammatory/oxidative stress processes, apparently depends on DNA damage followed by accumulation of p53, an event that can be inhibited by using antioxidants like NAC, a GSH precursor, or PDTC.

XI. Phosphorylation and Redox Considerations

For decades, phosphorylation of tyrosine, serine, and threonine residues has been known as the central means of controlling the behavior of intracellular proteins. However, in recent years, a number of new and exciting aspects of redox regulation have given to these mechanisms a strong reputation as "alternative" mechanisms in the autoregulation of the cell physiopathologic functions. Nonetheless, the routes of phosphorylation have checkpoints at which redox regulation plays a determinant role.

MAPKs are serine/threonine kinases that include the ERKs, JNKs, and p38MAPK, key mediators of extracellular signals, including neuroinflammatory responses. Key transcription factors in neuroinflammation, such as NF-κB, AP-1, CREB, and C/EBP, are often the downstream targets of MAPK signaling cascades. Neuronal dysfunction and neuron loss during long-term glial activation in neurodegenerative diseases has been attributed to ERK, JNK, and p38MAPK signaling pathways (100, 587).

Both phosphorylation and redox regulation may converge on the p38MAPK pathway, which can be selectively activated under the GSSG-mediated oxidative stress through upregulation and phospho-activation of p38 MAPK in a process in which the decrease in GSH content maybe an amplifying cofactor (147). However, GSSG is hydrophilic, not able to enter the cells; thus, it is speculated that the activation of the p38 MAPK pathway may be mediated by thiol/disulfide exchange reactions with membrane protein thiols, by means of a molecule able to sense and transduce the alterations of the extracellular redox environment. The best candidate to do this is Ask-1 (147), whose response to redox alterations depends on its association with Trx. Once uncomplexed from Trx, which undergoes oxidation, Ask-1 dimerizes and activates MKK4, which in turn releases JNK and p38 MAP kinase pathways (542) (Fig. 11).

Even though redox regulation of MAPK has not been well characterized, some evidence has accumulated. For example, phosphorylation of p38MAPK may be abrogated by using antioxidant GSH precursors, such as NAC, or the GSH analogue γ-glutamylcysteinyl-ethyl ester (γ-GCE), which means that p38MAPK phosphorylation could be a redox-dependent process (179). Conversely, it has been demonstrated that the overexpression of Trx negatively regulates p38MAPK activation and the subsequent expression of IL-6 in TNF-α–stimulated cells (187).

ERK2, in turn, possesses a cysteine residue (Cys^{166}, within its ATP-binding site) that plays a crucial role in the inhibitory activity for these MAPKs (Fig. 17). It is known that ATP-antagonist compounds bind covalently to this Cys residues and inactivate ERK, although it remains to be determined whether this residue is redox reactive (385).

MEKK1 (MAPK/ERK kinase kinase) has a Cys residue at the position 1238 of the kinase domain within a glycine-rich loop, critical for optimal binding and ATP coordination. Cys^{1238} is susceptible to attack by H_2O_2 under oxidative stress conditions, and its alkylation is relevant for the loss of MEKK1 catalytic activity. When Cys^{1238} is mutated to valine, the inhibitory effects of H_2O_2 become abrogated (104). In this context, GSH could play a critical role in the regulation of the kinase activity of this stress-activated protein kinase by interfering sterically with the function of the glycine-rich loop (12).

Phosphorylation/dephosphorylation cycles modulate the activity of kinases, and the redox regulation of phosphatase activity could influence the activity of kinases (545). Protein tyrosine phosphatases (PTPs) have in common readily oxidizable cysteine residues in their active sites, which are targets for reversible oxidation and inactivation of the enzymes (86) (Fig. 17). Quinolinic acid, an NMDAR agonist as well as a prooxidant agent, is able to induce a significant reduction in PTP activity in synaptosomes prepared from striata, along with a sustained increase in c-src and lyn activity, probably through the formation of reactive radical species or NMDAR overactivation or both (345). Conditions of oxidative stress lead to changes in the activity of signaling enzymes, including PTPs and PTKs (248). H_2O_2, acting as a second messenger, appears to oxidize active-site cysteines in PTPs, thereby inactivating them, while activating PTKs such as p38 or ERK

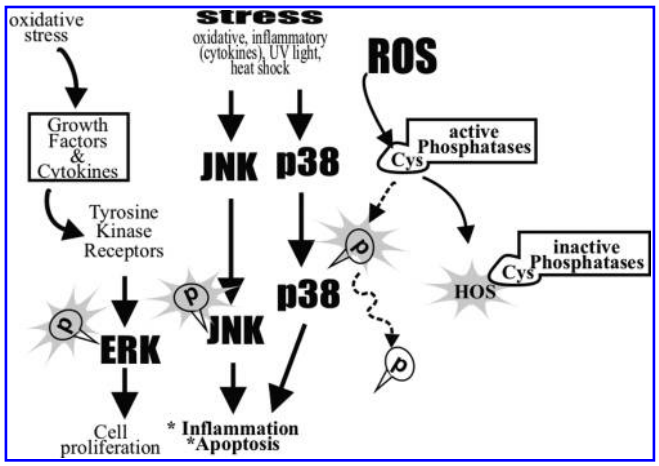

FIG. 17. Oxidative pressures activate MAPK, and the redox environment inside the cell modulates their activities. Stimulated tyrosine kinase receptors activate ERK, and this activation is modulated by phosphatases and, possibly, by direct oxidation of a cysteine residue corresponding to Cys^{166} of ERK. JNK and p38 MAPK phosphorylation is modulated by phosphatases, which contain a reactive and redox-regulated cysteine, which controls the hydrolysis of phosphotyrosine residues through a cysteinyl phosphate intermediate that catalyzes the dephosphorylation reaction of protein tyrosine kinases (PTPs). Oxidation of this cysteine residue to sulfenic acid by ROS renders PTPs inactive.

(150). However, the regulation of PTPs by ROS is rapid and reversible, but the receptor protein-tyrosine phosphatase (RPTP) has conformational and more-stable changes in response to H_2O_2 that oxidizes a Cys^{723} residue in the RPTPα-D2 and leads to a change in rotational coupling in RPTPα dimmers (555). Inhibition of SOD1, conversely, protects PTPs from oxidation by preventing the formation of H_2O_2 (235), which in turn would inhibit p38 or ERK phosphorylation through the previously mentioned mechanisms (Fig. 17).

A. Concurrent pathways

Major proinflammatory/redox-dependent routes, such as NF-κB or AP-1 or the apoptotic p53 pathway associated with neuroinflammation and redox variations, are connected to several other pathways that are part of the main MAPK pathway. These concurrent pathways represent key mechanisms of survival or adaptive responses to oxidative stress and neuroinflammation and, importantly, they also are candidates for redox regulation.

From an overall perspective, the different neuroinflammatory signaling pathways constitute a complex web of interconnected routes with a set of redox-regulation checkpoints (Fig. 18).

Ras protein, attached to the inner cell surface associated with the tyrosine kinase receptor, activates the Ras/Raf/MEK/ERK pathway that leads to AP-1 (339). From the same superfamily of small GTPases as Rho, Ras has a Cys residue at the −118 position (195). As evaluated in rat cultured vascular smooth muscle cells, after NOX activation, H_2O_2 is overproduced and attacks Cys^{118}, forming a thiyl radical (3). GSH reacts with the thiyl radical to form mixed disulfide bonds, yielding a GSS-Ras complex. The thiol modification of Ras by thiol oxidants going from H_2O_2, *S*-nitrosoglutathione, diamide, or GSSG (323), is related to an increase in Ras GTP-binding activity. The activation of Ras decreases by using diphenyleneiodonium chloride, a NOX inhibitor. Once activated, Ras leads to the phosphorylation of p38 and Akt (159, 592). Conversely, •NO also reacts with Ras, probably at the same Cys^{118} residue and, depending on its level of expression, modulates ERK (10) (Figs. 14 and 18).

PKC, a family of closely related Ca^{2+}/phospholipid-dependent phosphotransferase isozymes, may also be inactivated by oxidant-induced *S*-glutathiolation (Fig. 18). For example, PKC-α is susceptible to inactivation by oxidant-induced *S*-glutathiolation, which was confirmed by using the thiol-specific oxidant diamide (1,1′-azobis-(*N*,*N*′-dimethylformamide); this effect was potentiated by adding GSH or was fully reversed by DTT (424). PKC-ε possesses a Cys^{452} residue (92) and plays a crucial role in oxidant-induced neuritogenesis, as demonstrated in PC12 cells subjected to oxidant agents, where, by direct redox activation of PKC-ε, a rapid and sustained activation of ERK was induced (168). The activation of ERK is necessary and sufficient for neurite outgrowth in PC12 cells (398).

Protein kinase A (PKA) is a cAMP-dependent protein kinase, and it is the major positive regulator of CREB in the brain (463). In AD brain, PKA is downregulated, and PKA phosphorylation of tau may be an early event in the development of neurofibrillary pathology in AD (598). PKA activation in macrophages stimulates PKC and p38 MAPK, which leads to IKK-dependent NF-κB activation and contributes to the induction of iNOS and IL-6 genes (88). PKA has a Cys residue at the position 199 in the activation loop of the catalytic C-subunit, which is susceptible to attack by H_2O_2 or diamide, serving as a catalyst in promoting the formation of protein–mixed disulfides with glutathione (210). This site, which is conserved in other kinases, such as AKT1/Raca, S6K (S6 kinase), and even PKCα, is protected from glutathionylation in the PKA tetramer (R2C2), becoming accessible only

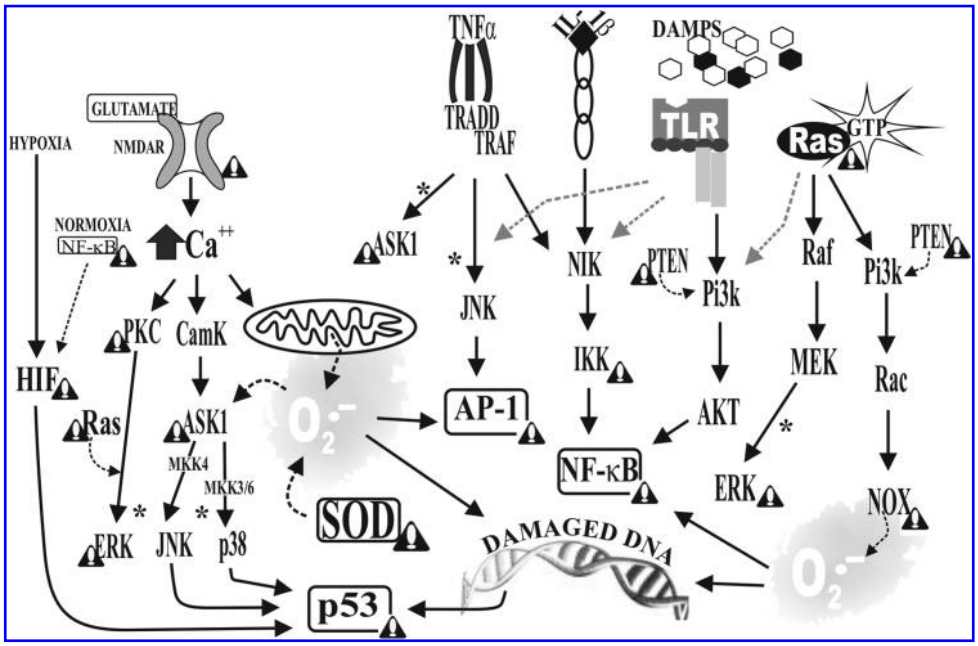

FIG. 18. Some of the major targets for direct redox regulation in neuroinflammatory pathways. Protein tyrosine phosphatases (*) fine-tune the extent, duration, and subcellular location of MAPK activation, and they also are susceptible to oxidative regulation. ⚠ = direct redox regulation through redox sensors.

after activation (12). Although Cys^{199} is not directly involved with the catalytic mechanism, its direct oxidation, glutathionylation, or alkylation turns the enzyme off, probably by changing protein–substrate interactions or by preventing activation-loop phosphorylation blocking kinase activation (210).

XII. Redox Changes Linked to the Pathophysiology of Neuroinflammatory/Neurodegenerative Diseases: A Tentative Classification

Throughout this article, we attempt to describe how redox alterations influence selected pathways, transcription factors, enzymes, and so on. We also provide one or several examples related to the phenomenon, as evaluated in one or another neuroinflammatory disease. As a result, the information about specific diseases appears scattered. Thus, to provide a clearer picture of how redox changes are linked to the pathophysiology of neuroinflammatory/neurodegenerative diseases, an integration of data is offered here.

Because neuroinflammation is a common denominator of a number of neurodegenerative pathologies and acute injuries linked to oxidative stress, redox regulation of neuroinflammatory processes associated with neurodegeneration can be classified in (a) particular disease-related alterations, (b) redox-regulatable processes common to neurodegenerative diseases, and (c) general stress-related pathophysiologic mechanisms, susceptible to redox regulation (Fig. 19). However, it is worth mentioning that by having the same physiopathologic background (neuroinflammation and oxidative stress in CNS tissue), some redox-regulated or redox-regulatory events now identified in one specific pathology, may in the future be found in another one. This is used as a justification of why our primary approach was from well-known mechanisms to some particular findings in specific diseases. Additionally, other processes are surely to be discovered.

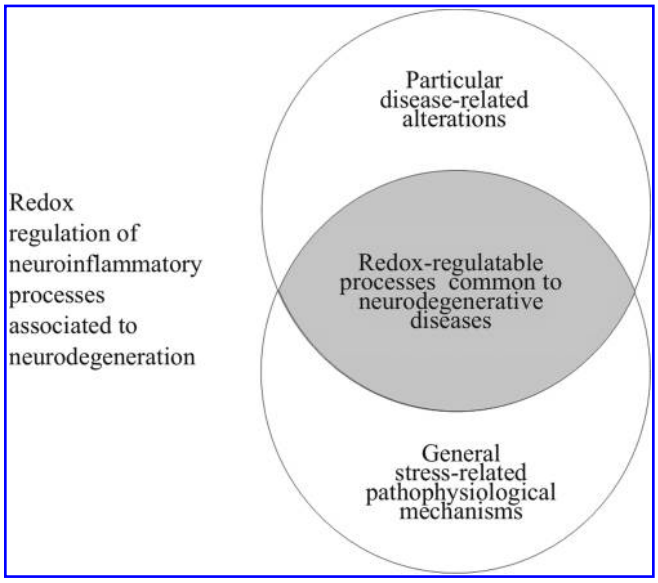

FIG. 19. According to some findings in neuroinflammatory pathologies associated with neurodegeneration, related to the mechanisms involved in redox regulation, a tentative classification is proposed.

A. Particular disease-related alterations

A number of thiol-sensitive proteins play key roles in the course of specific diseases. Thus, a specific glutathione *S*-transferase, hGSTM3, which protects cellular macromolecules from reactive electrophilic compounds, has been revealed to be *S*-glutathionylated in sections of the hippocampus obtained from patients with AD (123). The same occurs with 12-LOX, which is implicated in the oxidative modification of lipoproteins and phospholipids, in addition to the activation of sGC (67, 401), whereas the neurofilament-related NFL has been found to be *S*-nitrosylated during *in vivo* experiments in mice that received Aβ injections (6). Until now, these redox-related alterations have been related only to the pathology of AD, the same as oxidation, glutathionylation, and/or *S*-nitrosylation of metallothionein (17, 71) and the energy metabolism–related enzymes GAPDH (110, 185, 375), α-ketoglutarate dehydrogenase complex (KGDHC), and malate dehydrogenase (MDH) (493).

Several proteins have been discovered to be *S*-nitrosylated in PD brains that could be specific disease-related alterations such as Parkin (94, 551), TH (15, 272) or DJ-1 (97, 221, 610). It is worth noting that, given the high affinity of some nitrosylated proteins for glutathione, glutathionylation may also be an alternative of nitrosylated proteins; thus, TH can be both *S*-glutathionylated and *S*-nitrosylated. GSH abundance, as mentioned earlier, is critical for *S*-glutathionylation, whereas the increasing oxidative pressures and •NO accumulation, in addition to depleting GSH, favor *S*-nitrosylation. This post-translational modification also preferentially occurs at Cys residues flanked by basic and acidic residues (514); but even without that condition, *S*-nitrosylation is possible because of the three-dimensional disposition of the Cys residues, as demonstrated in the propensity of DJ-1 to be *S*-nitrosylated at Cys^{46} and Cys^{53} residues (221). The mitochondrial complex I multimeric enzyme has also been reported to undergo *S*-glutathionylation or *S*-nitrosylation or both in models of PD (31, 249, 417), and finally, an *S*-nitrosylated X-linked inhibitor of apoptosis protein (XIAP) was recently uncovered in PD pathogeny in both animal models and human brains (548). XIAP inhibits the caspase-induced apoptosis, and XIAP *S*-nitrosylation impairs its ability to inhibit caspase-3 activity.

In ALS, oxidative modifications and misfolding of wild-type SOD1 are selectively toxic to motor neurons, and they are as significant as the ALS-linked SOD1 mutant in its physiopathologic consequences (238). FALS cases are indistinguishable on the basis of clinical and pathologic criteria, suggesting that the two forms share similar or converging pathogenetic mechanisms. However, an extensive evaluation of protein nitration in the soluble fraction of spinal cord homogenates in a mouse model of FALS revealed 32 proteins nitrosylated *in vivo* under physiologic or pathologic conditions. This important protein nitration in ALS might lead to *S*-nitrosothiols depletion, disrupting the function or subcellular localization or both of proteins that are regulated by *S*-nitrosylation, such as GAPDH, and thereby contributing to ALS pathogenesis (482).

In acute injuries, such as brain ischemia, *S*-nitrosylation of PTEN is increased significantly after 12 h of reperfusion compared with sham controls. However, pretreatment with a NOS inhibitor may inhibit the activity of PTEN by decreasing *S*-nitrosylation of PTEN (412). Another specific redox-related

alteration in ischemia could be the *S*-nitrosylation of L-type Ca^{2+} channels during severe hypoxia, a pathologic event that enhances the influx of Ca^{2+} (540).

B. Redox-regulatable processes common to neurodegenerative diseases

1. Neurofilament-related alterations. In the category of redox-regulatable processes common to neurodegenerative diseases is the tau protein, which normally provides stability and works in the assembly of axonal microtubules in neurons. It is susceptible to *S*-nitrosylation and *S*-glutathionylation, which could be the underlying mechanism of insoluble paired helical filaments formation, as observed in AD brains (6, 448, 450). However, the assembly of tau proteins into paired helical filaments is a common feature of many "taupathies" with an important oxidative stress component, such as AD, dementia pugilistica, Down syndrome, prion diseases, ALS/parkinsonism-dementia complex, argyrophilic grain dementia, corticobasal degeneration, diffuse neurofibrillary tangles with calcification, frontotemporal dementia/parkinsonism linked to chromosone-17, Hallervorden-Spatz disease, multiple system atrophy (MSA), Niemann-Pick disease type C, Pick disease, progressive supranuclear palsy, and subacute sclerosing panencephalitis (166).

2. Protein aggregate–related alterations. The α-synuclein that works in the maintenance and transport of synaptic vesicles, particularly modulating dopamine transport, is susceptible to *S*-nitrosylation, and it is characteristic of PD, linked to several independent familial cases in which this protein accumulates in the Lewy bodies. However, a peptide derived from α-synuclein forms an intrinsic component of amyloid plaque in AD as well as in dementia with Lewy bodies (DLB) and multiple system atrophy (MSA). These entities are grouped as α-synucleinopathies (166).

S-Nitrosylation of the protein disulfide isomerase, which normally catalyzes thiol-disulfide exchange and facilitates disulfide bond formation and rearrangement reactions, which leads to improperly folded proteins and RE stress, is a common feature found in ALS, PD, and AD (98, 375, 551).

Oxidation of the carbonic anhydrase-II is also a common feature in both AD and ALS. This is a Zn^{2+} metalloenzyme that catalyzes reversible hydration of CO_2 to HCO_3^-; its oxidation in the brain might be related to abnormal aggregation of α-synuclein (482, 521).

3. Mitochondrial dysfunction and energy failure–related alterations. In ALS, some of the previously mentioned 32 nitrosylated proteins could enter into the category of redox-regulatable, common to neurodegenerative disease processes. For example, *S*-nitrosylation of GAPDH and α-enolase, involved in altered glucose metabolism, also are found in brains of AD and MS patients (45, 375), whereas Drp, which regulates mitochondrial fission in normal cells and mediates mitochondrial fragmentation during programmed cell death, may undergo *S*-nitrosylation in both AD and ALS (89, 506, 575).

Functional alterations of the ATP synthase are found in AD (328) and ALS (482). In the first case, it is probably due to *S*-nitrosylation on its α-chain (521), whereas in ALS, *S*-nitrosylation of the ATP synthase has been found in its β-chain (482). In acute injuries, *S*-nitrosylation of the α-chain of ATP synthase also was recently reported (446).

Respiratory chain complex I is reported mainly as a target for *S*-nitrosylation during nitrosative stress in PD (249), but *S*-nitrosylation also occurs in acute injuries such as ischemic stroke and TBI (355, 388, 428).

C. General stress-related pathophysiologic mechanisms

1. Processes related to the neuroinflammatory response, including signaling pathways. This section includes a number of redox-regulatable signaling pathways as well as transcription factors (Fig. 18) that feed back on their own redox state. These redox-regulation checkpoints on signaling pathways are significant because they may define the course of a neuroinflammatory response. It is worth noting that redox-linked extracellular danger signals also play a key role as general stress-related pathophysiologic mechanisms.

In the same context, heat-shock proteins are commonly nitrosylated during oxidative-stress responses, as reported in AD models (6), ischemia (372), or TBI (446).

2. Antiapoptotic or proapoptotic responses. The activation of caspase-3 (96, 193, 261, 336, 353, 386, 396) and p53 (123, 196, 534, 552) correspond to general stress-related pathophysiologic mechanisms. They are described in all the neuroinflammatory/neurodegenerative pathologies and possess thiol-based redox sensors functioning as on/off switches that make these proteins particularly vulnerable to redox regulation.

Activation of MMP-9 by *S*-nitrosylation as described in AD, PD, MS, ALS, and ischemia (267, 286, 413, 479, 582) is also a general stress-related pathophysiologic mechanism involved in neuronal apoptosis.

3. Related to the antioxidant response. Through mechanisms of redox regulation, CNS cells mount vigorous responses to counterbalance the oxidative stress in both chronic neurodegenerative diseases and acute injuries. Nrf2 and the Keap1/Nrf2/ARE pathway (108, 497, 509, 539), in addition to SOD enzyme (51, 90, 186, 250, 370), are redox-sensitive protagonists in all neuroinflammatory processes.

Conversely, oxidative stress is enhanced when peroxiredoxins become *S*-nitrosylated or undergo oxidative damage to sulfhydryl groups, an eventuality described in AD, PD (109, 142, 551), ALS (226), and ischemia (581).

4. Related to synaptic transmission and excitotoxic stress. Oxidation or nitrosylation of NMDAR with all its consequences (Fig. 18) is a common feature for all neuroinflammatory/neurodegenerative processes in which oxidative stress plays a significant role (306, 473, 519, 529, 578).

5. Common alterations related to lipids. Several processes derived from neuroinflammatory signaling pathways, such as mobilization of membrane lipids and oxidation and metabolism of PUFAs, are commonly associated with redox-regulatory mechanisms and are widely described in all neurodegenerative conditions and acute brain injuries (13, 35, 129, 266, 443, 494, 516, 549, 597). Thus, these phenomena are general stress-related pathophysiologic mechanisms.

XIII. Concluding Remarks

A. Major progress

The notion that both reactive oxygen and nitrogen species are primarily harmful to cells has changed, and now it is widely recognized that both regulate cellular signaling pathways by redox-dependent mechanisms. From this knowledge, alternative pathophysiologic pathways are being discovered, and new redox-based therapies are being proposed. The objective of these therapies is to affect key redox-related molecules in neuroinflammatory signal-transduction pathways, such as NF-κB, AP-1, Keap1, thioredoxin, Ras, or PPAR-γ, as well as neuronal survival regulators, such as NMDA receptors, caspase-3, Parkin, or PDI. Several examples are explained briefly in this review.

B. Problems

H_2O_2 and •NO modulate signal-transduction pathways by modifying tyrosine residues in phosphotyrosine signaling or by electrophilic attack to key cysteine residues. Thus, the redox environment is relevant to determine the final fate of a neuroinflammatory cascade. For example, *S*-nitrosylation of proteins is a key posttranslational event, including receptors, enzymes, and transcription factors. However, many reported experimental results are experiments *in vitro* based on purified proteins or were achieved by using high concentrations of exogenous •NO donors. Thus, the findings do not necessarily reveal functional changes in cells under physiologic conditions, and even less under pathologic conditions. Moreover, redox regulation implies collaborative efforts between neurons and glia to achieve homeostasis, and the results may vary in function of time as well as dose and persistence of the stimulus or stimuli.

S-Thiolation and *S*-nitrosylation of protein sulfhydryls are pleiotropic events. First, which event gains priority in a given physiologic or pathologic situation remains to be clarified. The precise correlation among different forms of cysteine modification is not clear. *S*-Thiolation and *S*-nitrosylation may choose the same cysteine residues, in which *S*-nitrosylation precedes *S*-thiolation, or modify different cysteine residues in the same protein, such as H-ras, with four reactive cysteines (323), whereas 15Δ-PGJ_2 competes for at least one Cys residue (387) on the same protein. *S*-Thiolation or *S*-nitrosylation of transcription factors, conversely, gives contrary results depending on the cell compartment where the protein modification occurs (Fig. 16). Thioredoxin catalyzes the denitrosylation of *S*-nitrosoamino acids and *S*-nitrosoproteins, but conflicting results regard the effects of *S*-nitrosylation on Trx antioxidant functions, depending on the cell type and its redox environment.

S-Glutathiolation of proteins is a mechanism to protect thiols from oxidation and irreversible modifications, while preserving their functionality. However, some other electrophile compounds—particularly those lipid derivatives—also are able to form covalent adducts at cysteine residues interfering with physiologic *S*-glutathiolation; whether these adducts hamper or become complementary to achieve homeostasis remains to be clarified (Figs. 6–9). To the complex relation between redox environment and posttranslational modifications to proteins with its functional consequences, as well as the particular cell microenvironment in glia and neurons and the intercommunication between them, an intricate network of signaling pathways is added. All the most important signaling pathways in neuroinflammation have specific checkpoints where redox sensors define the continuity or the interruption of a signal (Fig. 18). It is worth mentioning that the existence of a regulatory pathway does not necessarily mean a significant biologic role for it. In any case, the study of a given type of cell, taking into account its biologic context, will aid in defining which of the many possible regulatory pathways may apply to each circumstance. Validation *in vivo* is always necessary.

C. Prospects in the field

Advances in this field depend on *in vivo* studies that allow evaluation of the specific role of distinct cells, preferably in real time, and here, technologic advances play an important role. Improved 2DGE-based redox proteomics (427) and the advent of quantitative assessment of the cellular redox status *in vivo* (288) to identify target proteins that use redox-sensitive thiol groups to modulate their protein activity, as well as redox fluorometry (121), are among the newest tools to clarify the extent of redox regulation during neuroinflammatory processes. In addition, the role of the distinct protagonists will be better delineated by using more reliable markers (named synthetic redox reporter molecules), which consist of tyrosine subunits bonded covalently to polyunsaturated fatty acids that can be further connected through an ester bond to a third unit, either to sterols or to 2′-deoxyguanosine (560).

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Abbreviations Used

AD = Alzheimer disease
Akt = protein kinase B
ALS = amyotrophic lateral sclerosis
APP = Aβ precursor protein
ARE = antioxidant response element
Ask-1 = apoptosis signal-regulating kinase 1
BSO = L-buthionine-*S*-*R*-sulfoximine
BTB = broad complex–tramtrack–bric-a-brac
BzATP = 3′-*O*-(4-benzoyl)benzoyl-ATP
C/EBP = CCAAT/enhancer-binding protein
cGMP = cyclic guanosine monophosphate
CN/NFAT = chronic neuroinflammation, through the calcineurin/nuclear factor of activated T cells
CREB = Ca^{2+}/cAMP response element–binding protein
Cul3 = Cullin-based E3-ligase
DAMPs = damage-associated molecular patterns
DGR = double glycine repeat or Kelch repeat domain
DOPA = L-tyrosine to dihydroxyphenylalanine
EAAT1-5 = excitatory amino-acid transporters
EGLN1 = Egl nine homologue 1
EPO = erythropoietin
EpRE = electrophile response element
ERK = extracellular signal-regulated kinases
fAβ = fibrillar Aβ
FAD = flavine-adenine dinucleotide
FADD = Fas-associated protein with death domain
FALS = transgenic mouse model of familial ALS
FLICE = DD-containing signaling molecule FADD interleukin-1β converting enzyme (ICE) family
FMN = flavin mononucleotide
Gα1 and Gα0 = tyrosine kinases and small G proteins
GDP = guanosine diphosphate
GEFs = guanine-nucleotide exchange factors
γGCSr = γ-glutamylcysteine synthetase
GLUT-1 = glucose transporter-1
Grx = glutaredoxin
GSH/GSSG = glutathione/oxidized glutathione disulfide
GSNO = *S*-nitrosogluthathione
GTP = guanosine triphosphate
HaCaT = human keratinocyte
HD = Huntington disease
HDGF = hepatoma-derived growth factor
HMGB1 = chromatin protein high-mobility group Box1
HNE = hydroxynonenal
HSP = heat-shock protein
IGF-1 = insulin-like growth factor 1
IκB = IκB protein
IL1R = IL-1 receptor
IRAK-1 = IL-1 receptor–associated kinase
IRF1 = interferon regulatory factor
IVR = intervening region
JAK/STAT = Janus kinase/signal transducers and activators of transcription
JNK = c-Jun NH_2-terminal kinase
Keap 1 = Kelch-like ECH-associated protein 1
LBD = Lewy body variant of AD
LOO• = reduction of peroxyl radical
LOX = lipoxygenases COX-2
MAdCAM-1 = mucosal vascular addressin cell-adhesion molecule
MAP2 = microtubule-associated protein-2
MAPK = mitogen-activated protein kinase
MCP-1 = monocyte chemotactic protein-1
MIF = macrophage migration-inhibitory factor
MKP-1 = mitogen-activated protein kinase phosphatase-1
MMP-9 = matrix metalloproteinase-9
MPP + = 1-methyl-4-phenylpyridinium
MPTP = 1-methyl-4-phenyl-1,2,3,6-tetra-hydropyridine
MS = multiple sclerosis
MSA = multiple system atrophy
NAC = *N*-acetylcysteine
NALP3 = Nacht domain-leucine-rich repeat and PYD-containing protein 3
NF-κB = nuclear factor kappa B
NFL = neurofilament light-chain polypeptide
NGF = nerve growth factor
NQO1 = quinine reductase
NT = 3-nitrotyrosine
NUDEL = nuclear distribution element-like
OL = oligodendrocyte
ONE = 4-oxo-2-nonenal
P2X = ionotropic ATP-gated ion channel receptors
P2Y = metabotropic G protein–coupled receptor
P2Y receptor = purinergic receptor stimulation
PAMPs = pathogen-associated molecular patterns
Panx-1 = hemichannel pannexin
PD = Parkinson disease
PDI = protein-disulfide isomerase
PDTC = pyrrolidine dithiocarbamate
PERK = pancreatic endoplasmic reticulum kinase
PGH2 = prostaglandin H_2
PI3K = phosphoinositide 3-kinase
PKC-δ = protein kinase C-delta
PLA2 = phospholipase A_2
PPAR-γ = peroxisome proliferator–activated receptor γ

Abbreviations Used (cont.)

PRRs = pattern-recognition receptors
Prx = peroxiredoxin
PTK = protein tyrosine kinase
PTP = protein tyrosine phosphatase
RAGE = receptor for advanced glycation end products
RPTP = receptor protein-tyrosine phosphatase
sGC = soluble guanylyl cyclase
SH3 = Src homology 3
SR = scavenger receptor
tBHQ = *tert*-butyl hydroxyquinone
TBK1 = activator–binding kinase 1
TGF-β = transforming growth factor β
TH = tyrosine hydroxylase enzyme
TNFR = tumor necrosis factor receptor
TNFR1 = TNF receptor 1
TRADD = TNFR-associated death domain
Trx = thioredoxin peroxidase
TrxR = selenoenzyme thioredoxin reductase
Tx-1 = thioredoxin 1
UPR = unfolded protein response
VEGF = vascular endothelial growth factor
XIAP = X-linked inhibitor of apoptosis protein

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