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OxInflammation in Alzheimer's disease

Carlo Cervellati*, Giovanni Zuliani, Giuseppe Valacchi

OxInflammation: definition and role in disease: OxInflammation has been introduced as a new term in the biomedical field to highlight the deep and mutual relationship between the dysregulation of redox and immune homeostasis (Valacchi et al., 2018). Regardless of which is the initial spark, inflammation cannot take place without a concomitant oxidative stress response and vice-versa. In other words, they are sides of the same coin.

The maintenance of a steady redox status is paramount for the cells, since any perturbations of the dynamic interactions between electron donors and acceptors lead to macroscopic consequences in metabolic activities, and potentially to biological damage and diseases (Ursini et al., 2016). Triggers of these effects are reactive species related to oxygen (ROS) and/or nitrogen (RNS) which play also a fundamental role in cell signaling. The result of ROS/RNS challenge depends on their levels and location. A moderate increase causes alteration of metabolism, and proliferation, but also regulated cell death (including apoptosis and necroptosis); an overt increase leads to severe damage of plasma membranes and organelles (in primis, mitochondria), and eventually to necrosis. The switch between these two antithetic roles, second messenger or pathogen, does not solely rely on the concentration of ROS/RNS. The final outcome depends on the "healthy" state of the antioxidant adaptive system which is the complex network of molecular pathways involved in the regulation of redox homeostasis, and in the defense against oxidative challenges (Ursini et al., 2016).

Inflammation response occurs via activation of several redox-sensitive transcription factors, such as nuclear factor 'kappa-light-chain-enhancer' of activated B-cells, activator protein-1, and components of multimeric protein complexes critically involved in innate immunity regulation, inflammasome (Volkman et al., 2019). Regardless of the activated pathways, the result is the secretion of cytokines that propagate and potentiate inflammation mostly by redox-related mechanisms. The secreted interleukin (IL)-6, IL-1, IL-18, IL-33, tumor necrosis factor-α, and IL-8 are all able to induce ROS/RNS levels by several enzymatic and non-enzymatic routes. In particular, there are enzymes in phagocytes able to "translate" pro-inflammatory stimuli into the formation of ROS/RNS; intuitively, the levels and the proportional effects of these species mainly depend on the initial stimuli. This is the case of reduced nicotinamide adenine dinucleotide phosphate-oxidases (NOXs) (in particular phagocytic NOX2 and NOX4) and Myeloperoxidase (MPO) that are crucial mediators of the intracellular microbicidal system of phagocytes and host innate immune system (Cervellati et al., 2020; Smyth et al., 2022) . More specifically, NOXs and MPO work synergically. Once assembled on the internal membrane surface, NOXs are able to directly generate superoxide anions (O_2^-•), which rapidly dismutate to hydrogen peroxide (H_2O_2) by the superoxide dismutase enzyme. MPO catalyzes the reaction of H_2O_2 with chloride ions to form hypochlorous acid, with the aim to counteract pathogens infection, but can also lead to host tissue damage (Smyth et al., 2022). In chronic sterile inflammation, the continuous over-activation of these enzymes results in an increase of collateral effects, that contribute to the onset and progression of a number of diseases, in primis cardiovascular, but also neurodegenerative disorders.

To make simple a very complex scenario, pro-inflammatory cytokines released by immune cells act as the instigators, and ROS/RNS are the mediators. In other words, the cells are the "killer" and ROS/RNS are the "bullets". This process leads to the perpetration of the initial inflammation state and the refuel of OxInflammation vicious cycle (Valacchi et al., 2017).

Another characteristic feature of OxInflammation is its systemic nature, as a physiological consequence of organ-to-organ interaction (Valacchi et al., 2018; Marsillach et al., 2020). In general, the OxInflammatory status, when chronic, can spread systemically as noticed for immune diseases affecting the brain. In particular, there is a wealth of experimental and clinical evidence clearly showed that this adverse crosstalk between the periphery and central nervous system (CNS) is an early and prominent feature of Alzheimer's disease (AD) (Cervellati et al. 2020)

OxInflammation in Alzheimer's disease: *Microglia as the main trigger of neuroinflammation in Alzheimer's disease*: The interconnection between inflammation and oxidative stress certainly plays a key role in AD pathogenesis. In particular, neuroinflammation is now universally regarded as one of the main neuropathological hallmarks of the disease, along with the classic amyloid-beta (Aβ) plaques and neurofibrillary tangles (Cervellati et al., 2020). Chronic activation of the glial cells, firstly microglia, is the initial event of inflammation in the CNS. Microglia is the first defensive line in the CNS, responding to neuronal damage and removing the damaged cells by phagocytosis. Aging and various persistent pathological insults induce the pre-activation, also referred to as "priming", of microglia, resulting in amplified response to a second inflammatory stimulation (praeludium to chronic neuroinflammation) (Cervellati et al., 2020). Causative agents of this pre-activated state are, among others, stroke, depression, trauma, toxins, infection, but also reactive species and proinflammatory cytokines.

Main sources of oxidative stress in Alzheimer's disease: It is well-known that the brain is the most oxidative vulnerable tissue, due to its physiological scarcity of endogenous antioxidants (that rarely can cross the blood-brain barrier, BBB), elevated levels of unsaturated fatty acid (the main target of peroxidation), high oxygen consumption, and high levels of pro-oxidant trace elements such as copper and iron (Salim, 2017). Thus, even a slight increase in ROS/RNS can cause considerable harms to the brain. As proof of concept, multiple signatures of oxidative damage to biomolecules have been found in the AD brain since the early stage of the disease. Indeed, there is abundant literature reporting elevated levels of redox biomarkers such as isoprostanes, 4-hydroxynonenal, nitrotyrosine, and protein carbonyls in the hippocampus and frontal cortex of patients affected by AD (Valacchi et al., 2018).

It is now widely acknowledged that progressive impairment of mitochondrial function, and related enhancement in ROS generation, is a key event in AD pathogenesis (Salim, 2017). Mitochondrial dysfunction is indeed the primary cause of glucose hypometabolism, which has been demonstrated to be a precocious feature of the AD brain. Notably, the AD typical aberrant peptides (Aβ oligomers and hyperphosphorylated tau) are both also effective generators of ROS/RNS, and therefore possible causes of mitochondrial dysfunction. In particular, the highly neurotoxic $Aβ_{1–42}$ oligomers can cause oxidative/nitrosative stress by several mechanisms, including direct interference with mitochondrial function and induction of metal dyshomeostasis (Salim, 2017; Valacchi et al., 2018).

A considerable contribution of brain redox imbalance in AD is given by NOX2 and NOX4, which are localized in several CNS cells, but to a greater extent in activated microglia and astrocytes (Ma et al., 2017; Luengo et al., 2022). Indeed, numerous studies demonstrated that subunits of these two NOX isoforms are heavily expressed in AD brain, especially in activated microglia surrounding Aβ-laden capillaries from patients with cerebral amyloid angiopathy, typical AD-related neurovascular disease caused by abnormal deposits within small- to medium-sized blood vessels of the brain (Ma et al., 2017). Notably, recent findings point to a causal role of NOX4 also in tau pathology (Park et al., 2021; Luengo et al., 2022). Overproduction of O_2^-• and H_2O_2 by NOX2 contributes directly (peroxidative damage to neuron membranes) or indirectly (formation of pro-inflammatory mediators such as isoprostanes and 4-hydroxynonenal) to neurotoxicity, leading to brain inflammation and degeneration (Ma et al., 2017).

Brain OxInflammation in Alzheimer's disease as a possible result of cross-talk between peripheral and central immune systems: The main inducers of glial NOX2 activation are two potent pro-inflammatory cytokines able to reach high levels in the AD brain, IL-1β, and tumor necrosis factor-α (Cervellati et al., 2020). Intriguingly, these two cytokines can derive from other reactive microglia and CNS cells, but also from peripheral immune cells. Relevant proofs of this cross-talk between the periphery and brain come from studies on humans and AD animal models (Cervellati et al., 2020; Bettcher et al., 2021). It has been reported indeed, the increased levels of IL-1β and tumor necrosis factor-α in blood, brain, and cerebrospinal fluid of AD patients. In addition, studies on AD-animal models gave insight into the underlying pathological mechanisms (Bettcher et al., 2021). It was shown that these and other cytokines possibly access to CNS by diffusion through neuroanatomical sites of increased BBB permeability (circumventricular organs) or by active transport (Cervellati et al., 2020). Thus, they appear to serve as a bidirectional bridge that allows peripheral–central immune communication

in AD. This also represents the most likely way by which systemic OxInflammation (resulting from several comorbidities, low-grade inflammation, and aging, **Figure 1**) spread to the brain (Cervellati et al., 2020). In addition, ROS/RNS generated in periphery may facilitate the cytokines-mediated cross-talk by damaging BBB.

Once activated, microglia and astrocytes release other cytokines that contribute to the expression of BBB endothelial adhesion molecules, that, in turn, can promote the entry of a higher amount of cytokines along with peripheral immune cells into the brain (Bettcher et al., 2021). BBB dysfunction, an early event in the disease, may represent the major mechanism by which neutrophils, monocytes, lymphocytes T and B, and natural killers infiltrate into the brain (Bettcher et al., 2021). In particular, peripheral T regulatory cells (Tregs), a subset of T lymphocytes, have been found in large amount in the AD brain. The exact mechanism linking the immune activity of these cells with AD pathology is not fully known, yet. However, there is intriguing evidence suggesting Tregs may contribute to Aβ formation and cognitive decline (Jevtic et al., 2017). For a detailed description of these process, the reader is referred to more focused reviews by Bettcher et al. (2021)

Convergent preclinical evidence suggests that infiltrating neutrophils, coming from both periphery and cerebral vessels, contribute to AD pathogenesis and cognitive impairment (Smyth et al., 2022). This contribution is at least partially exerted through MPO (which however is also produced by neurons and glial cells). Indeed, it has been shown that MPO deficiency improves cognitive impairment in the AD animal model (Smyth et al., 2022). This enzyme has been reported to localize to Aβ plaques and neurofibrillary tangles (Smyth et al., 2022), where participate in exacerbating the OxInflammatory cycle.

In conclusion, the OxInflammation phenomenon is a self-perpetuating misalignment of redox and immune homeostasis involved in a multitude of diseases, including AD. This condition starts as a subclinical event, preceding the appearance of the first signs of cognitive decline. Being a thriving source of biomarkers, the identification of this condition in preclinical AD patients could represent a possible way for an early diagnosis and a potential therapeutical target.

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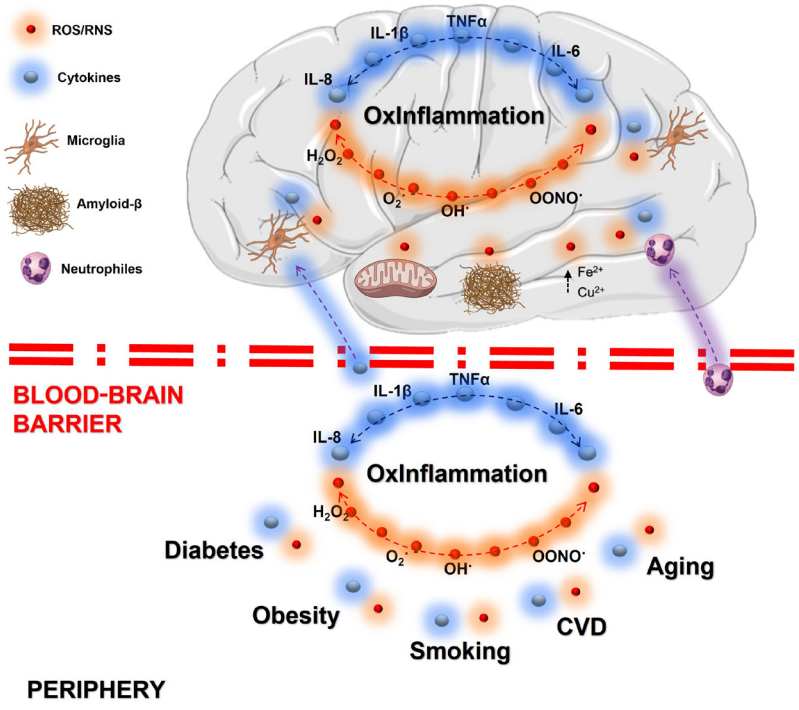

Figure 1 | **OxInflammation in AD: potential triggers.**
Oxinflammation in the AD brain can be triggered by dysregulation of redox homeostasis and immune system which can start locally and/or in the periphery. OxInfllamation vicious cycle in the brain can be initiated by various sources of reactive species and/or pro-inflammatory cytokines, such as reactive microglia, dysfunctional mitochondria, amyloid-β aggregates, and metal dyshomeostasis. Triggers of this condition can also come from the periphery, as result of a systemic OxInflammation, which. in turn, leads to the generation of the high amount of reactive species and pro-inflammatory cytokines. The latter, can cross the blood-brain barrier and enter the brain, where can activate resident microglia. Moreover, a damaged barrier can allow the brain infiltration of peripheral immune cells (such as the displayed neutrophils) that can exacerbate the OxInflammation process. AD: Alzheimer's disease; CVD: cardiovascular disease; H_2O_2: hydrogen peroxide; IL: interleukin.

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