

# Iron, the endolysosomal system and neuroinflammation: a matter of balance

Daniel A. Bórquez, Pamela J. Urrutia, Marco T. Núñez\*

Iron accumulation and neuroinflammation are cardinal features of many neurodegenerative diseases (Urrutia et al., 2021). However, the hierarchy in the pathogenic mechanisms and the molecular connections between these processes are still obscure. In this perspective, we will discuss current evidence showing that aging, the main risk factor for neurodegenerative diseases, negatively impacts lysosomal function by triggering a paradoxical condition of iron accumulation accompanied by functional iron deficiency. This condition is self-sustaining in the neuroinflammatory process.

**Iron and the endolysosomal system:** Iron in its reduced state mediates the non-enzymatic conversion of hydrogen peroxide to the highly reactive hydroxyl radical, which is associated with lipid peroxidation, protein oxidation and aggregation, and DNA damage. Conversely, iron is an essential cofactor in numerous physiological processes, including mitochondrial energy metabolism, hydroxylation reactions, DNA replication and myelination. Therefore, iron content and distribution at the subcellular level and between the several cell types present in the brain must be finely regulated to satisfy iron requirements while avoiding iron toxicity (Urrutia et al., 2021).

The three key steps in the cellular uptake of extracellular transferrin-bound iron depend on the acidic environment of the endolysosomal system: the dissociation of  $Fe^{3+}$  from transferrin; the subsequent reduction of  $Fe^{3+}$  to  $Fe^{2+}$  by the metalloreductase STEAP3, and finally the translocation of iron to the cytoplasm mediated by the divalent metal transporter 1. The endolysosomal system is also essential for the recycling of intracellular iron, through autophagy of iron-rich mitochondria (mitophagy) or of the iron storage protein ferritin (ferritinophagy) (Yambire et al., 2019).

At the cellular level, the endolysosomal system plays a key role in achieving an adequate supply of iron to the mitochondria and the cytoplasm, the main compartments where iron is required (Weber et al., 2020).

Interestingly, impaired endolysosomal acidification, a hallmark of aging (Colacurcio and Nixon, 2016), has been recently associated with iron dyshomeostasis and neuroinflammation *in vivo* (Halcrow et al., 2021). Pharmacological or genetic-targeting of the vacuolar (v)-ATPase, which that maintains the acidic pH inside the lysosomes, results in functional iron deficiency in both the cytoplasm and the mitochondria. In the cytoplasm, iron deficiency triggers the accumulation of the hypoxia inducible factor-1 $\alpha$ , whose degradation depends on an iron-dependent hydroxylation reaction. A decrease in the synthesis of deoxyribonucleotides triphosphate, catalyzed by a ribonucleotide reductase whose activity is dependent on iron, is also observed. Moreover, mitochondrial iron deficiency generates inefficiency in the electron transport chain, with lower oxygen consumption and higher generation of reactive oxygen species (ROS). Limited availability of cytosolic deoxyribonucleotides triphosphate disrupts mtDNA homeostasis and triggers a necrotic-like cell death mechanism. Furthermore, iron sequestration in lysosomes increases the expression of pro-inflammatory cytokines (like tumor necrosis factor- $\alpha$ ) and interferon-responsive genes in cultured cortical neurons, triggering inflammatory signatures in the brain *in vivo* (Yambire et al., 2019).

**Microglia in brain iron homeostasis:** The above results show an intimate connection between inadequate intracellular iron distribution, which results in functional iron deficiency, and the onset of neuroinflammatory processes.

Inflammatory responses are mainly mediated by microglia, the innate immune cells that reside in the central nervous system. Microglia, like macrophages, can polarize along a continuum between a detrimental (M1) and a beneficial (M2) state. Both phenotypes differ markedly in their iron homeostatic mechanisms (Urrutia et al., 2021). Iron loading triggers polarization towards the proinflammatory M1 state and switches the M2 phenotype into M1. These changes could represent an adaptive survival

mechanism developed by microglia exposed to high iron levels.

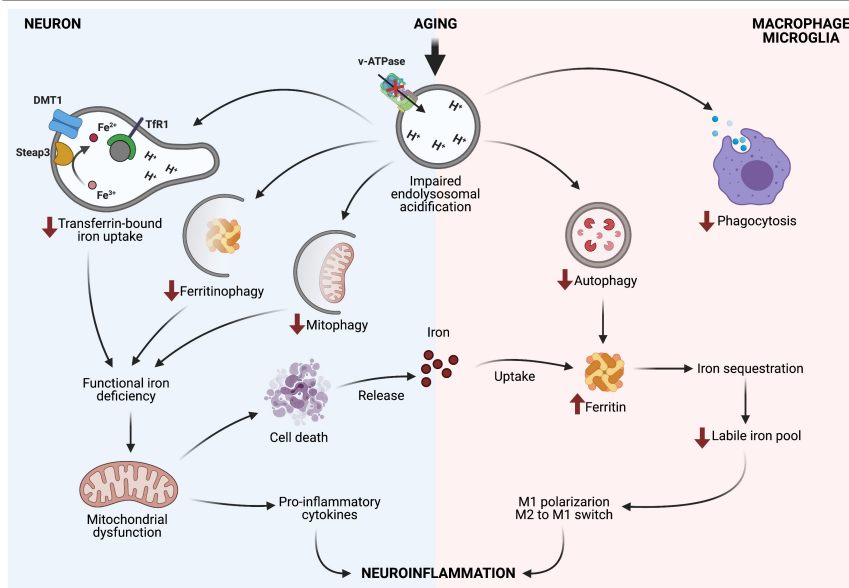
M1 macrophages/microglia have high levels of ferritin light chain, which help to maintain low levels of the cytosolic labile (redox-active) iron pool. Additionally, they support ATP production by glycolysis, reducing the ROS-producing mitochondrial energy metabolism. These changes confer resistance to ferroptosis, a ROS- and iron-dependent cell death process (Kapralov et al., 2020).

Recent evidence suggests that the microglia present in the brain of patients with neurodegenerative diseases contribute to sequester iron in a non-bioavailable form, generating the paradox of a functional iron deficiency in the presence of iron accumulation at the tissular level (Urrutia et al., 2021). Remarkably, like M1 microglia, senescent cells accumulate large amounts of intracellular iron stored in ferritin. Due to a defect in ferritinophagy, this iron cannot become bioavailable, and these cells turn resistant to ferroptosis (Masaldan et al., 2018).

Numerous studies indicate that a subpopulation of dystrophic microglia, characterized by high levels of L-ferritin, is present in the brains of patients with Alzheimer's disease and not in the brains of healthy aging people (Shahidehpour et al., 2021). Dystrophic microglia, which accumulate around amyloid plaques and tau-positive structures, are exhausted in their futile attempts to degrade protein aggregates and have decreased phagocytic capacity (Streit et al., 2009; Kenkhuis et al., 2021). Arguably, this decreased phagocytic ability could be due to decreased lysosomal acidification, would explain the prominent accumulation of ferritin in these patients.

**A link between iron dysregulation, endolysosomal acidification and inflammation:** Based on the evidence discussed above, we propose a model that links iron dysregulation, both in neurons and microglia, with endolysosomal acidification, mitochondrial dysfunction and neuroinflammation (**Figure 1**).

As stated above, iron dyshomeostasis in the neurodegenerative aging brain not only involve iron accumulation, but also its abnormal distribution at the subcellular and tissular levels. As iron becomes unavailable, several mechanisms must be activated to ensure new iron entry into the brain, worsening the iron load. Hence, treatments that help to redistribute iron making it available for cellular processes, as low affinity iron chelators (Sohn et al., 2008), could be suitable.



**Figure 1 | Aging- and disease-associated endolysosomal malfunction generates functional iron deficiency associated with iron accumulation and neuroinflammation.**

Impaired endolysosomal acidification is a cellular hallmark of aging and profoundly impacts neuronal iron homeostasis. The failure to obtain iron from the extracellular environment, added to a deficient intracellular iron recycling from mitochondria and ferritin, both dependent on acidic lysosomal pH, trigger mitochondrial dysfunction, a proinflammatory transcriptional signature and neuronal death. In dystrophic or senescent microglia, the same endolysosomal defect support phagocytosis and autophagy impairment, associated with iron sequestration in ferritin and conversion into a detrimental M1 state. Created with BioRender.com.

Lysosomal dysfunction results in a deterioration of mitochondrial functions and oxidative stress; hence, the existence of a lysosome-mitochondrial axis with a functional role in cell senescence has been proposed. Thus, enhancing the functioning of lysosomes, for example through the activation of the master regulator of lysosomal homeostasis, the transcription factor TFEB (Bajaj et al., 2019), would offer a therapeutic alternative that targets the founding problem.

The model depicted in **Figure 1** may have profound implications for the pathophysiology of neurodegenerative diseases and foster future therapeutic developments.

*This work was supported by Fondo de Enlace UDP 2021 (to DAB) and FONDECYT Initiation in Research, No. 11201141 (to PJU).*

**Daniel A. Bórquez, Pamela J. Urrutia, Marco T. Núñez\***

Center for Biomedical Research, Faculty of Medicine, Universidad Diego Portales, Santiago, Chile (Bórquez DA)

Department of Biology, Faculty of Sciences, Universidad de Chile, Santiago, Chile (Urrutia PJ, Núñez MT)

\*Correspondence to: Marco T. Núñez, PhD, mnunez@uchile.cl.

<https://orcid.org/0000-0002-1967-8570> (Marco T. Núñez)

**Date of submission:** April 28, 2021

**Date of decision:** June 17, 2021

**Date of acceptance:** July 13, 2021

**Date of web publication:** September 17, 2021

<https://doi.org/10.4103/1673-5374.324847>

**How to cite this article:** Bórquez DA, Urrutia PJ, Núñez MT (2022) Iron, the endolysosomal system and neuroinflammation: a matter of balance. *Neural Regen Res* 17(5):1003-1004.

**Copyright license agreement:** The Copyright License Agreement has been signed by all authors before publication.

**Plagiarism check:** Checked twice by iThenticate.

**Peer review:** Externally peer reviewed.

**Open access statement:** This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

**Open peer reviewer:** Nuno Raimundo, Penn State College of Medicine, USA.

## References

Bajaj L, Lotfi P, Pal R, Ronza AD, Sharma J, Sardiello M (2019) Lysosome biogenesis in health and disease. *J Neurochem* 148:573-589.

Colacurcio DJ, Nixon RA (2016) Disorders of lysosomal acidification-The emerging role of v-ATPase in aging and neurodegenerative disease. *Ageing Res Rev* 32:75-88.

Halcrow PW, Lakpa KL, Khan N, Afghah Z, Miller N, Datta G, Chen X, Geiger JD (2021) HIV-1 gp120-induced endolysosome de-acidification leads to efflux of endolysosome iron, and increases in mitochondrial iron and reactive oxygen species. *J Neuroimmune Pharmacol* doi: 10.1007/s11481-021-09995-2.

Kapralov AA, Yang Q, Dar HH, Tyurina YY, Anthony-muthu TS, Kim R, St Croix CM, Mikulska-Ruminska K, Liu B, Shrivastava IH, Tyurin VA, Ting HC, Wu YL, Gao Y, Shurin GV, Artyukhova MA, Ponomareva LA, Timashev PS, Domingues RM, Stoyanovsky DA (2020) Redox lipid reprogramming commands susceptibility of macrophages and microglia to ferroptotic death. *Nat Chem Biol* 16:278-290.

Kenkhuis B, Somarakis A, de Haan L, Dzyubachyk O, ME IJ, de Miranda N, Lelieveldt BPF, Dijkstra J, van Roon-Mom WMC, Höllt T, van der Weerd L (2021) Iron loading is a prominent feature of activated microglia in Alzheimer's disease patients. *Acta Neuropathol Commun* 9:27.

Masaldan S, Clatworthy SAS, Gamell C, Meggyesy PM, Rigopoulos AT, Haupt S, Haupt Y, Denoyer D, Adlard PA, Bush AI, Cater MA (2018) Iron accumulation in senescent cells is coupled with impaired ferritinophagy and inhibition of ferroptosis. *Redox Biol* 14:100-115.

Shahidehpour RK, Higdon RE, Crawford NG, Neltner JH, Ighodaro ET, Patel E, Price D, Nelson PT, Bachstetter AD (2021) Dystrophic microglia are associated with neurodegenerative disease and not healthy aging in the human brain. *Neurobiol Aging* 99:19-27.

Sohn YS, Breuer W, Munnich A, Cabantchik ZI (2008) Redistribution of accumulated cell iron: a modality of chelation with therapeutic implications. *Blood* 111:1690-1699.

Streit WJ, Braak H, Xue QS, Bechmann I (2009) Dystrophic (senescent) rather than activated microglial cells are associated with tau pathology and likely precede neurodegeneration in Alzheimer's disease. *Acta Neuropathol* 118:475-485.

Urrutia PJ, Bórquez DA, Núñez MT (2021) Inflaming the Brain with Iron. *Antioxidants (Basel)* 10:61.

Weber RA, Yen FS, Nicholson SPV, Alwaseem H, Bayraktar EC, Alam M, Timson RC, La K, Abu-Remaileh M, Molina H, Birsoy K (2020) Maintaining Iron homeostasis is the key role of lysosomal acidity for cell proliferation. *Mol Cell* 77:645-655.e7.

Yambire KF, Rostosky C, Watanabe T, Pacheu-Grau D, Torres-Odio S, Sanchez-Guerrero A, Senderovich O, Meyron-Holtz EG, Milosevic I, Frahm J, West AP, Raimundo N (2019) Impaired lysosomal acidification triggers iron deficiency and inflammation in vivo. *Elife* 8:e51031.

*P-Reviewer: Raimundo N; C-Editors: Zhao M, Liu WJ, Wang L; T-Editor: Jia Y*