Amyotrophic lateral sclerosis, a neurodegenerative motor neuron disease with retinal involvement

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that damages the motor neurons, the spinal cord, the cerebellum, and some areas of the brain. However, more recent studies show that it can also affect the visual system, for example, through oculomotor and visual pathways. ALS patients do not usually complain of visual problems, so studies focusing on the visual pathway are scarce. Early work on ALS and the eye was related to oculomotor function and visual pathway analysis, with visually evoked potentials being used to study the disease. Subsequently, some works have appeared that analyze visual function, with tests such as visual acuity, visual field, and contrast sensitivity. Furthermore, in neurodegenerative diseases, it is observed that the changes that occur in the brain also occur in the retina, with this nervous tissue being considered as a "window to the brain" (MacCormick et al., 2015). The changes in the retina can be detected by a widely used diagnostic test in ophthalmology, namely optical coherence tomography (OCT). Recently, this technique has been used for the analysis of the retinal and optic nerve changes that occur in various neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, and even ALS, and can serve to help in their diagnosis and follow-up (Salobrar-García et al., 2016; Rojas et al., 2019). In the retina of ALS patients, OCT has demonstrated retinal thinning in the peripapillary retinal nerve fiber layer, inner nuclear layer, and outer nuclear layer, which may be related to neurodegenerative processes (Volpe et al., 2015; Rohani et al., 2018; Rojas et al., 2019). This fact has been confirmed by histopathological studies performed by Volpe et al. on retinas of ALS patients and they observed a loss of retinal ganglion cell axons, which would explain the macular thinning observed by OCT (Volpe et al., 2015). In addition, a study in early spinal-onset ALS patients without ocular disease demonstrated retinal thickening, through significant increases in the macular thickness in the temporal and inferior areas of the inner macular ring, suggesting that the thickening may be due to microglial activation during the neuroinflammatory process (Rojas et al., 2019).

Among the pathogenic mechanisms of neurodegenerative diseases (e.g., Alzheimer's disease and Parkinson's disease, glaucoma), neuroinflammation plays an important role (Ramirez et al., 2017). In the inflammatory process of the nervous system, microglial cells are activated in response to damage, undergoing morphological changes, and proliferating and migrating. In addition, activated microglia can exist within a continuum between two different activation extremes, namely M1 and M2 phenotypes. The M1, or proinflammatory, phenotype is characterized by the release of inflammatory mediators such as nitric oxide and reactive oxygen species, as well as proinflammatory cytokines, including interferon gamma, tumor necrosis factor-α, interleukin (IL)-1β, IL-6, and IL-12. All of these factors produce an intense inflammatory response, which, if chronic, can lead to neuronal death. However, the M2 phenotype produces the opposite effect, releasing neurotrophic factors such as brain-derived neurotrophic factor, neurotrophins, and glial cell derived neurotrophic factor, as well as anti-inflammatory cytokines, mainly IL-4, IL-10, IL-13, and TGF-β, which could control the inflammatory process, contributing to neuronal survival (Ramirez et al., 2017).

As in other neurodegenerative diseases, the activation of microglial cells has also been observed in ALS, which could induce alterations in motor neurons. This has been found both in motor cortex of ALS patients and in animal models of ALS (superoxide dismutase 1 (SOD1) mutant mice (Zhang et al., 2018; Jara et al., 2019). In motor neuron cultures, it has been observed that the administration of exogenous mSOD1(G93A) does not cause motor neuron death. However, when it is added to microglia cultures, it causes the activation of these cells by promoting the release of free radicals and proinflammatory cytokines (Zhao et al., 2010). Furthermore, when both cells are cultured together (motor neurons and microglia), exogenous mSOD1(G93A) causes alterations in the motor neurons, which would indicate that exogenous mSOD1(G93A) is only detrimental to motor neurons in the presence of activated microglia (Zhao et al., 2010). In addition, in murine transgenic models of ALS (SOD1), it has been observed that the microglia undergo several phenotypic changes during disease progression. In pre-symptomatic stages, the microglia express anti-inflammatory phenotypic markers of the M2 type, but, when the disease progresses, the main phenotype is M1 or inflammatory–neurotoxic, which promotes neuronal death (Geloso et al., 2017).

The activation of microglial cells in retinas with ALS has been poorly studied. There are only three works on this topic, and they are performed in different animal models of ALS (Ringer et al., 2017; Cho et al., 2019; Rojas et al., 2021). In the mouse model of ALS, devoid of ran2-binding protein (Ranbp2), activation of $CD11b^+$ and $CD45^+$ microglial cells was observed. In addition, an increase in the number of $F4/80^+$ microglia and the presence of amoeboid microglial forms were also found (Cho et al., 2019). In the SOD1G93A mouse model, using anti-Iba-1 (microglia-specific marker) in the retinal sections, Ringer et al. (2017) found no activation of microglial cells in either the early or late stages of the disease. However, in a recently published study in the SOD1G93A mouse model at an advanced time of disease (120 days), signs of microglial activation were found. The difference between this study with respect to the one mentioned above is that it was performed on retinal whole-mounts, which allow for a more detailed morphological study of microglial cells. Microglial changes were recorded in the main retinal layers where the microglia were located, such as the outer plexiform layer and the inner retinal layer complex (comprising the inner plexiform layer and the nerve fiber layer–ganglion cell layer). These changes were characterized by a significant increase in the area of microglial arborization, a significant increase in cell area, the appearance of cells with retracted processes, and cell displacements and clustering (**Figure 1**; Rojas et al., 2021). Furthermore, in this study, microglial cells were shown to be of the M1 activation phenotype, as they expressed interferon gamma and IL-1β, which are typical markers of the M1 (proinflammatory-cytotoxic) response (**Figure 1**), while they were not labeled with antibodies typical of the M2 (anti-inflammatory-neuroprotective) response, such as arginase-I and IL-10 (Rojas et al., 2021). In addition, the loss of retinal ganglion cells was also observed in these animals (**Figure 1**), consistent with the damage observed in these cells in other ALS models (Volpe et al., 2015; Cho et al., 2019). All this would indicate that not only motor neurons, but also retinal neurons, are affected by ALS (Rojas et al., 2021). The results of this study demonstrate that, in the SOD1G93A ALS model, at an advanced stage of the disease, the microglial cells are of the proinflammatory-cytotoxic M1 phenotype,

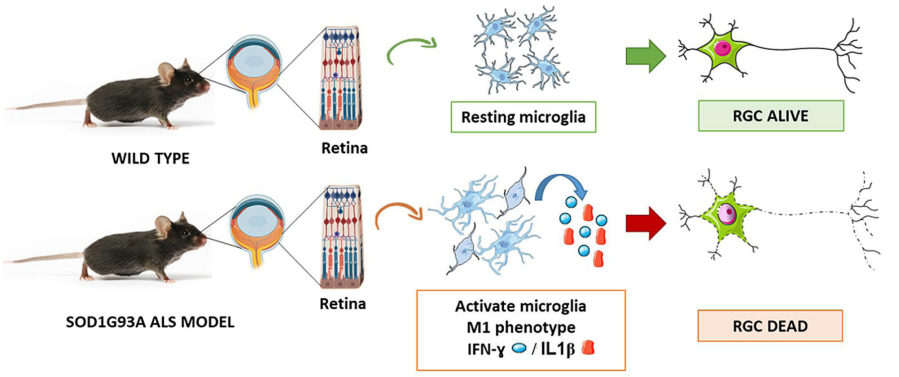

Figure 1 | **Main changes observed in microglial cells and RGC in the ALS model SOD1G93A at a very advanced stage of the disease (120 days).**
ALS: Amyotrophic lateral sclerosis; IFN-γ: interferon gamma; IL-1β: interleukin-1β; RGC: retinal ganglion cells.

which could induce neuronal death, as evidenced by the loss of retinal ganglion cells (**Figure 1**). This would be in line with the findings with regard to the spinal cord, where microglia in the late stages of the disease are present in an M1 phenotype (Liao et al., 2012), thus indicating that the changes occurring at the spinal cord or brain level are occurring in parallel in the retina.

Conclusion: In this work, we showed that ALS is a neurodegenerative disease that not only damages motor neurons but also affects retinal tissue, with microglial activation and loss of retinal ganglion cells. These retinal changes have been recorded by OCT in patients with ALS and could serve as biomarkers that could help in the diagnosis and follow-up of ALS patients, as well as to test the efficacy of different treatments by monitoring them in a simple way using an easy-to-use, non-invasive, and inexpensive technique, such as OCT.

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