PRO- AND ANTI-INFLAMMATORY STATE OF MICROGLIA IS AFFECTED BY THE PARTIAL DELETION OF METABOTROPIC GLUTAMATE RECEPTOR TYPE 5IN SOD1G93A MICE DURING DISEASE PROGRESSION

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Introduction: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that results from the selective death of upper and lower motor neurons (MNs). Despite its aetiology is not completely understood, it is known that ALS is a multifactorial disease, in which different causes concur to the disease progression. Glutamate(Glu)-mediated excitotoxicity is considered one major cause for MN dysfunction and degeneration. In particular, in an our previous study we demonstrated that the partial deletion of the subtype 5of metabotropic Glu receptors (mGluR5) in mice carrying the $SOD1^{G93A}$ mutation, the most widely used animal model for human ALS, obtaining the double mutant $SOD1^{G93A}mGluR5^{+/-}$ mouse, produced delay of the disease onset, increase of survival probability, amelioration of clinical symptoms, and normalization of cellular and biochemical readout of the pathology. ALS is also a multicellular disease, in which motor neuron death is the ultimate clinical cause, although astrocytes and microglia play an important role, for instance by promoting an inflammatory environment contributing to motor neuron damage. Founding on this setting, the aim of this study was to investigate whether dampening the mGluR5in $SOD1^{G93A}$ mice could affect microglia phenotype.

Materials and methods: Microglia was acutely purified by Percoll gradient (75%-25% in PBS) from total brain, motor cortex and spinal cord of WT, $SOD1^{G93A}$ and $SOD1^{G93A}mGluR5^{+/-}$ mice. The balance between the pro-inflammatory (M1) and the protective (M2) microglia phenotype was investigated at different stages of the disease (30, 90 and 120 days, representing the pre-, early-, and late-symptomatic stage, respectively) by flow cytometry. Microglia was stained with the specific microglia marker TMEM 119by means of a Alexafluor488-labelled rabbit monoclonal antibody. The M1phenotype was characterized by using a phycoerythrin-labelled anti-CD86(B7-2) mouse monoclonal antibody (GL1), while the M2phenotype was characterized by using an allophycocyanin-labelled anti-CD206(MMR) mouse polyclonal antibody. Expression of mGluR5in total brain, motor cortex and spinal cord of WT, $SOD1^{G93A}$ and $SOD1^{G93A}mGluR5^{+/-}$ mice was measured by Western blot using an anti-mGluR5rabbit polyclonal antibody.

Results: No alterations of the balance between M1and M2phenotype were observed in $SOD1^{G93A}$ when compared to WT mouse microglia from total brain and motor cortex, even at the late stage of the disease. On the contrary, M1polarization prevailed in microglia isolated from 120 day-old $SOD1^{G93A}$ mouse spinal cord; whereas, no significant modifications were observed at the pre-symptomatic (30 days) stage or at the onset (90 days) of the disease symptoms. To verify the impact of the reduction of mGluR5on the microglia phenotype, we compared WT and $SOD1^{G93A}$ mouse microglia to that purified from $SOD1^{G93A}mGluR5^{+/-}$ mice at the same time point. The results showed that pro-inflammatory microglia polarization was greatly increased in the spinal cord of 120 day-old $SOD1^{G93A}mGluR5^{+/-}$ respect to both WT and $SOD1^{G93A}$ mice; whereas, no differences were present in total brain and motor cortex. Western Blot analysis proved that mGluR5was overexpressed (about 100% increase) in microglia from 120 day-old $SOD1^{G93A}$ mouse spinal cord and much less so in the brain.

Discussion and conclusion: Our data show that the partial ablation of mGluR5in $SOD1^{G93A}$ mice drives microglia toward a pro-inflammatory phenotype, at least in spinal cord, and suggest that mGluR5sustains the anti-inflammatory state of this cells. Therefore, the previously observed in-vivo disease amelioration in double mutant $SOD1^{G93A}$mGluR5+/- mice, does not seem to be supported by a protective shift of microglia phenotype.