NG2, expressed by immune and neural cells, displays multiple roles in development of Experimental Autoimmune Encephalomyelitis

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INTRODUCTION AND OBJECTIVES

Nerve/glial-antigen 2 (NG2) is expressed in the central nervous system (CNS) on oligodendrocyte precursor cells (OPCs), involved in myelination and remyelination [1], and on pericytes, implicated in the maintenance of blood-brain barrier (BBB) integrity [2]. In the periphery, NG2 was shown to be expressed on macrophages and increased expression of NG2 is apparently also associated with activated microglia during neuroinflammation [3]. This project aims at investigating the role of NG2 in the development of experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis, through the utilization of a transgenic knockout (NG2KO) mouse. Our objectives are therefore to:

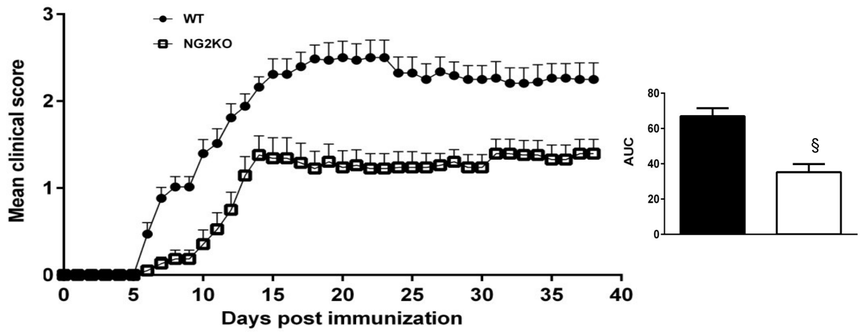
1. Monitor differences in EAE in NG2KO vs naïve mice at both clinical and neuropathological levels;
2. Investigate the cyto-architecture of NG2KO CNS in naïve and EAE-affected mice at the histological level, focusing on the BBB;
3. Evaluate the role of NG2 in the immune response in naïve and EAE-affected mice.

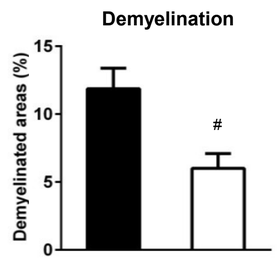
METHODS

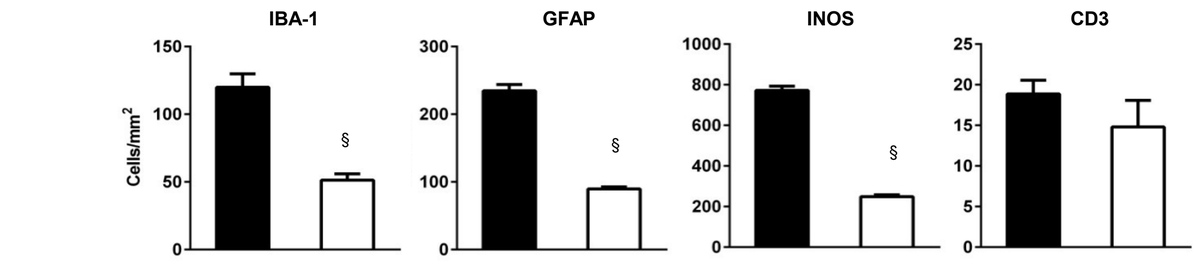
Chronic EAE was induced in female C57Bl/6J wild-type (WT) and NG2KO mice (6–8 weeks of age) by subcutaneous immunization with MOG 35-55 (myelin oligodendrocyte glycoprotein) peptide (200 μg) supplemented with *Mycobacterium tuberculosis* (300 μg) and pertussis toxin i.v.. Dendritic cells (DCs) were obtained from bone marrow cells upon stimulation with GM-CSF (20ng/ml) and intracellular cytokine staining was performed on CD11c-positive cells using conjugated anti-IL12. To create bone marrow chimera, recipient mice were lethally irradiated (9.5 Gy) and transplanted with 5 x 10^6 syngeneic donor whole bone marrow (BM) cells.

RESULTS

NG2KO mice develop milder EAE with decreased demyelination and inflammation





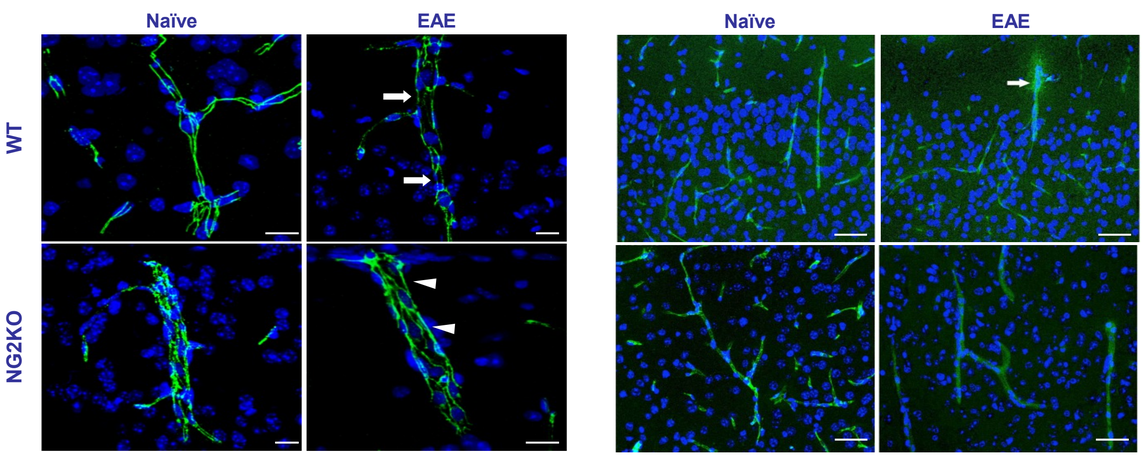


WT
NG2 KO

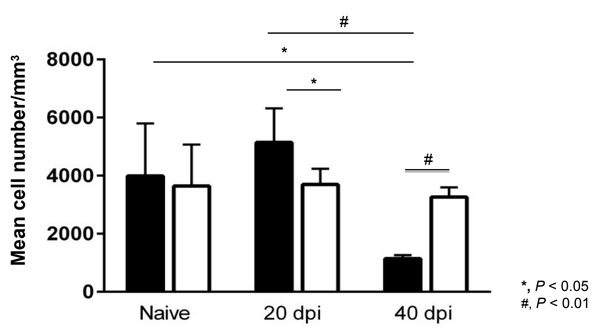
*, $P < 0.05$
#, $P < 0.01$
§, $P < 0,0001$

Effect of NG2KO at CNS level

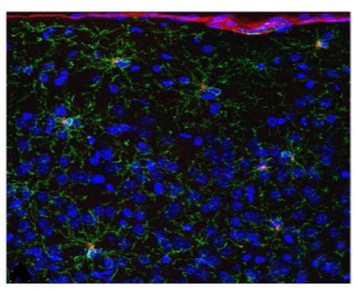
NG2KO affects the organization of the BBB



In contrast to WT, the number of OPCs in NG2KO mice does not change throughout EAE



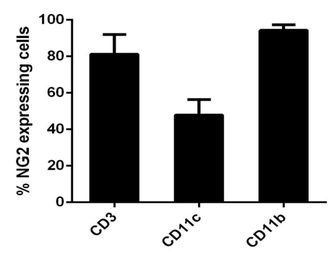
*, $P < 0.05$
#, $P < 0.01$



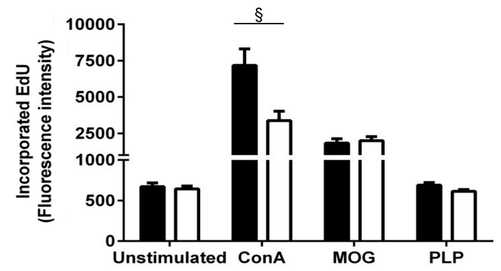
OPCs were quantified as per their expression of PDGFrα

Effect of NG2KO at immune system level

In WT mice, NG2 is also expressed in immune cells

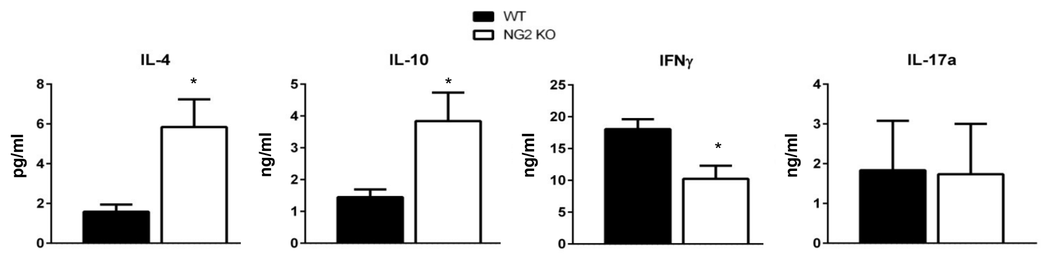


Ex vivo proliferative response to MOG does not differ between WT and NG2 KO mice



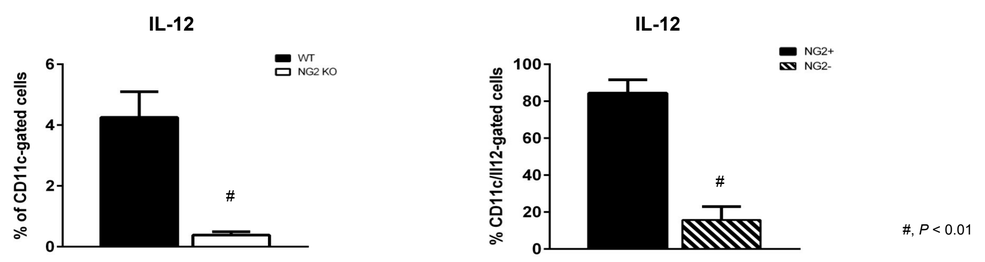
§, $P < 0.0001$

Ex vivo cytokine response of lymph node cells to MOG suggests a less inflammatory profile of MOG-specific T cells from NG2KO mice



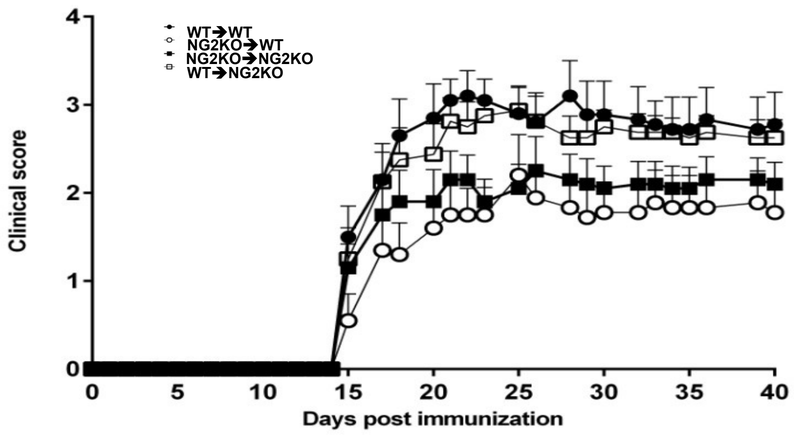
*, $P < 0,05$

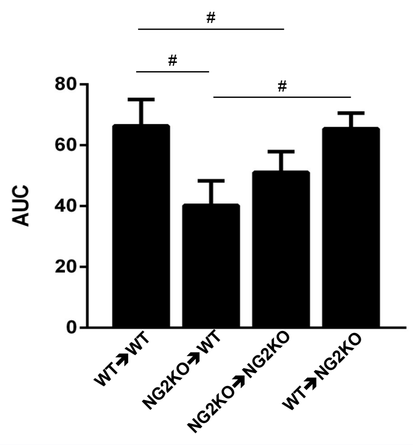
MOG-primed NG2KO dendritic cells express lower amounts of IL-12 and in WT mice the proportion of IL-12-expressing cells is lower in CD11c+ cells that do not also express NG2.

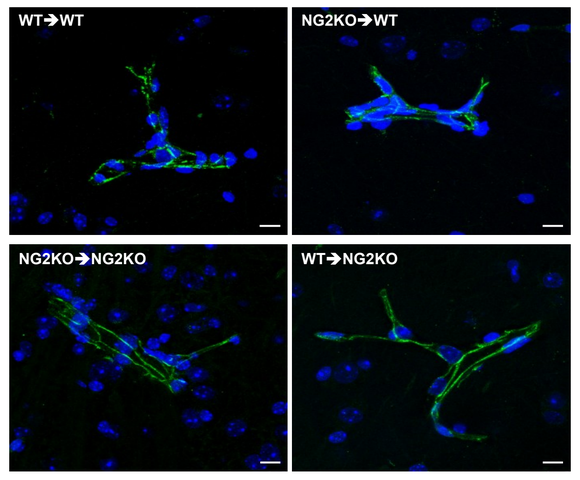


#, $P < 0.01$

Chimera mice reconstituted with NG2KO BM cells (NG2KO➔WT and NG2KO➔NG2KO) develop a less severe EAE than mice reconstituted with WT BM cells (WT➔NG2KO and WT➔WT)







The BBB status remains that of the inherent mouse genotype regardles of the BM cell origin

CONCLUSIONS

We suggest that the milder EAE in NG2KO mice is not due to a functional defect in T cells per se, but results mainly from a skewed T-cell response likely due to less inflammatory NG2KO DCs, in combination with a less leaky BBB and an apparently reduced proliferation or apoptosis of OPCs in NG2KO mice, or their increased differentiation to pre-myelinating oligodendrocytes.

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1. *Nishiyama, A., NG2 cells in the brain: a novel glial cell population. Hum Cell, 2001. 14(1): p. 77-82.* ***2***. *Muramatsu, R. and T. Yamashita, Pericyte function in the physiological central nervous system. Neurosci Res, 2014.* ***3***. *Zhu, L., et al., Induced NG2 expressing microglia in the facial motor nucleus after facial nerve axotomy. Neuroscience, 2010. 166(3): p. 842-51. The authors declare no conflict of interest.* *This project was supported by FISM (Fondazione Italiana Sclerosi Multipla), Grant nr. 2001/R/37.*