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Microglial-axon contacts at the Node of Ranvier in Multiple Sclerosis and EAE

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Background

Microglia (MG), the resident immune cells of the central nervous system, are major modulators of myelination and remyelination processes (Miron et al, 2013, Wlodarczyk et al, 2017). **The nodes of Ranvier (NR)**, small excitable domains allowing the regeneration of action potentials along myelinated axons, are **affected early in Multiple Sclerosis (MS)** and can recluster prior to remyelination.

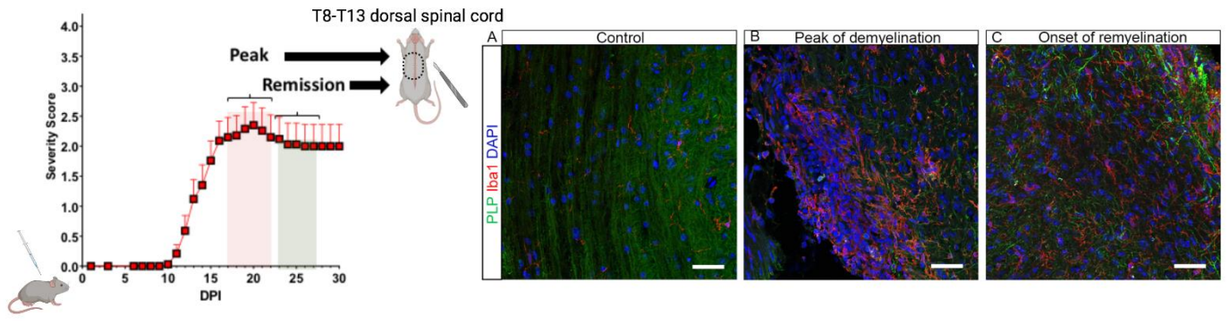
Our recent work in a lysophosphatidylcholine (LPC) model of focal demyelination in mice showed both *in vivo* and *ex-vivo* that **MG-NR interaction is altered in normal appearing tissue** surrounding the demyelinating lesion **and is increased during remyelination** compared to control condition. **Disrupting this interaction after demyelination further leads to a decrease of pro-regenerative MG and to impaired remyelination** (Ronzano, Roux et al, 2020).

Objective

Further explore **the impact of adaptative immunity on MG-NR interaction** in order to account for the inflammatory component of MS using an inflammatory animal model of demyelination, the experimental autoimmune encephalomyelitis (EAE), as well as study various hemispheric brain MS lesions.

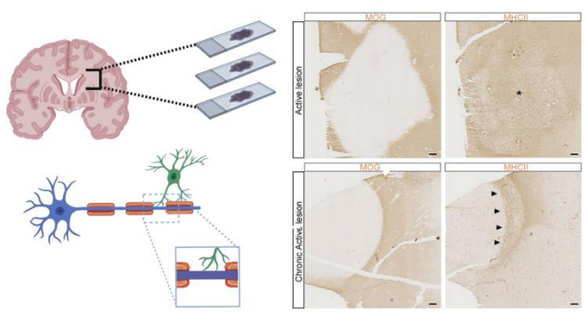
Experimental Design

I – *In vivo* evaluation of microglial and monocyte derived macrophage interaction with the node contact during MOG35-55 induced EAE



MOG-induced EAE in C56BL6/J mice (n=26) and control (n=14) littermates were followed up daily for a month. Interactions of resident MG (TMEM119+) and infiltrating monocyte derived macrophages (MDM, Iba1+/TMEM119-) with the was assessed by immunostainings of fixed mouse dorsal spinal cord in (A) control, (B) during the peak of disease severity (DPI17) and (C) at onset of remission (DPI21). Scale bars : 20 µm.

II – Evaluation of axo-microglial contact in human post mortem MS tissue



We used hemispheric human brain snap frozen tissue of MS patients (n=8) and controls (n=5). Lesion characterization was done using MOG or MHCII staining associated with hematoxylin coloration. We proceeded in analyzing the axo-microglial interaction in resident (TMEM119+), homeostatic (P2Y12r+) and activated MG-macrophages using pro-inflammatory (iNOS, p22phox) and pro-regenerative (CCL22, CD206) markers in various MS lesions. Scale bars : 500 µm.

Results

1. Dynamic changes of microglial cells and MDM during EAE

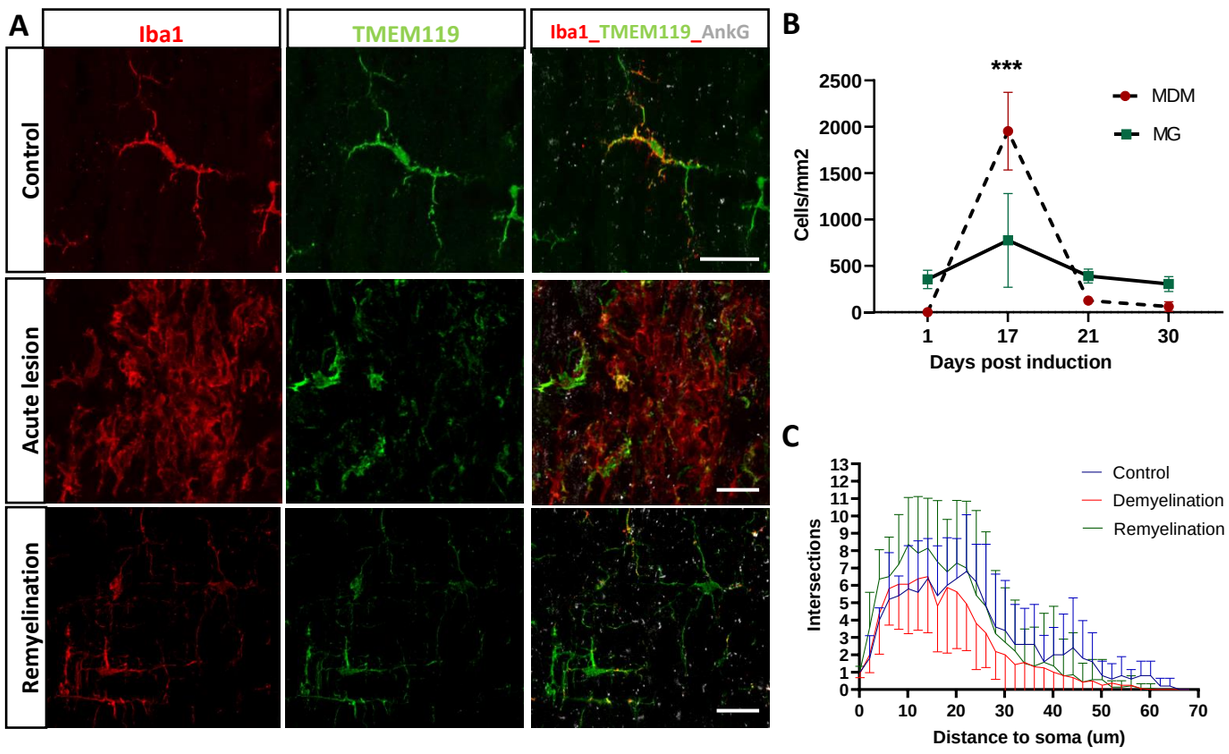


Figure 1. (A) Immunostainings of control and EAE induced C57BL6/J dorsal spinal cord during acute inflammation and at onset of remyelination. During remyelination, as in control conditions, most Iba1+ cells (red) express TMEM119 (green), indicating resident MG cells. At the peak of the disease, acute lesions contain mainly MDM cells (Iba1+/TMEM119-) while MG cells (Iba1+/TMEM119+) are found perilesionally. Scale bar : 10 µm. (B) Mean MG and MDM density. MDM infiltration peaks at D17 while MG numbers are only slightly modified during the course of the disease. (C) Sholl analysis of MG morphology. Return to homeostatic state during remyelination. Statistical analysis : Two way ANOVA with Tukey post-hoc test, ***p<0.001.

2. During EAE both microglial cells and MDM interact with the node

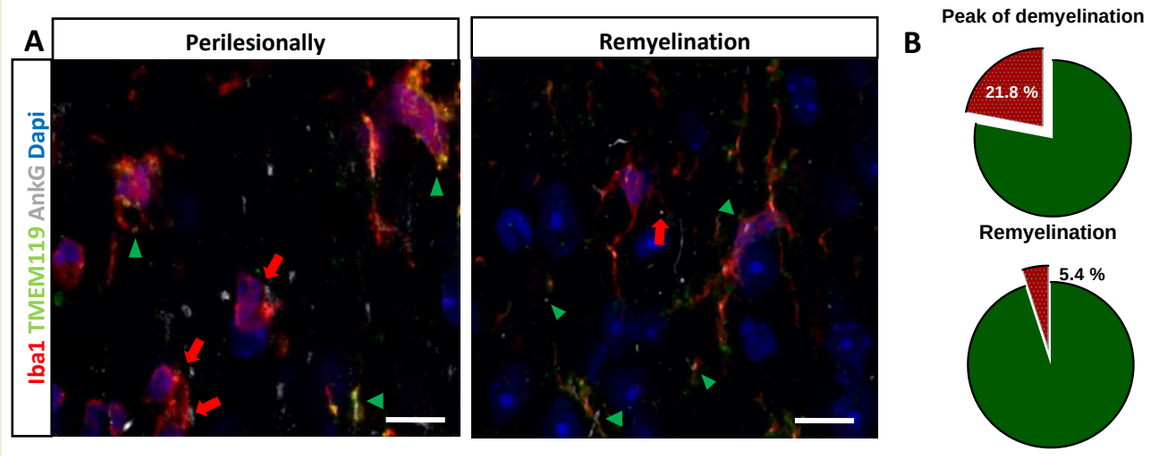


Figure 2. (A) Immunostainings of EAE induced C57BL6/J mice. The nodes of Ranvier (AnkG, grey) are contacted by both resident MG cells (TMEM119+/Iba1+, green arrow heads), and MDMs (TMEM119-/Iba1+, red arrows). Scale bar : 10 µm. (B) Chart pie representing the proportion of nodes contacted by MG cells (green) and MDM (red) at peak (perilesionally) and during remyelination.

3. Microglia-node interaction is enhanced during remyelination and correlates with clinical recovery

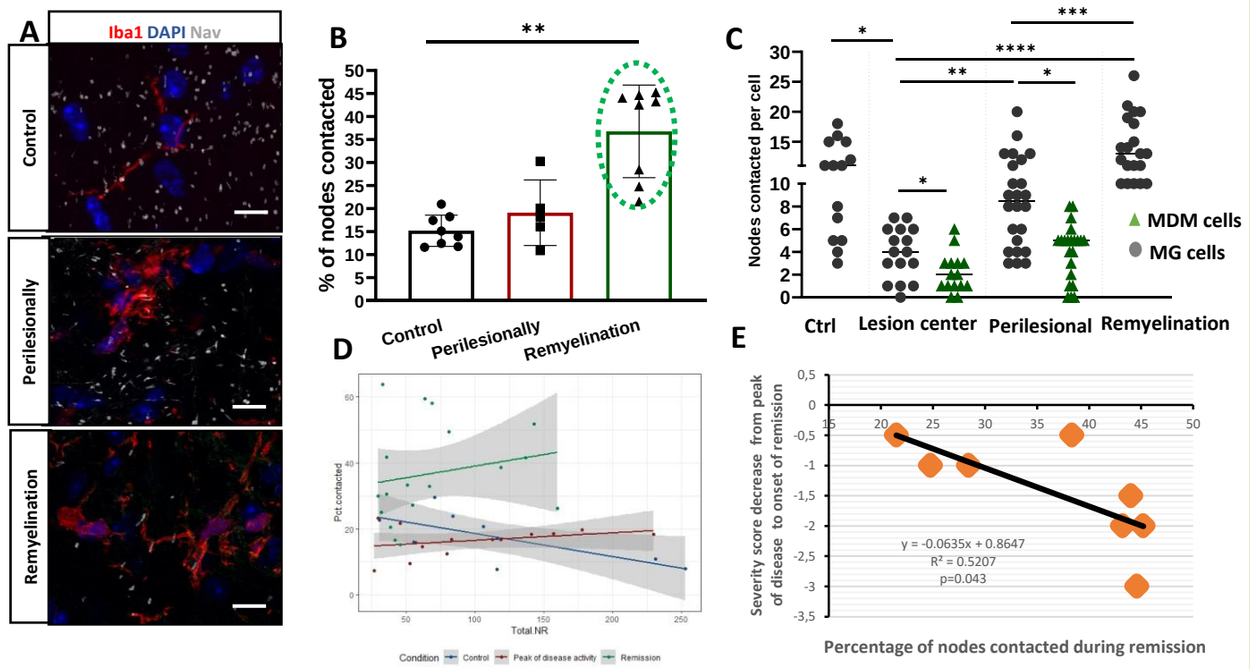


Figure 3. (A) Immunostainings of control and EAE mice showing interactions of microglia/MDM cells with the nodes. (B) Percentage of nodes contacted. (C) Number of nodes contacted by individual MG (grey) and MDM (green) cells. (D) Multivariate linear regression model of the percentage of nodes contacted corrected by the number of microglial cells to the total nodal density in the area analyzed. (E) Univariate linear regression model of the percentage of nodes contacted by microglial cells during remyelination and the change of EAE Severity Score from peak of disease activity to remission. **During remyelination MG-NR interaction is reinforced independently of nodal and microglial density and correlates with clinical recovery status.** Statistical analysis for A and B: ANOVA with Tukey post-hoc test, *p<0.05, **p<0.01, ***p<0.001.

4. Increased microglial-node interaction is observed in shadow plaques in MS

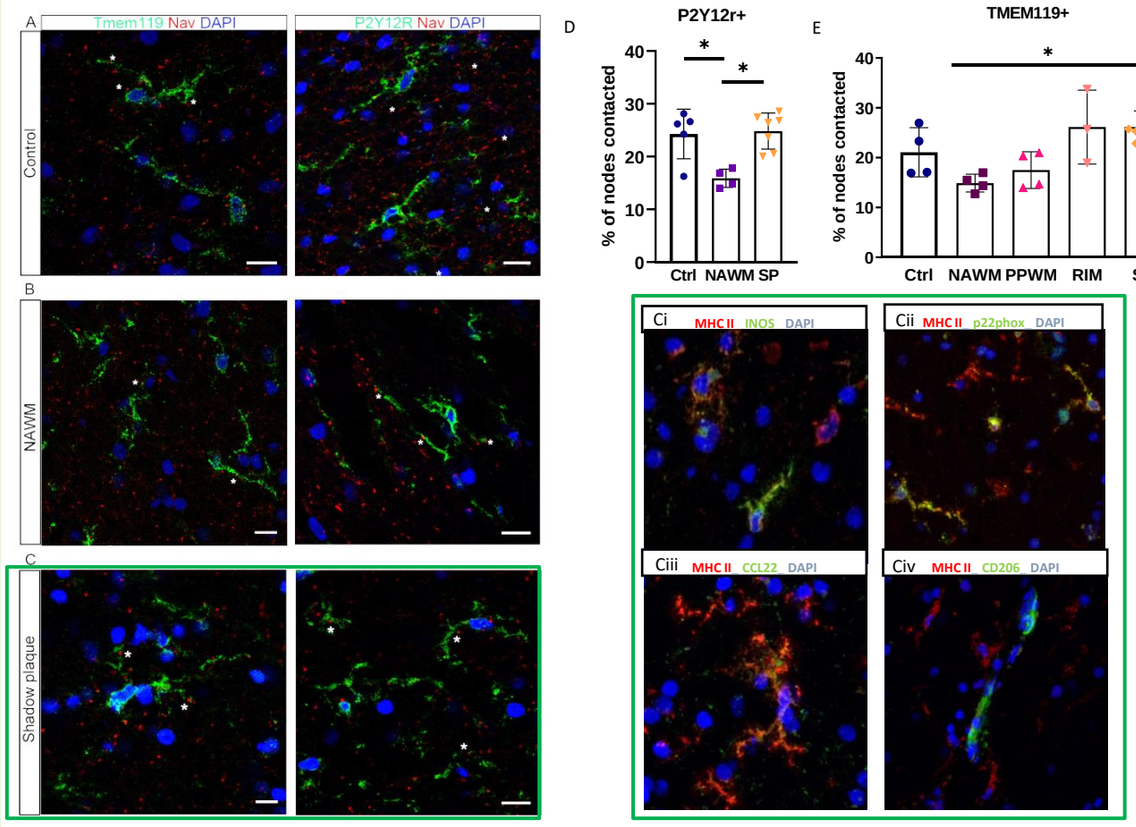


Figure 4. Resident MG cells (TMEM119+, green) and homeostatic MG cells (P2Y12r+, green) contact nodes of Ranvier (Nav, red) in (A) control, (B) NAWM and (C) shadow plaques (asterisks). Characterisation of MG phenotype in shadow plaques using pro-inflammatory (Ci, INOS; Cii p22phox) and pro-regenerative markers (Ciii, CCL22; Civ CD206). Scale bars 10 µm. (D) Diagrams showing the percentage of contacted nodes of Ranvier by P2Y12r+ cells and (E) TMEM119+ cells. One-way ANOVA with Tukey's post-hoc comparison tests, *p<0.05.

Conclusions

- During EAE, MDMs engage the node at peak of the disease while MGs are the main contacting cells during remission.
- Compared to control condition, microglial cells retract their processes and disengage from the nodes during inflammation.
- During repair, the microglial-node interaction is enhanced, even though microglial cell number, morphology and nodal density is restaured. Similar observations are made in shadow plaques of human MS patients.
- Increased microglial-node contact in repair correlates with a better clinical outcome in EAE mice.

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