Immunophenotyping and Characterization of PLP and MOG Induced Relapsing/Remitting and Chronic Mouse Models of Experimental Autoimmune Encephalomyelitis

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1 OVERVIEW

Multiple sclerosis (MS) is an autoimmune disease causing a wide range of symptoms by demyelination in central nervous system (CNS). To study mechanisms of MS by using preclinical in vivo models, one of the most used one is MOG_{35-55} induced experimental autoimmune encephalomyelitis (EAE) in mice. EAE is an inflammatory demyelinating disease of the central nervous system characterized by infiltration of immune cells into the CNS and subsequent demyelination by activated myelin-specific T-cells. During the disease progression weakening of animals is seen, which requires visual daily scoring based on the observed manifestations (Table 1).

We have characterized here two models of EAE; the chronic myelin oligodendrocyte glycoprotein (MOG) induced model in C57BL/6J mice and the relapsing/remitting proteolipid protein (PLP) induced model in SJL mice, but in absence of pertussis toxin (PTX), widely used immune response stimulator used in model induction. Normally MOG or PLP models require PTX to induce robust phenotype (Fig 1C and 1D) and immunological profile characterized by infiltration of inflammatory cells to CNS, which we have evaluated earlier. In this study we sought to understand if the phenotype is significantly milder from behaviour and immunophenotype point of view, in the absence of PTX.

Table 1. Used scoring criteria of mice over disease progression.

Score	Manifestations	Score	Manifestations
0.5	Partial tail weakness	2.5	Partial paralysis in one hind limb
1.0	Complete tail paralysis	3.0	Complete paralysis in both hind limbs
1.5	Flaccid tail and abnormal gait	4.0	Complete paralysis in hind limbs and weakness in forelimbs
2.0	Flaccid tail and clear weakness of hind limbs	5.0	Complete paralysis in all limbs, moribund

2 IN VIVO METHODOLOGIES

All animal experiments were carried out according to the National Institute of Health (NIH) guidelines for the care and use of laboratory animals, and approved by the National Ethics Committee (Regional State Administrative Agency of Southern Finland). All *in vivo* experiments were carried in AAALAC accredited facilities.

Altogether 20 female C57BL/6J and SJL/J between 9-10 weeks of age were inoculated with MOG35-55/CFA or PLP139-151/CFA, respectively, without PTX for the induction of the chronic and relapsing/remitting EAE models. Mice were then monitored for clinical signs of disease development and changes in body weight for three weeks before sample collection (DLN), brain and spinal cord samples for characterization using flow cytometry.

C57BL/6J mice were inoculated by using commercially available ready to use inoculum (EK-0115, Hooke Laboratories, USA) containing 100 µg of MOG_{35-55}, 200 µg of heat inactivated Mycobacterium tuberculosis in mineral oil in 100 µL of inoculum. Inoculation was done by giving each mouse 2 x 100 µl injections subcutaneously to lower and higher aspect of the back.

SJL/J mice were inoculated by using a commercially available ready to use inoculum (EK-0120, Hooke Laboratories, USA) containing 100 µg of PLP139-151, 100 µg of heat inactivated Mycobacterium tuberculosis in mineral oil in 100 µL of inoculum. Inoculations were done by giving each mouse 4 x 50 µl injections subcutaneously to lower and higher aspect of the back. 2 x 50 µl was injected in the upper back of the mouse (one over each shoulder) and 2 x 50 µl was injected in the lower back (one injection over each hip/base of the tail).

Clinical scoring and body weight were followed daily until the end of the study until day 24 post EAE induction. At the end-point, all animals were deeply anesthetized with pentobarbital, perfused with heparinised saline and dissected. Collected samples were stored in HBSS on ice until processing.

3 TISSUE PROCESSING AND FACS

Brains were processed using Adult Brain Dissociation Kit (Miltenyi, #130-107-677) according the manufacturer's instructions. Cell counting was performed using haemocytometer.

Pooled DLN samples were kept on wet ice during the entire isolation phase. The DLNs were manually dissociated by crushing through a 70 µm strainer and simultaneously rinsed with 30 ml of HBSS. After cell dissociation cells were centrifuged 450 x g for 5 minutes, supernatant was removed and the cells were suspended in 2 ml of complete RPMI medium. 10 µl of the cells were used for staining with propidium iodide (89 µl HBSS, 10 µl Sample, 1 µl propidiumiodide), cell number and viability were determined using flow cytometer (MACSQuant Analyzer 10).

After cells were processed, they were stained with desired dyed antibodies against targets of interest and calculated using flow cytometry. Applied details are described in Table 2.

Table 2. Summary of used detection channels and antibodies.

Used channel	Laser (nm)	Filter	Antibody and dye	Clone
V1	405	450/50	CD4 VioBlue	REA604
V2	405	525/50	Zombie Aqua	N/A
B1	488	525/50	CD3 FITC	REA641
B2	488	585/40	CD49b PE	REA541
B4	488	750 LP	CD11b PE-Vio770	REA592
R1	638	655 – 730	F4/80 APC	REA126
R2	638	750 LP	CD45 APC-Vio770	REA737

4 RESULTS

The chronic myelin oligodendrocyte glycoprotein (MOG) induced model in C57BL/6J mice and the relapsing/remitting proteolipid protein (PLP) induced model in SJL mice were studied. As a summary, clinical symptoms were less severe in these models as compared to previous model including also dosing of PTX (Figure 1C and 1D). However, clear difference between healthy controls and induced mice in cell population analysis was observed from both analysed tissues, brain (Figure 2) and draining lymph nodes (Figure 3). Flow cytometry analysis of the brain tissue showed elevated T-cell populations, significant invasion of CNS-associated monocytes from periphery as well as upregulation of the microglial population in both models. Finally, the analysis of the draining lymph nodes (DLN) showed that the levels of NK-cells seem to be more elevated in the MOG model than in the PLP model.

Based on the work described here, we can show that despite milder behavioural phenotype was in both models, immunophenotype was resembling the model previously seen when PTX was used. This type of basic phenotypic profiling allows to understand the immunophenotype with different types of immunization protocols which results in differences in behavioural or disability phenotypes.

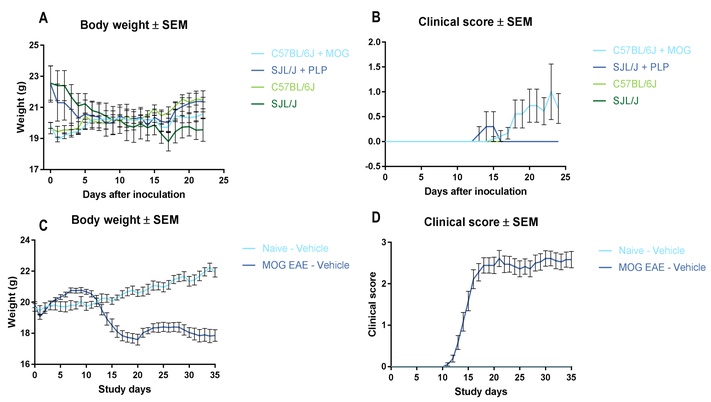

Figure 1. Body weight (A) and clinical score (B) in chronic MOG induced model in C57BL/6J mice and the relapsing/remitting proteolipid protein (PLP) induced model in SJL mice. In this study pertussis toxin was not given as compared to traditional model. As a result, no decrease in body weight over time was observed and also the clinical status remained better as compared to model with pertussis toxin (C and D).

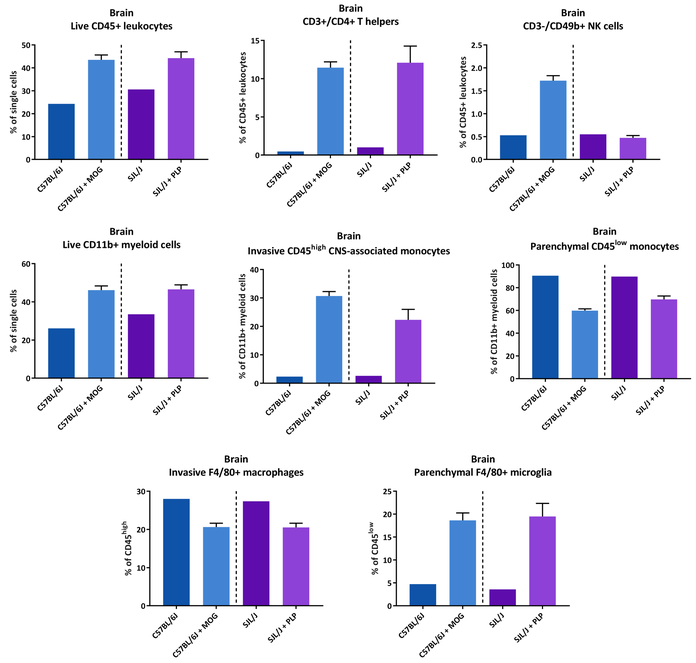

Figure 2. Immunophenotyping of cells extracted from the brain on day 24 after the induction of chronic MOG induced model in C57BL/6J mice and the relapsing/remitting proteolipid protein (PLP) induced model in SJL mice. Data shows differences in immunophenotypes, both resident and infiltrating cells. Clear phenotype difference was seen between healthy and EAE model

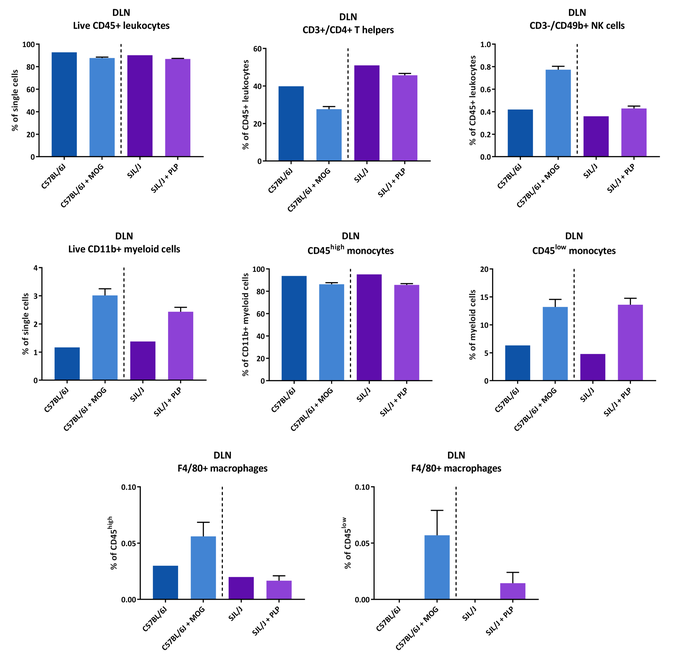

Figure 3. Immunophenotyping of cells extracted from draining lymph nodes on day 24 after the induction of chronic MOG induced model in C57BL/6J mice and the relapsing/remitting proteolipid protein (PLP) induced model in SJL mice. Data shows differences in immunophenotypes, both resident and infiltrating cells. Clear phenotype difference was seen between healthy and EAE model