Neuroinflammation in the EAE Model of Multiple Sclerosis

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BACKGROUND

Multiple sclerosis (MS) is one of the most common neurological disorders among young adults. Key hallmarks are demyelination, neuroinflammation, and neurodegeneration resulting in persistent invalidity. Experimental autoimmune encephalomyelitis (EAE) shows many pathological similarities to MS and is therefore used as model to mimic MS by injecting myelin-oligodendrocyte-glycoprotein (MOG) in combination with pertussis toxin (PTX).

MATERIALS and METHODS

C57BL/6 mice were s.c. injected with MOG and 2 hours later i.p with PTX. PTX was again injected on the next day. After two weeks, animals were treated daily with fingolimod or vehicle by oral gavage for three weeks. During the last treatment week, animals were tested for motor changes in the wire hanging and RotaRod tests. Spinal cord was analyzed for pathology such as demyelination by Luxol fast blue staining and neuroinflammation by immunofluorescence. Plasma was evaluated for neurofilament light chain (NF-L) levels using a kit from UmanDiagnostics AB.

RESULTS

EAE-induced animals presented strong deficits in muscle strength and motor behavior compared to vehicle controls. Quantitative analysis of the spinal cord detected focal demyelination accompanied by severe neuroinflammation, as indicated by increased GFAP and Iba1 levels. The number of leukocytes and macrophages was strongly increased as analyzed by CD45 and CD68 labeling, respectively. NF-L as marker of neurodegeneration was severely increased in EAE-induced animals, while fingolimod was able to partially prevent the observed pathologies.

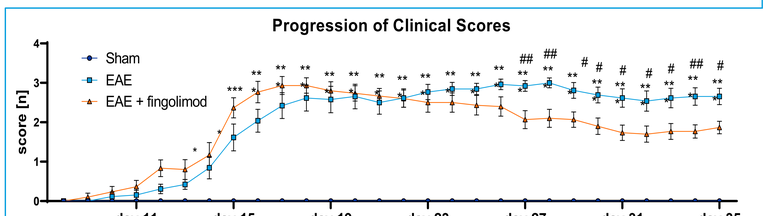


Figure 1 ▲. Clinical signs of EAE-treated mice compared to sham-treated mice and fingolimod-treated EAE mice from day 8 until 35 after MOG treatment. Mean + SEM; Mixed effects ANOVA followed by Bonferroni's multiple comparison *post hoc* test; *p<0.05, **p<0.01, ***p<0.001 compared with sham-treated mice, #p<0.05 ##p<0.01 compared with fingolimod-treated mice.

RESULTS

Wire Hanging

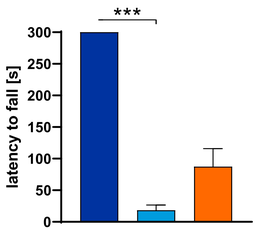


Figure 2 ▲. Wire Suspension. Mean latency to fall off the wired grid was severely shortened in EAE-treated mice compared to sham-treated mice and fingolimod-treated EAE animals. Kruskal-Wallis test followed by Dunn's *post hoc* test; ***p<0.001. Mean ± SEM.

RotaRod

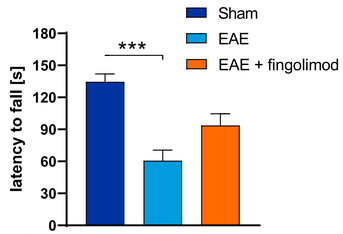


Figure 3 ▲. RotaRod test. Mean latency to fall off the rod was shortened in EAE-treated mice compared to sham-treated mice and fingolimod-treated EAE animals. One-Way ANOVA followed by Bonferroni's *post hoc* test or Kruskal-Wallis test followed by Dunn's *post hoc* test; ***p<0.001. Mean ± SEM.

Neurofilament Light Chain in Plasma

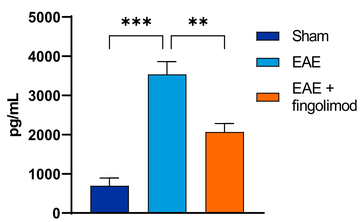


Figure 4 ◄. Quantification of neurofilament light chain in plasma. NF-L levels in pg/ml in the plasma of vehicle-treated EAE mice compared to sham-treated animals and fingolimod-treated EAE mice. One-way ANOVA and Dunnett's *post hoc* test; **p<0.01, ***p<0.001 Mean ± SEM.

Demyelination

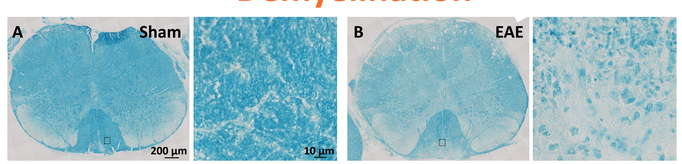
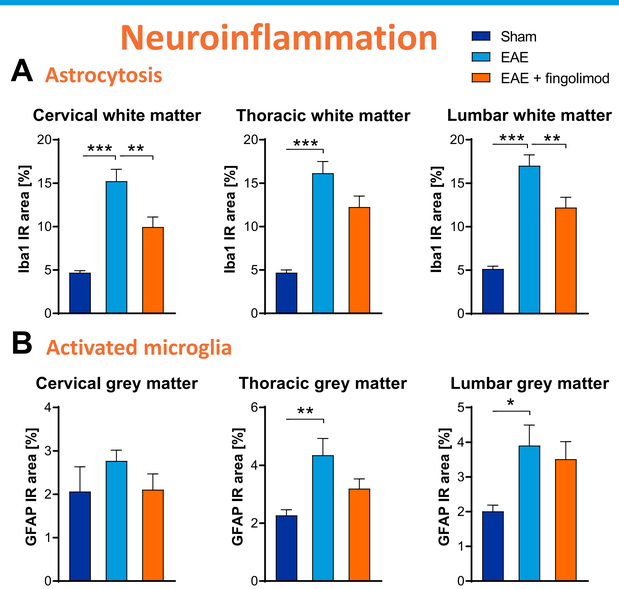


Figure 5 ▲. Luxol Fast Blue staining. Representative images of Luxol Fast Blue staining in sham-treated mice (**A**) and EAE-treated mice (**B**).

Figure 6 ▶. Quantitative analysis of immunofluorescent labeling of neuroinflammation markers. IBA-1 (**A**), GFAP (**B**), CD45 (**C**) and CD68 (**D**) immunoreactive (IR) area in percent in the cervical, thoracic and lumbar spinal cord of vehicle-treated EAE mice compared to sham vehicle treated mice and fingolimod-treated EAE mice. White matter showed in IBA1, CD45 and CD68; grey matter in GFAP. One-way ANOVA and *post hoc* test; *p<0.05, **p<0.01, ***p<0.001. Mean ± SEM.

Neuroinflammation



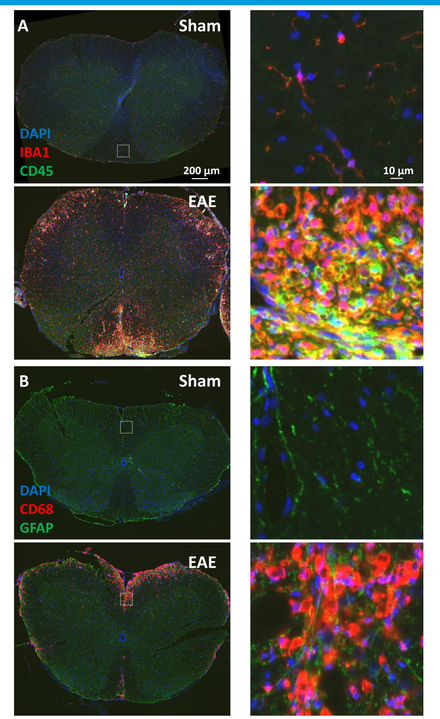


Figure 7 ▲. Representative images of IBA1 (**A**), CD45 (**A**), GFAP (**B**) and CD68 (**B**) in the white matter of sham- and EAE-treated mice.

SUMMARY and CONCLUSION

EAE induces a MS-like pathology 5 weeks after MOG treatment. Muscle strength is reduced resulting in motor deficits as well as strong neuroinflammation and neurodegeneration. The EAE model thus mimics the key phenotypic and pathological hallmarks of multiple sclerosis and can be used to test new compounds against the observed pathologies. Fingolimod can be used as reference compound.