LB-ECTRIMS-2021-01631

Targeting BTK in chronic CNS autoimmunity inhibits activation of microglia

Anastasia Geladaris[1], Sebastian Torke[4,5], Roland Grenningloh[6], Ursula Boschert[7], Wolfgang Brück[1], Martin S. Weber[1,2,3]

[1] Department of Neuropathology, University Medical Center, Göttingen, Germany, [2] Department of Neurology, University Medical Center, Göttingen, Germany, [3] Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, Göttingen, Germany, [4] Experimental and Clinical Research Center, University Medical Center Berlin, [5] Max-Delbrück Center for molecular medicine Berlin, [6] EMD Serono Research & Development Institute, Inc. Billerica, MA, USA, an affiliate of Merck KGaA, [7] Ares Trading SA, Eysins, Switzerland, an affiliate of Merck KGaA

Background

Therapeutically controlling chronic progression remains a major challenge in multiple sclerosis (MS) therapy. Small molecules inhibiting the enzyme Bruton's tyrosine kinase (BTK) may be promising candidates to target innate immunity within the CNS associated with disease progression. BTK is expressed in B cells where it has a well-established role in mediating B-cell receptor signaling. BTK is also expressed in monocytes, macrophages, and microglia regulating signalling downstream of a variety of receptors including Fc, integrin, chemokine and toll-like receptors. In the present study, we aimed at analyzing the effect of the BTK inhibitor evobrutinib on microglial cells in vitro and in vivo in an animal model of chronic MS.

Methods

Primary microglia were generated from newborn C57BL/6 mice and activated by IFN-gamma and/or GM-CSF and/or LPS. C57BL/6 mice were immunized with MOG peptide 35-55. After 11-12 days, immunization draining lymph nodes were isolated and cultivated for 3 days in the presence of anti-IFN-gamma antibody, IL-12 and MOG Peptide 35-55. Subsequently, the pathogenic T cells were purified by the magnetic-bead associated removal of B cells and intraperitoneally injected into recipient mice. Recipients received evobrutinib or vehicle control starting 3 days prior to transfer. Microglial activation/modulation was assessed by ELISA and flow cytometry.

Results

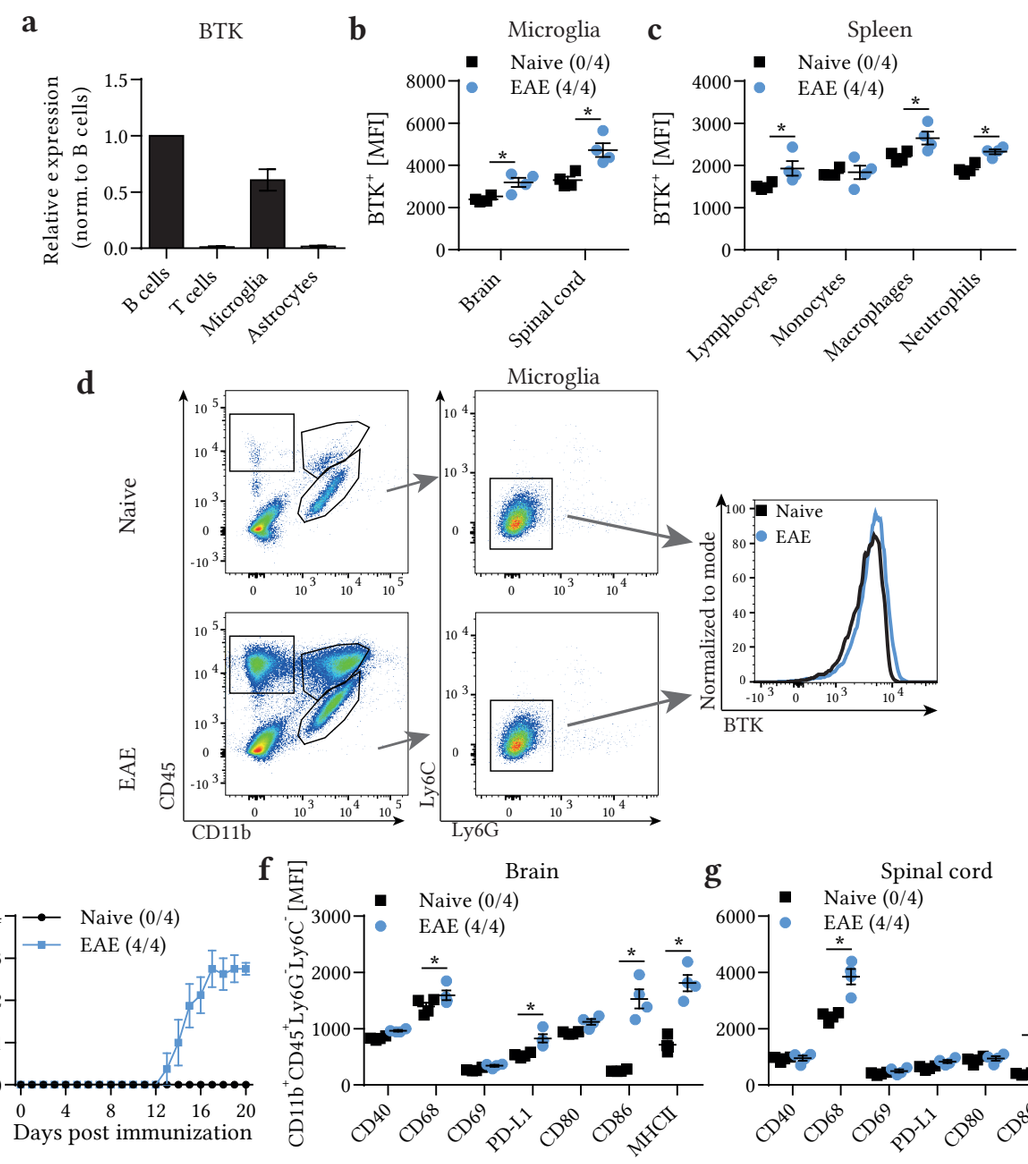

Figure 1: BTK is expressed in microglia but not astrocytes and upregulated under inflammation. a) Primary cell cultures were harvested and lysed for RNA extraction; relative expression normalized to GapDH ± SEM **b-f)** Mice were left untreated or immunized with MOG_{35-55}. On day 20, cells were isolated and stained for flow cytometry, **b, c, f)** Mean fluorescence intensity ± SEM; n=4; Mann-Whitney U test *p<0.05. **d)** Representative histograms and gating strategy. **e)** Mean clinical score ± SEM

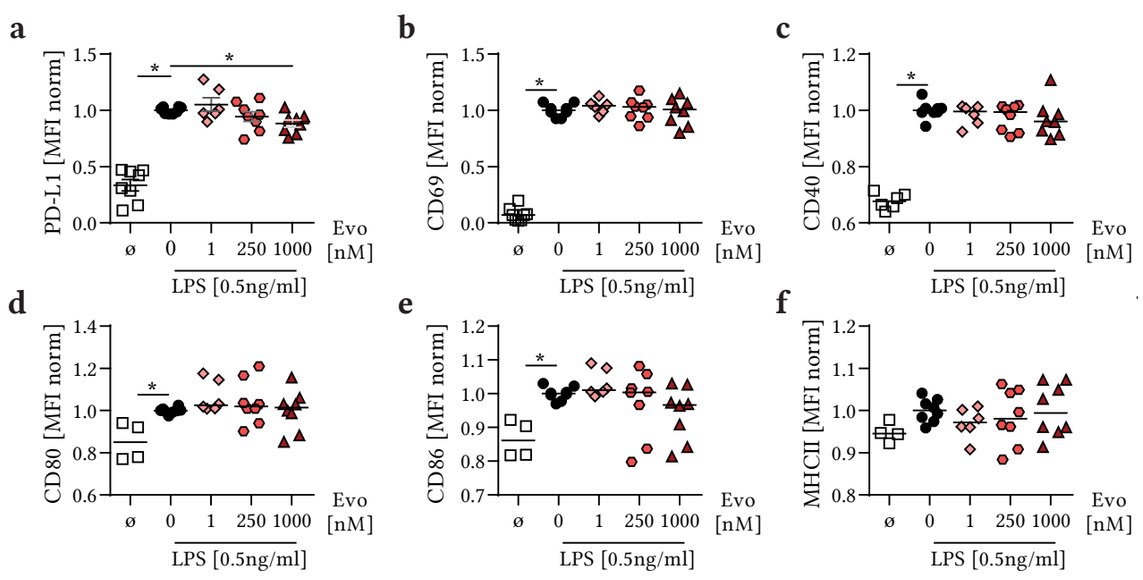

Figure 2: Evobrutinib affects LPS-induced microglial PD-L1 expression in vitro. Primary microglia were pre-treated with the indicated evobrutinib concentrations for 30 minutes prior to stimulation with 0.5ng/ml LPS. After 6h, the cells were harvested and stained for flow cytometry analysis. **a-f)** Mean fluorescence intensity ± SEM; n=4-8, pooled from 2-3 independent experiments; Kruskal-Wallis with Dunn's post hoc test; *p<0.05.

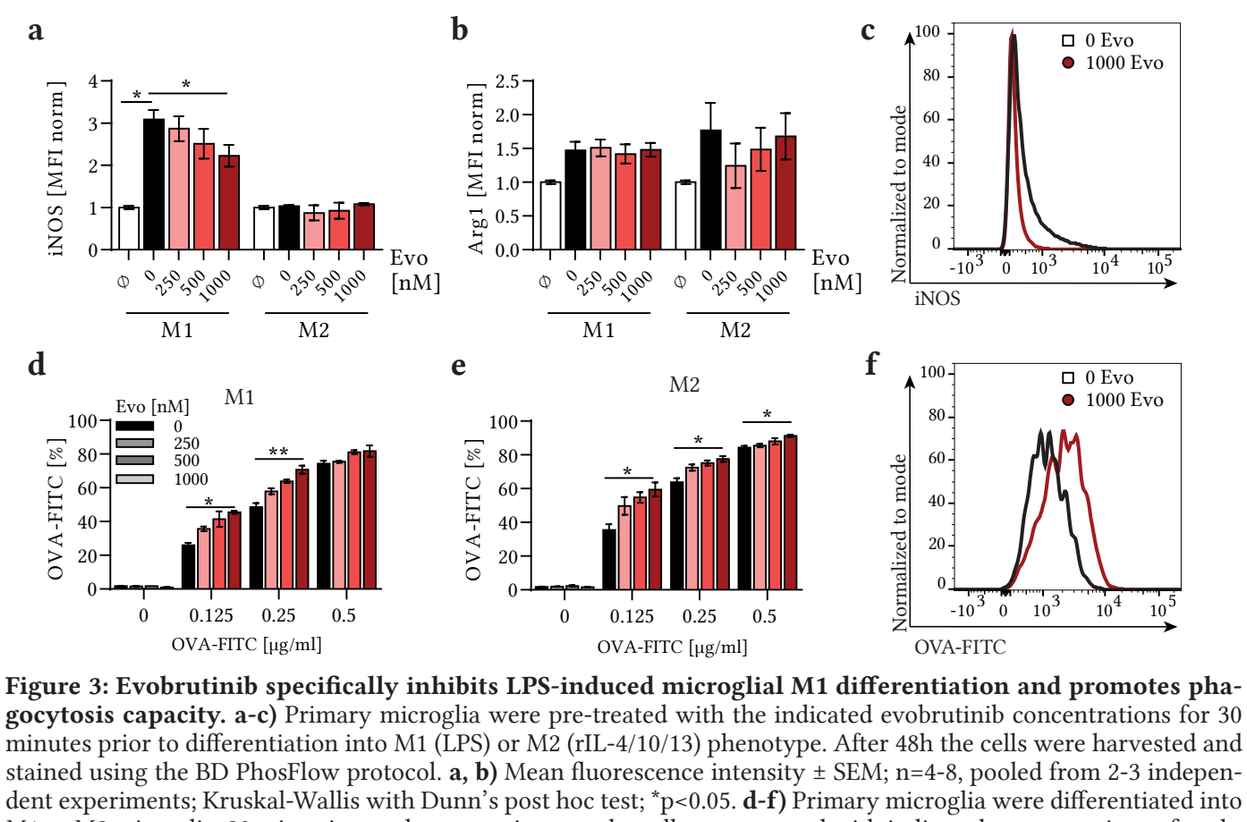

Figure 3: Evobrutinib specifically inhibits LPS-induced microglial M1 differentiation and promotes phagocytosis capacity. a-c) Primary microglia were pre-treated with the indicated evobrutinib concentrations for 30 minutes prior to differentiation into M1 (LPS) or M2 (rIL-4/10/13) phenotype. After 48h the cells were harvested and stained using the BD PhosFlow protocol. **a, b)** Mean fluorescence intensity ± SEM; n=4-8, pooled from 2-3 independent experiments; Kruskal-Wallis with Dunn's post hoc test; *p<0.05. **d-f)** Primary microglia were differentiated into M1 or M2 microglia. 30 min prior to phagocytosis assay the cells were treated with indicated concentrations of evobrutinib. Thereafter, the cells were incubated for 2h with indicated concentrations of OVA-FITC, harvested and stained for flow cytometry analysis. **d, e)** Frequency of OVA-FITC+ microglia cells ± SEM; n=3, Kruskal-Wallis with Dunn's post hoc test; *p<0.05.

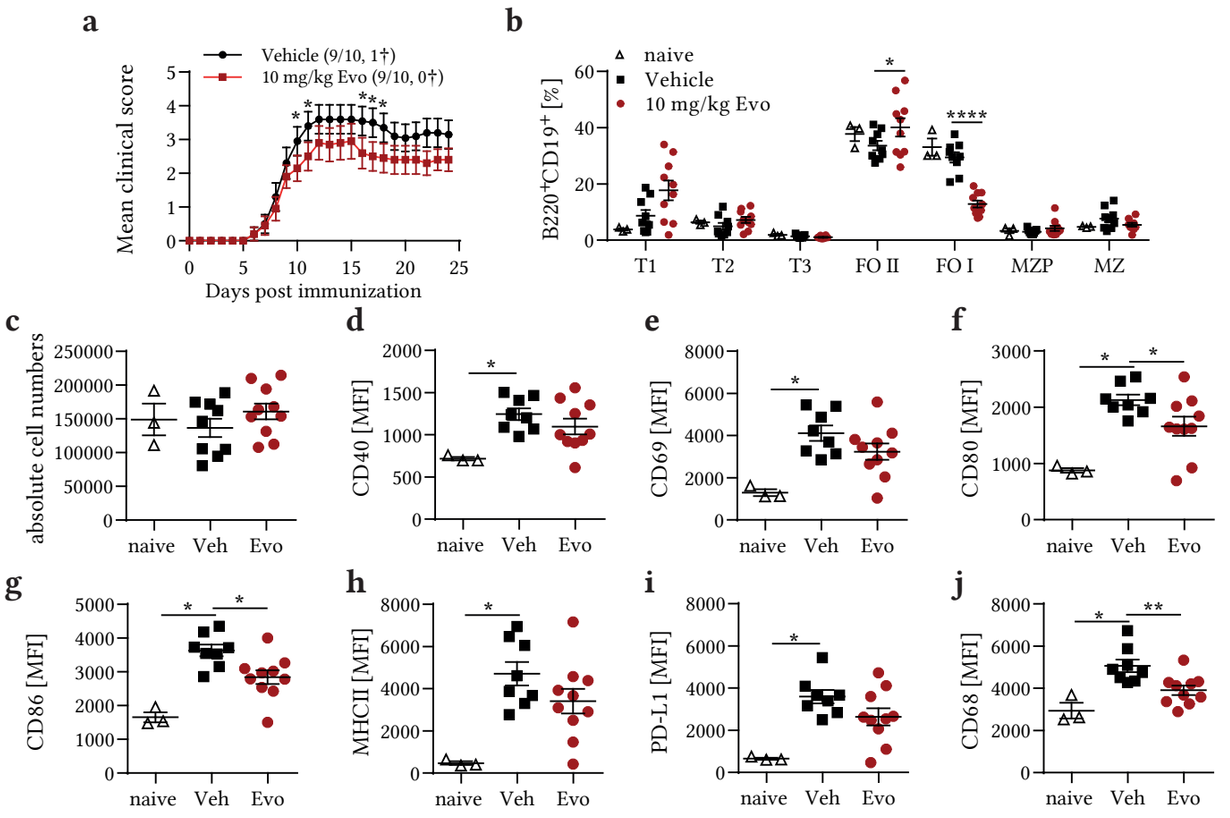

Figure 4: Adoptive transfer of pathogenic T cells lead to a strong microglia activation, a process that can be dampened by evobrutinib. C57BL/6 mice were immunized with 200 µg MOG35-55. After 11-12 days, the inguinal lymph nodes were isolated and cultivated for 3 days at the density of 2-2.5x10^6 cells in the presence of 20 µg/ml anti-IFNγ, rIL-12 and 25 µg/ml MOG35-55. Subsequently, T cells were purified by a magnetic-bead associated removal of B cells. Recipient mice, pre-treated for 3 days with evobrutinib or vehicle control, received 1.83x106 cells intraperitoneally. **a)** Mean clinical score ± SEM **b)** B cell maturation **c)** Absolute cell numbers ± SEM; **d-j)** Microglia activation in the brain, mean fluorescence intensity ± SEM; n=9-10. Mann-Whitney U test *p<0.05 or Unpaired T test; *p<0.05, **p<0.01.

Conclusion

BTK-dependent inflammatory signaling in microglial cells can be modulated by evobrutinib. These findings highlight the therapeutic potential of BTK inhibition in counteracting chronic progression of MS.

Disclosure: A.G has nothing to disclose. **S.T.** recieves an intramural grant of the University Medical Center, Göttingen ("Startförderung") and travel support from EMD Serono Research & Development Institute, Inc., Billerica, MA, USA, an affiliate of Merck KGaA. **M.S.W.** is serving as an editor for PLoS One. He receives research support from the National Multiple Sclerosis Society (NMSS; PP 1660), the Deutsche Forschungsgemeinschaft (DFG; WE 3547/4-1), from Novartis, EMD Merck Serono, TEVA, Biogen-Idec, Roche and the ProFutura Programm of the Universitätsmedizin Göttingen. The authors declare no conflicts of interest.