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Evobrutinib, a Bruton's tyrosine kinase inhibitor, acts on microglia: implications in treatment of progressive mechanisms in multiple sclerosis

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Background

In multiple sclerosis (MS), persisting disability can derive from acute relapses or alternatively, from slow and steady progression independent of relapse activity, termed chronic progression. Therapeutically controlling progression independent of relapses (PIRA) in MS, remains a major challenge. One promising strategy may be to reduce chronic neuroinflammation by the inhibition of the enzyme Bruton's tyrosine kinase (BTK), which is centrally involved in the activation of both B cells as well as myeloid cells, such as macrophages and microglia.

In the present study, we analysed the potential of the BTK inhibitor evobrutinib as a therapeutic strategy in halting progression in MS.

Methods

Primary micorglia were generated from newborn C57BL/6 mice. Furthermore, C57BL/6 mice were immunized with MOG peptide 35-55. Mice were treated with 10 mg/kg evobrutinib or vehicle control starting at day 40 post immunization. On day 70 cells were isolated and analyzed by flow cytometry. In a second approach mice were immunized with MOG peptide 35-55 and 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

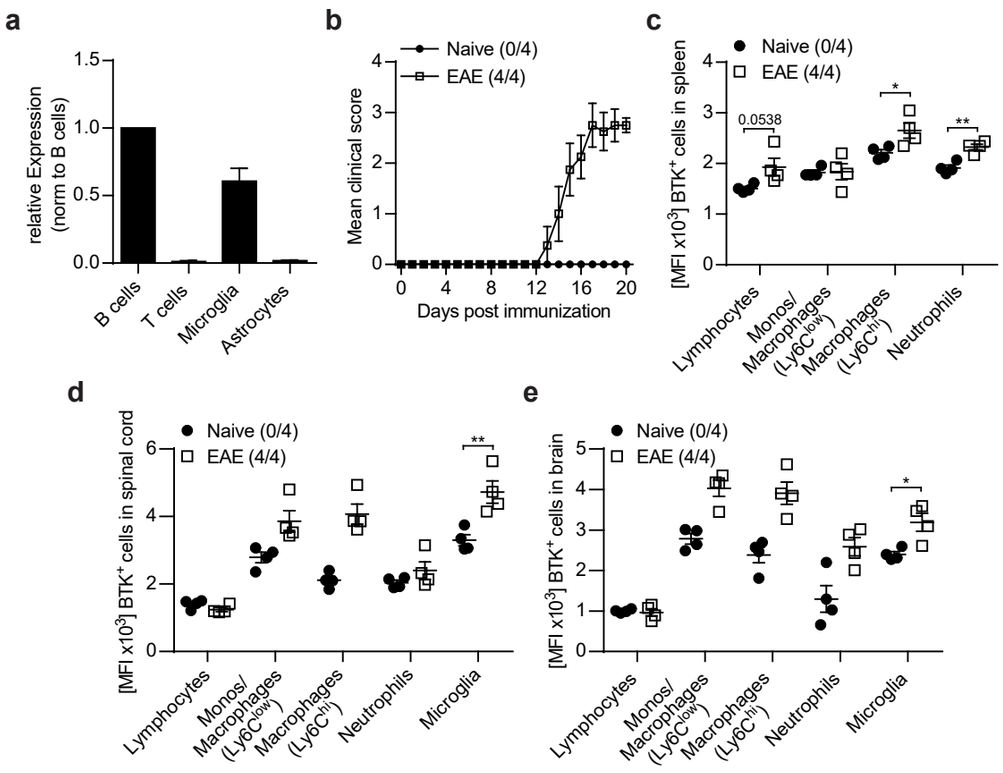

Figure 1: BTK is expressed in microglia but not astrocytes and upregulated under inflammation. a) Primary cell cultures were harvested and lysed for RNA extraction; relative expression normalized to GapDH ± SEM **b-f)** Mice were left untreated or immunized with MOG_{35-55}. On day 20, cells were isolated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

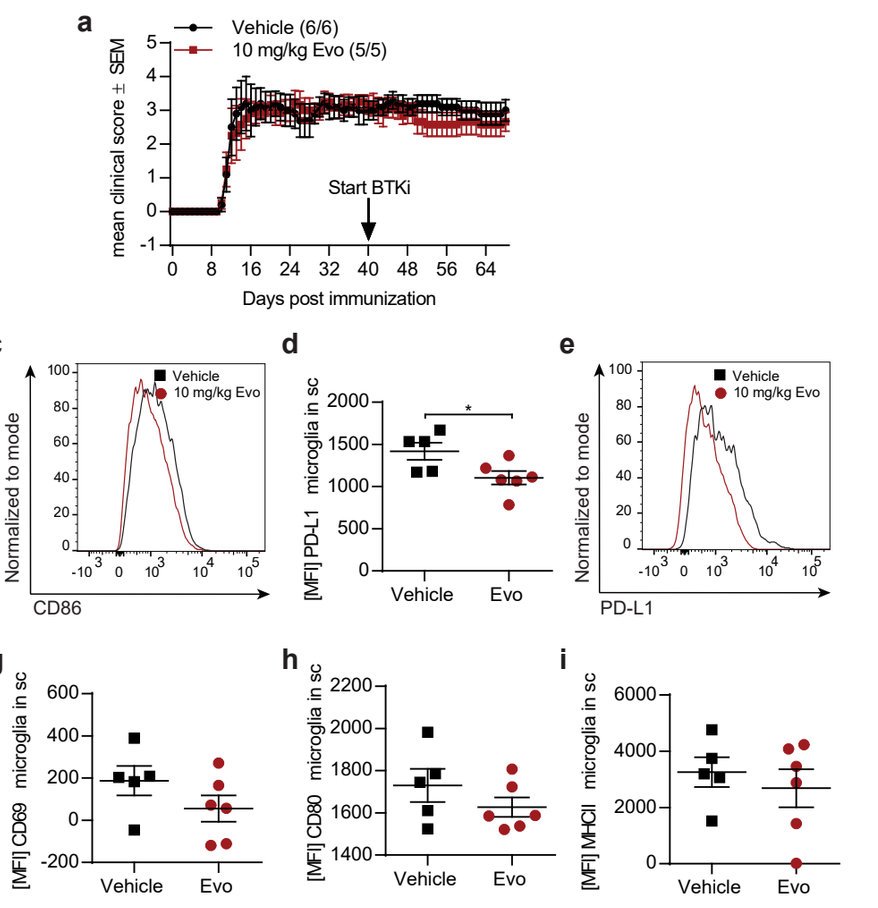

Figure 2: Evobrutinib alter the microglia phenotype in chronic EAE. C57BL/6 mice were immunized with 75 µg MOG_{35-55} peptide. Mice were treated with evobrutinib or vehicle starting at day 40 post immunization. On day 70 cells were isolated from the spinal cord and analyzed by flow cytometry. **a)** Mean clinical score ± SEM. **b,d,f-i)-** Microglia activation in the spinal cord; mean fluorescence intensity ± SEM; n=5-6. t test; *p<0.05, **p<0.01. **c, e)** Representative histograms.

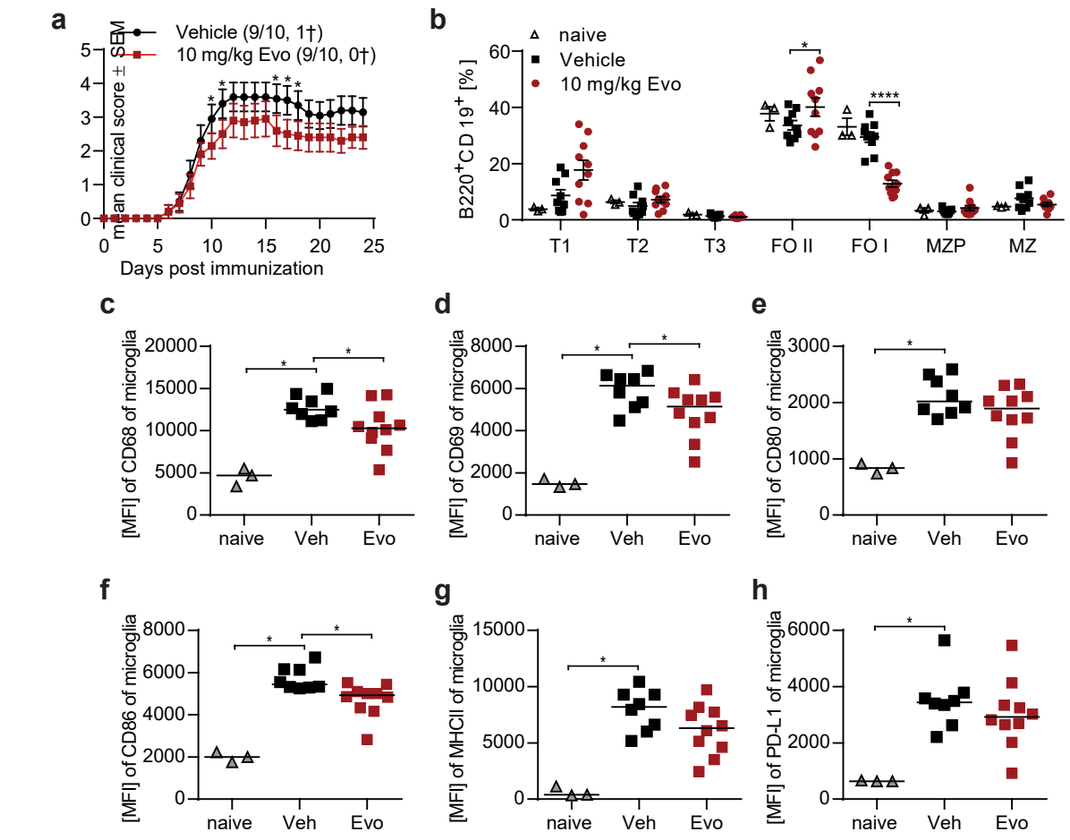

Figure 3: Adoptive transfer of pathogenic T cells lead to a strong microglia activation, a process that can be dampened by evobrutinib. C57BL/6 mice were immunized with 200 µg MOG_{35-55}. After 11-12 days, the inguinal lymph nodes were isolated and cultivated for 3 days at the density of 2-2.5x10^6 cells in the presence of 20 µg/ml anti-IFNγ, rIL-12 and 25 µg/ml MOG_{35-55}. 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-h)** Microglia activation in the spinal cord, mean fluorescence intensity shown an median; n=9-10, unpaired t test; *p<0.05, **p<0.01.

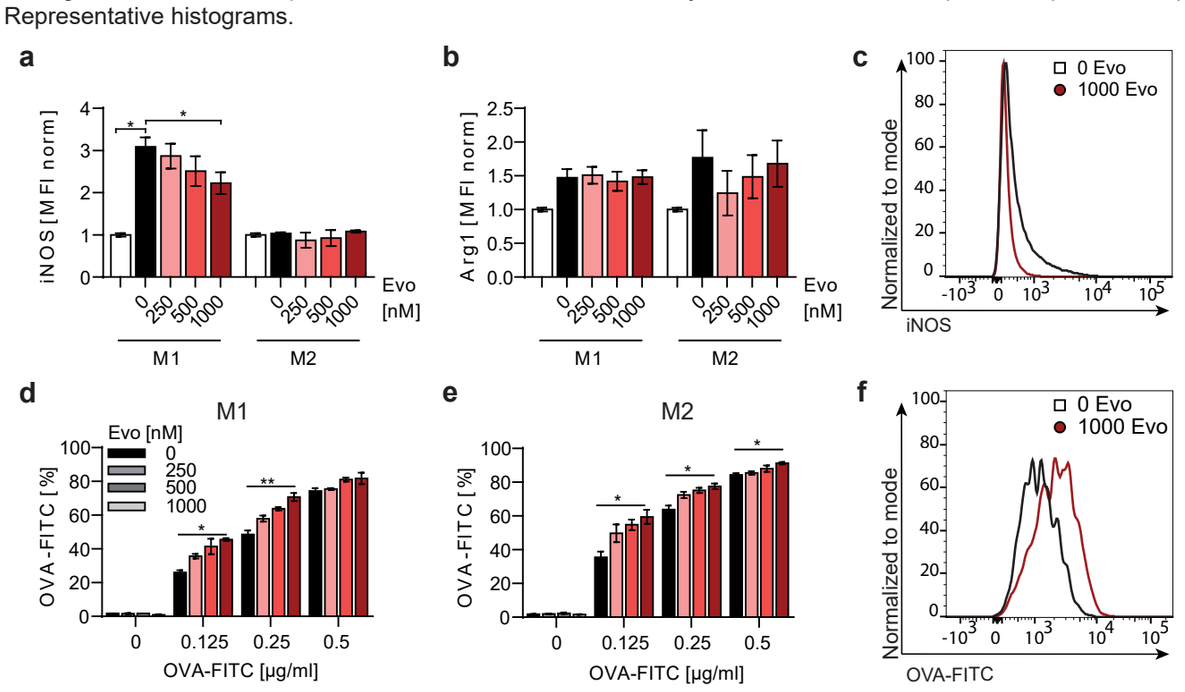

Figure 4: Evobrutinib specifically inhibits LPS-induced microglial M1 differentiation and promotes phagocytosis capacity. a-c) Primary microglia were pre-treated with the indicated evobrutinib concentrations for 30 minutes prior to differentiation into M1 (LPS) or M2 (rIL-4/10/13) phenotype. After 48h 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.

Conclusion

BTK inhibition by evobrutinib downregulates inflammatory properties of microglia. These data highlight the therapeutic potential of BTK inhibition with evobrutinib in ameliorating underlying processes associated with chronic progression in MS.

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