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Expression of Bruton's tyrosine kinase (BTK) in B cell-rich meningeal infiltrates in two models of progressive MS

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MS Virtual 2020 — 8th Joint ACTRIMS-ECTRIMS Meeting
September 11-13, 2020

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Disclosures & Funding

- Hania Kebir holds a postdoctoral fellowship from the Fonds de Recherche du Québec — Santé (FRQS) and received an Educational Grant Award from the Americas Committee for Treatment and Research In Multiple Sclerosis (ACTRIMS).
- Guadalupe Ceja, Miles C. Miller, Cen Li, Michael J. May, Charles H. Vite, and Molly E. Church have nothing to disclose.
- Roland Grenningloh is an employee of EMD Serono and Ursula Boschert is an employee of Ares Serono Switzerland, a subsidery of Merck KGaA
- Jorge I. Alvarez receives research support from EMD Serono, the National Institutes of Health (NIH)-National Institute of Neurological Disorders and Stroke (NINDS), and the Institute for Translational Medicine and Therapeutics (ITMAT) of the University of Pennsylvania.
- This study is supported by funding from EMD Serono.

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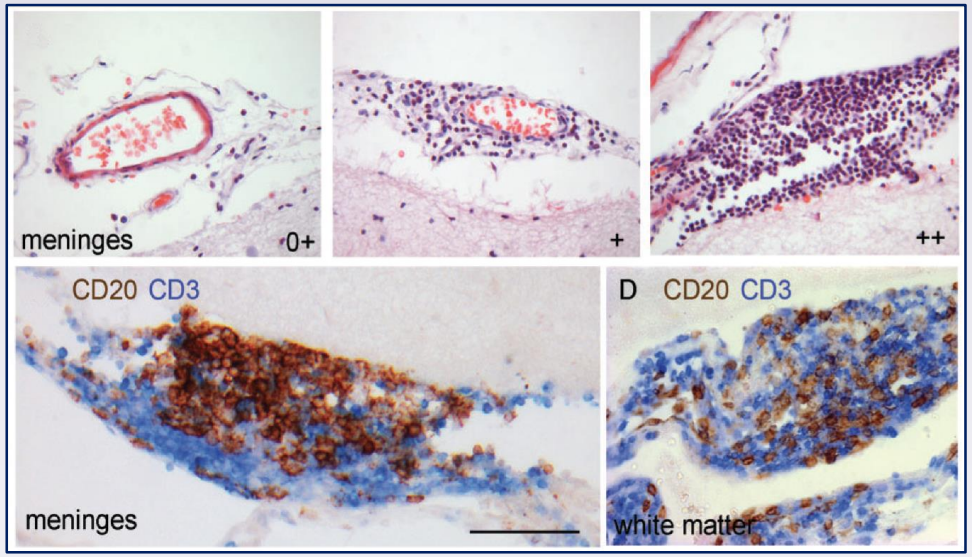
Background

- Compartmentalized CNS inflammation may be a source of progressive disease in MS:
  - **Meningeal inflammation** consisting of B cell-rich aggregates, linked with adjacent cortical subpial pathology, is predictive of an aggressive disease with rapid, unrelenting **progression of disability** (Howell *et al.*, 2011, Magliozzi *et al.*, 2018).



Choi et al., Brain 2012

- Challenges in treatment of progressive MS:
  - Few animal models recapitulate pathology of progressive MS.
  - CNS B cells are protected from the direct effects of anti-CD20 therapies.
- Alternative: **Bruton's tyrosine kinase (BTK)** inhibition
  - BTK is expressed by B cells, macrophages/microglia, but not by T cells (Lopez-Herrera *et al.*, 2014).
  - BTK is critically involved in B cell and myeloid cell activation and survival (Lopez-Herrera *et al*., 2014).
  - The BTK inhibitor Evobrutinib has been described to enter the CNS (Boschert *et al*., 2017, ECTRIMS).
- **Overall goal** □ Evaluate BTK expression in the CNS of two animal models recapitulating determining aspects of progressive MS and test efficacy of Evobrutinib in a mouse model of progressive disease.

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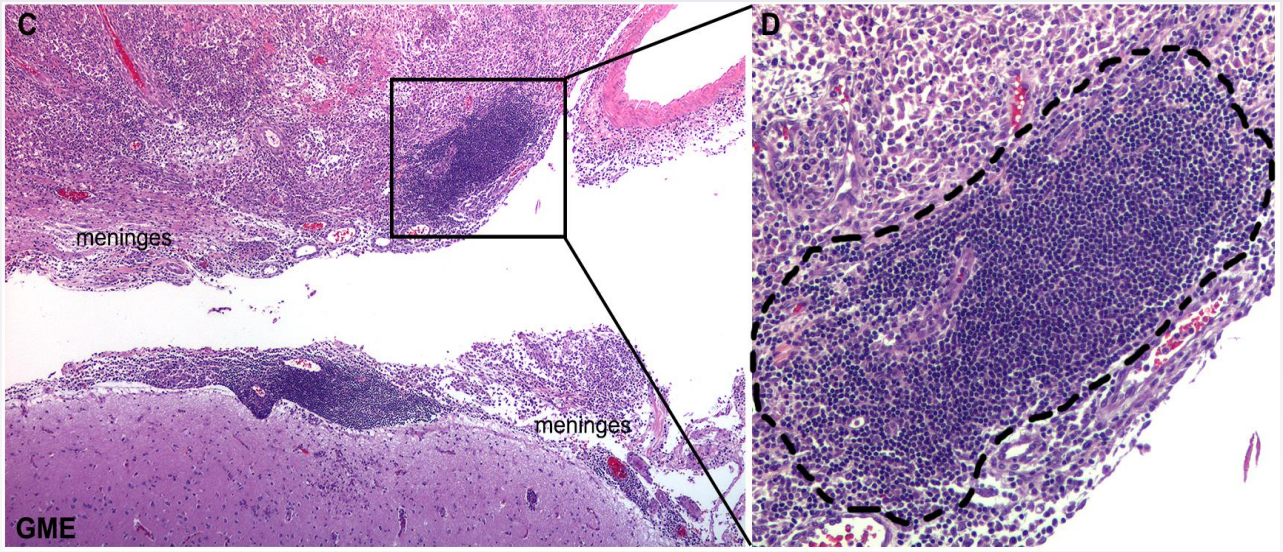
Granulomatous meningoencelphalomyelitis (GME)

- GME is the most common idiopathic neuroinflammatory disease in dogs that prevails in young females (Coates and Jeffery, 2014).
- GME is marked by by leukocyte infiltration in the parenchyma and meninges (Vite, 2005; Cherubini *et al.*, 2006).

A. Hematoxylin & eosin (H&E) staining



B. Immunofluorescence staining (IF)

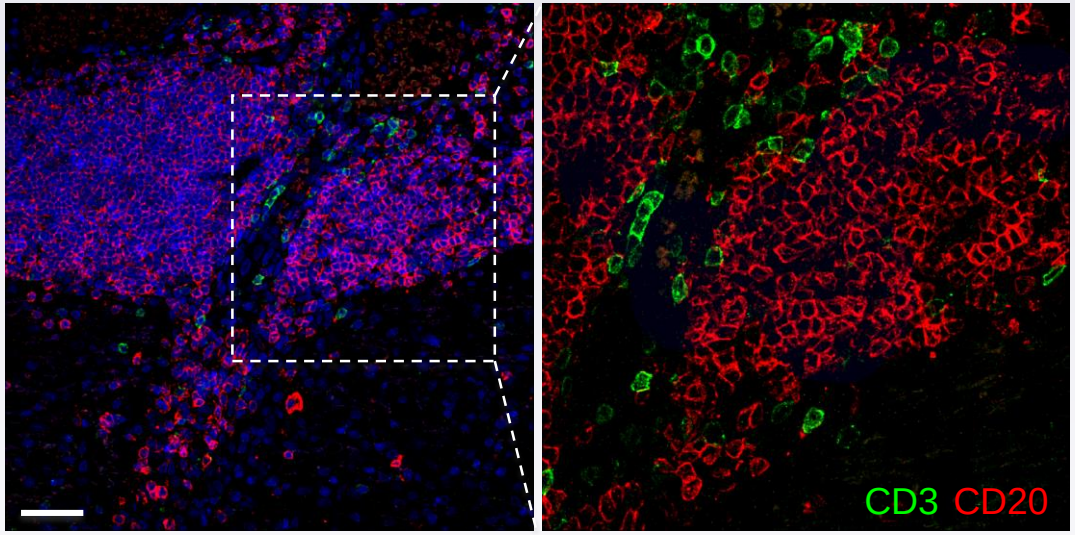


Figure 1. Neuropathology of GME. (**A**) Hematoxylin & eosin (H&E) staining showing heavy immune cell infiltration in the meninges. (**B**). $CD20^+$ B cells (red) are the predominant leukocyte population within the meningeal infiltrate, as compared to $CD3^+$ T cells (green). Nuclei are stained in blue. Scale bar: 50 μm.

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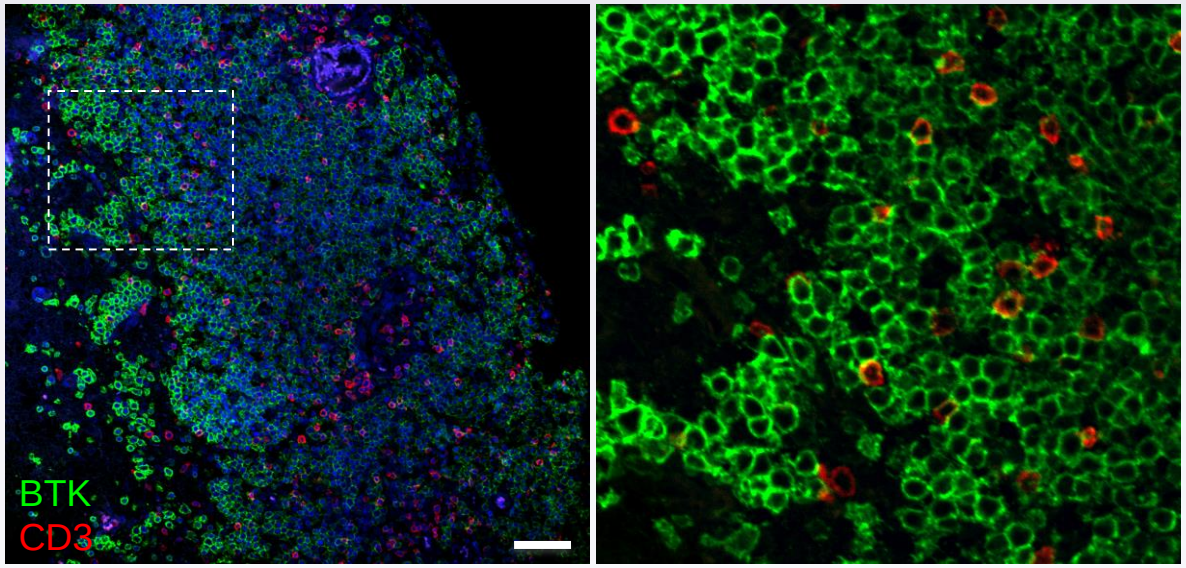
Meningeal infiltrates in GME express high levels of BTK

- $CD3^+$ cells stain negative for BTK, whereas Iba-1 co-localizes with BTK staining
- The primary antibodies directed against canine CD20 and BTK are both raised in the same species, preventing us from performing double immunofluorescent staining.

A. Staining of BTK with T cells (no co-localization)



B. Staining of BTK with myeloid cells (co-localization)

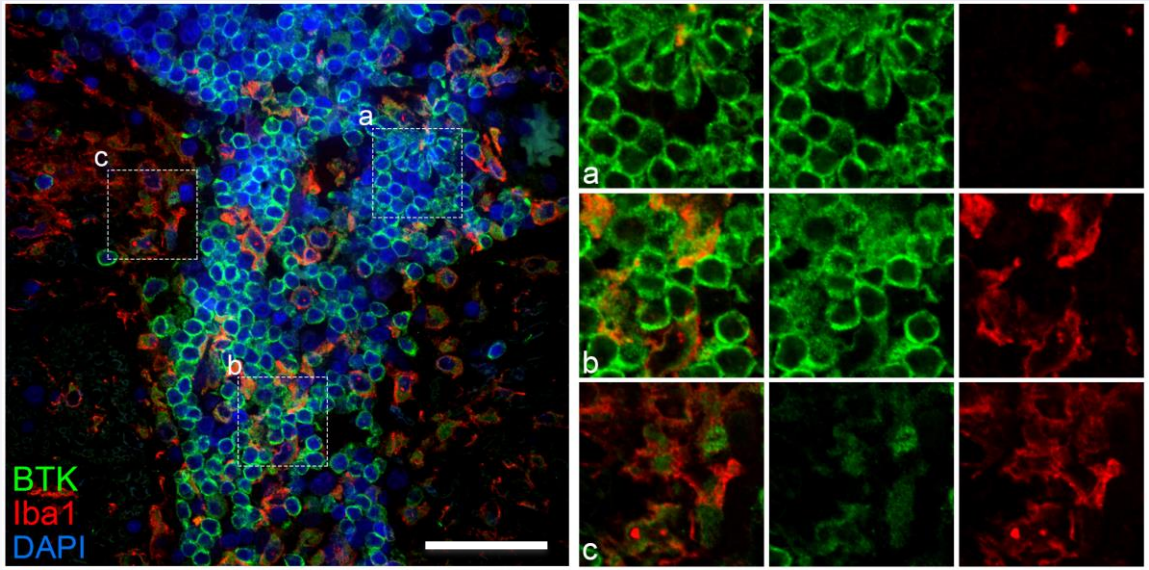


Figure 2. Expression of BTK in the GME brain. (**A**) Immunofluorescent staining revealing a lack of co-localization between BTK (green) and CD3 T cells (red) staining in a meningeal infiltrate. (**B**) Myeloid cells (Iba-1^+, red) express BTK within meningeal infiltrates. The majority of BTK^+ cells are more likely B cells. Nuclei are stained in blue. Scale bar: 50 μm.

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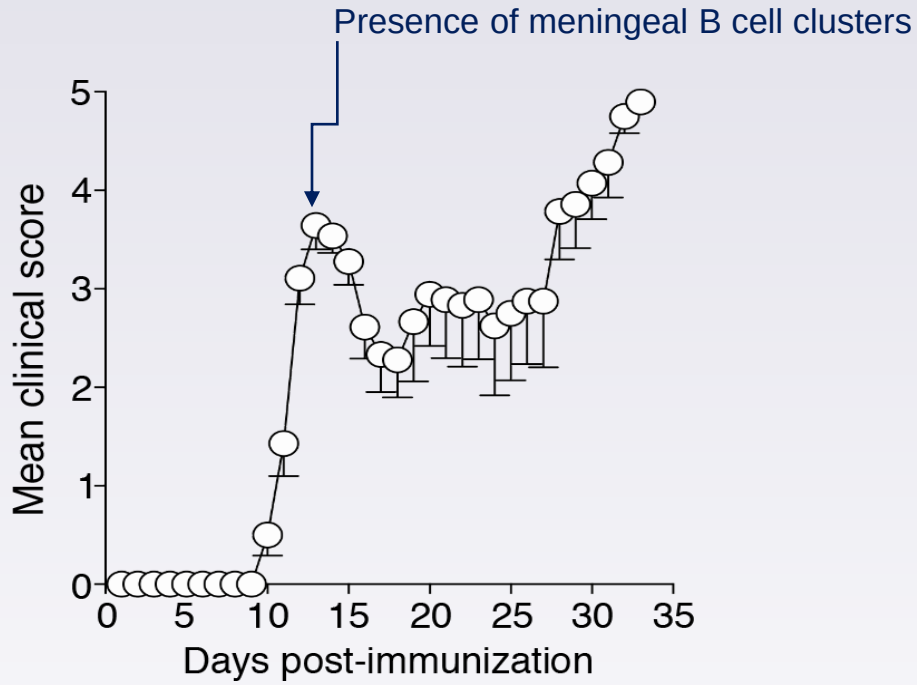
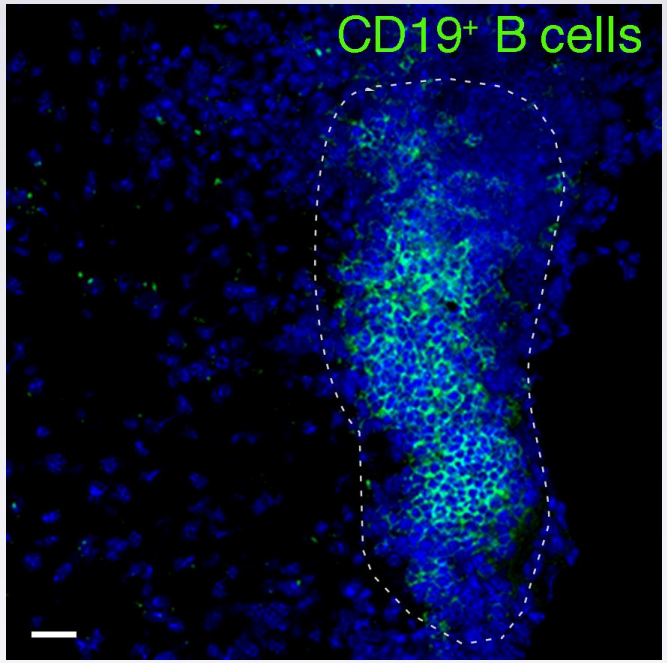
Experimental autoimmune encephalomyelitis model of progressive-like disease (pEAE)

A. Clinical score curve of pEAE



B. Neuropathology of pEAE



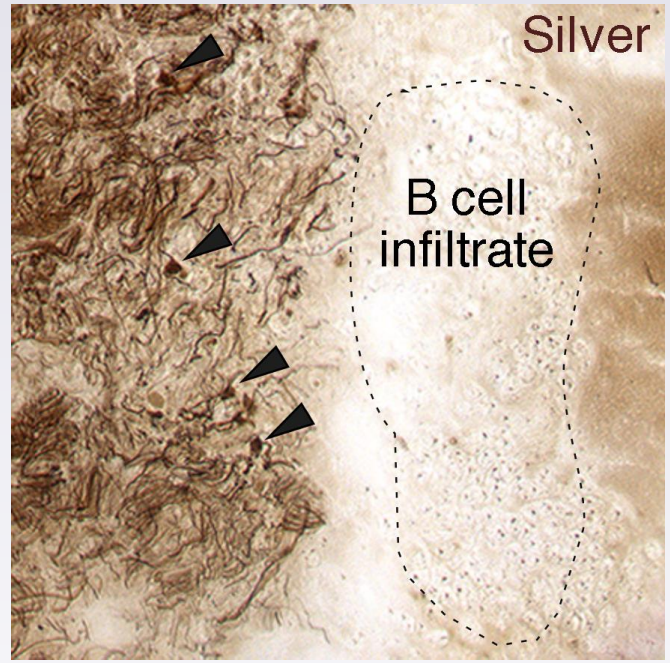


Figure 3. EAE in a model of progressive-like disease (pEAE). 10 wk-old C57BL/6×FVB mice were immunized with $MOG_{35–55}$ peptide. (**A**) Representative disease course with mean clinical score (± SEM). Scoring scale: 0, no clinical signs; 1, limp tail; 2, impaired righting reflex; 3, hindlimb weakness; 4, paralysis of both hindlimbs; 5, moribund/death. *n* = 15. (**B**) Meningeal inflammation consisting of $CD19^+$ B cell-rich clusters (green) that persist until day 34 and are found adjacent to areas of subpial neuronal damage (silver staining) that involve axonal swelling and blebbing (arrowheads). Scale bar: 20 μm.

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Meningeal infiltrates in pEAE express high levels of BTK

- CD19^{+} B cells and Iba-1^{+} microglia/macrophages express BTK, while CD4 T cells are BTK negative

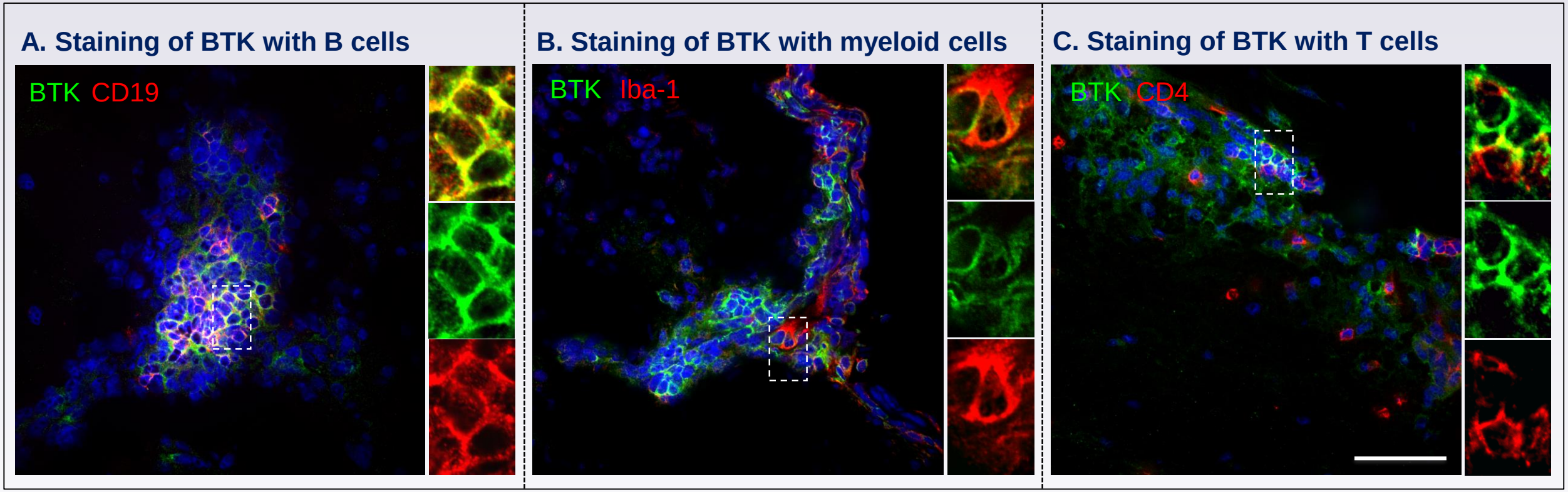

Figure 4. Expression of BTK in the CNS of pEAE mice at day 16. (**A**) CD19^{+} B cells (red) and (**B**) Iba-1^{+} myeloid cells (red) express BTK (green) within meningeal infiltrates in the brain of pEAE mice. (**C**) Lack of co-localization between BTK (green) and CD4 T cells (red) in a spinal cord meningeal infiltrate. Nuclei are stained in blue. High power views (dashed rectangle) are shown to the right. Scale bar: 50 μm.

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Prophylactic treatment with Evobrutinib (BTKi) ameliorates the clinical parameters of EAE in a model of progressive-like disease (pEAE)

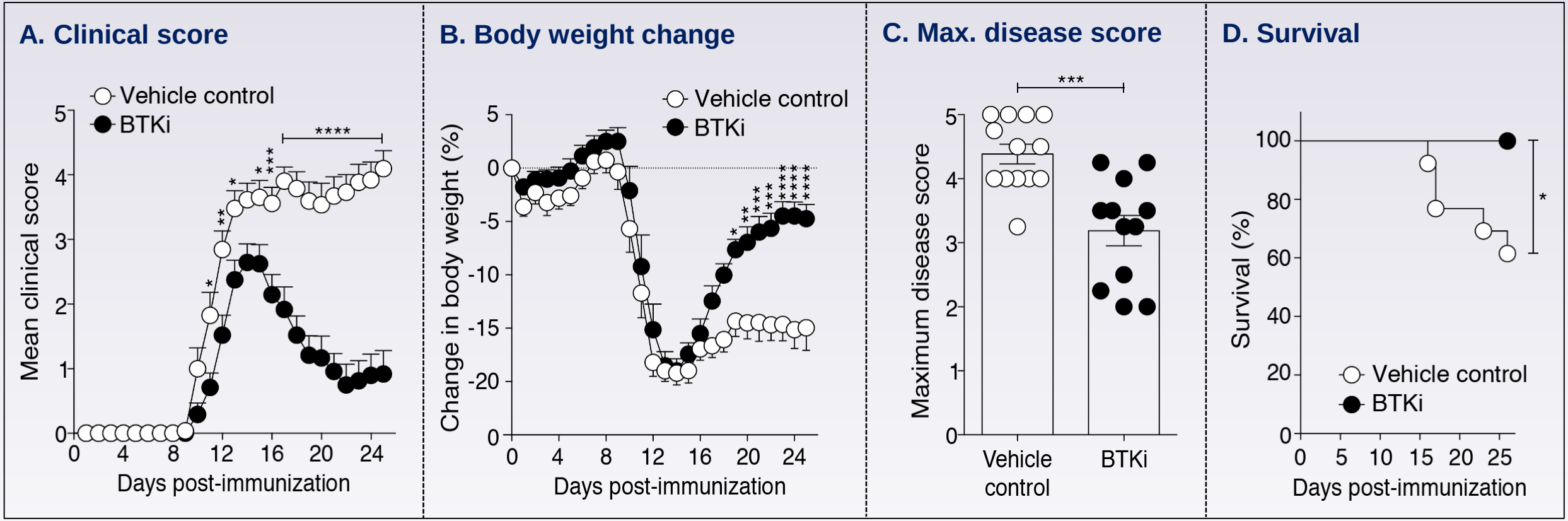

Figure 5. Effect of prophylactic treatment with Evobrutinib on pEAE. (**A**) Clinical disease score (mean ± SEM), (**B**) percentage body weight change, (**C**) maximum disease score, and (**D**) percentage survival rate in control and Evobrutinib (BTKi)-treated mice. Evobrutinib (10mg/kg - orally) was given daily starting the day of immunization.

Statistics: (A and B) Two-way ANOVA with repeated measures and Bonferroni's multiple comparisons test, (C) Mann-Whitney, and (D) Logrank (Mantel-Cox) test. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

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Evobrutinib (BTKi) reduces neuropathology in pEAE

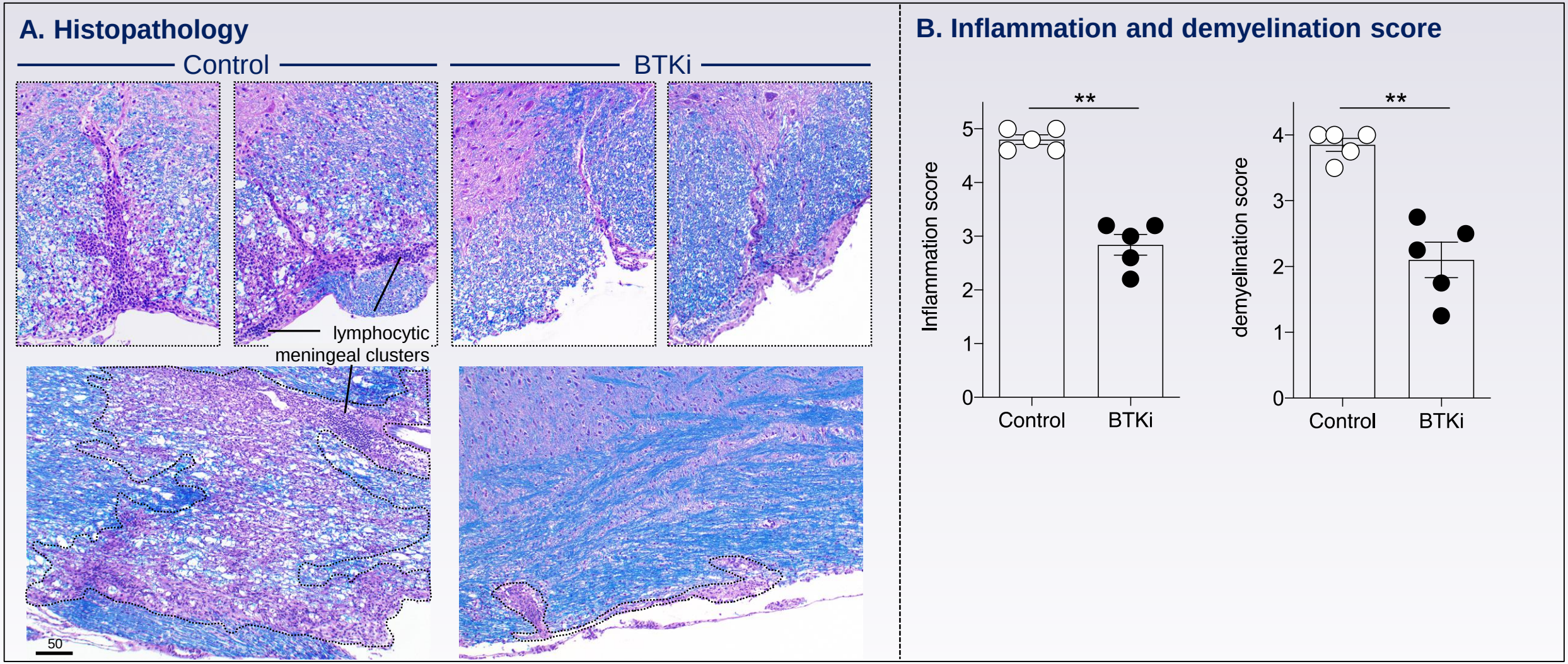

Figure 6. Evobrutinib (BTKi) reduces inflammation and demyelination in pEAE. (**A**) Luxol fast blue and H&E staining of spinal cords from control (*n*=5) and Evobrutinib (*n*=5) pEAE mice at day 25. (**B**) Quantification of inflammation and demyelination in both groups. ***P* < 0.01.

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Conclusions & acknowledgments

- The GME and the pEAE models are characterized by prominent B cell infiltrates in the leptomeninges, associated with subpial neuronal damage in the adjacent tissue, reminiscent of the meningeal inflammation seen in cases of human MS.
- We found robust BTK expression within B cell-rich leptomeningeal infiltrates in both the GME and pEAE models.
- T cells do not express BTK. However, B cells (most likely in the case of GME), and macrophages/microglia express BTK.
- BTK inhibition with Evobrutinib (prophylactic regimen) reduces disease severity and the neuropathology of pEAE, including immune cell infiltration in meninges and parenchyma and the extent of demyelination.

Acknowledgments

- We wish to thank EMD Serono.
- We are grateful to the MSVirtual2020 Congress Planning Committee for the Educational Grant Award to H.K.
- We also acknowledge the Comparative Pathology Core of the School of Veterinary Medicine at the University of Pennsylvania.

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