Title: Inhibition of Bruton's Tyrosine Kinase Selectively Prevents Antigen-Activation of B cells and Ameliorates B cell-Mediated Experimental Autoimmune Encephalomyelitis

Short title: BTK inhibition ameliorates B cell-mediated EAE

Authors

Sebastian Torke[1], Roxanne Pretzsch[1,2], Darius Häusler[1], Roland Grenningloh[3], Ursula Boschert[4], Wolfgang Brück[1] and Martin S. Weber[1,2]

Affiliations

[1]Institute of Neuropathology, University Medical Center, Göttingen, Germany

[2]Department of Neurology, University Medical Center, Göttingen, Germany

[3]EMD Serono Research and Development Institute, Inc. (an affiliate of Merck KGaA, Darmstadt, Germany), Billerica, MA, USA

[4]Ares Trading S.A., an affiliate of Merck Serono S.A., Eysins, Switzerland

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BACKGROUND: B cells are key mediators of inflammatory processes in multiple sclerosis, a notion substantiated by the success of B cell depletion. However, depletion does not only target pathogenic B cells but can also affect regulatory B cell properties. An alternative strategy is the specific inhibition of Bruton´s tyrosine kinase (BTK), an enzyme centrally involved in B cell receptor (BCR) signaling and subsequent activation and differentiation.

METHODS: Mice received oral evobrutinib or vehicle starting 7 days prior to immunization with conformational MOG_{1-117} (a B-cell-mediated model of EAE). EAE severity was assessed using a standard scale (0–5). The B cell phenotype and activation markers on B and T cells were analyzed 12 days post immunization. T cell proliferation and differentiation were assessed after 3-day co-culture with BTKi-treated B cells. Intracellular calcium flux was analyzed using calcium sensitive dyes and anti-IgM BCR stimulation. Cytokine production after BCR stimulation was analyzed by quantitative PCR. Peripheral blood mononuclear cells of healthy individuals were stained for surface markers and/or stimulated using anti-IgM or CpG.

RESULTS: Intermediate and high-dose evobrutinib showed a dose-dependent amelioration of EAE. Evobrutinib led to an accumulation of follicular II B cells and a corresponding reduction of follicular I B cells, a BTK-dependent transition. The expression of activation markers on B and T cells was reduced.

Evobrutinib inhibited the proliferation and pro-inflammatory differentiation of T cells while increasing FoxP3$^+$ regulatory T cells. Evobrutinib reduced the BCR-mediated mobilization of excitatory calcium and the production of interferon-gamma after BCR stimulation. We observed increased expression of BTK and enhanced inducibility of BTK phosphorylation in activated and matured human B cell subsets.

CONCLUSIONS: Evobrutinib efficiently reduces BTK-dependent signaling after BCR stimulation, preventing B cell activation, pro-inflammatory differentiation and function. This translates into reduced CNS inflammation and clinical amelioration in a B cell-mediated EAE model.