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Inhibition of Bruton's tyrosine kinase selectively prevents antigen-activation of B cells and ameliorates B cell-mediated experimental autoimmune encephalomyelitis

UNIVERSITÄTSMEDIZIN GÖTTINGEN UMG

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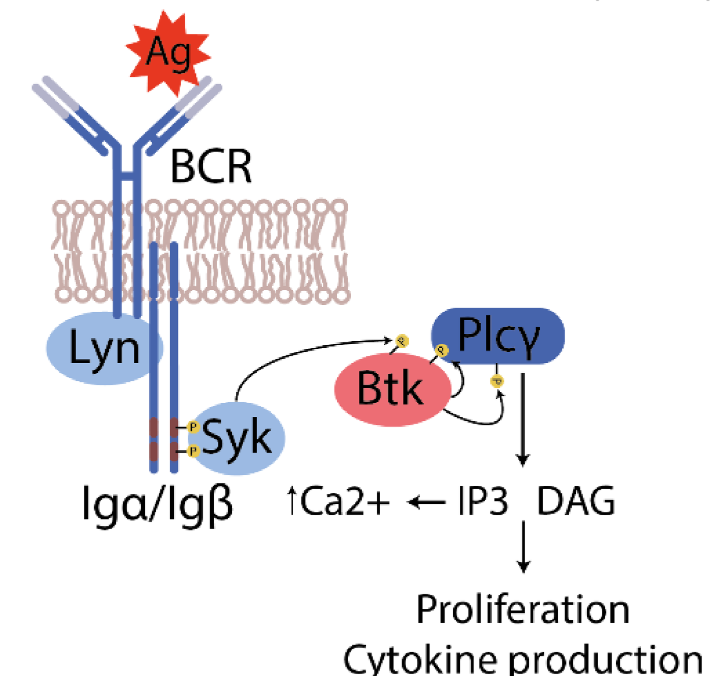
Background and Methods

The role of B cells as key mediators of inflammatory processes in multiple sclerosis (MS) has been increasingly recognized in recent years. This notion was substantiated by the success of pan B cell depletion by anti-CD20 monoclonal antibodies (Mab). However, anti-CD20 Mab not only target pathogenic B cells but can also affect regulatory B cell properties. An alternative strategy may be the therapeutic abrogation of pro-inflammatory B cell functions by Bruton´s tyrosine kinase (BTK) inhibition. BTK is centrally involved in B cell receptor (BCR) signaling and subsequent activation and differentiation of B cells. BTK inhibition (BTKi) could thereby be a promising new strategy in MS to control pathogenic B cell differentiation and function.

Daily treatment with the BTK inhibitor evobrutinib started 7 days prior to immunization with conformational myelin oligodendrocyte glycoprotein (MOG) 1-117 protein, a B cell-mediated model of experimental autoimmune encephalomyelitis (EAE). EAE severity was assessed using a standard scale (0–5). Flow cytometric analysis of the B cell maturation and B and T cell activation was performed 12 days post immunization. Intracellular calcium flux was performed using the calcium-sensitive dyes Fluo-3 and Fura Red and αIgM or αCD3/αCD28 stimulation for B and T cells, respectively. IFNγ production was assessed after 3 hours of αIgM stimulation via qPCR. Evobrutinib treated B cells were co-cultured with naïve MOG-reactive 2D2 T cells for 72h and T cell proliferation and differentiation were assessed via CFSE dilution or positivity for IFNγ, IL17 or FoxP3. Peripheral blood mononuclear cells (PBMCs) of healthy controls were directly used after preparation or thawed from frozen storage, stained for surface markers and/or stimulated using αIgM or CpG.



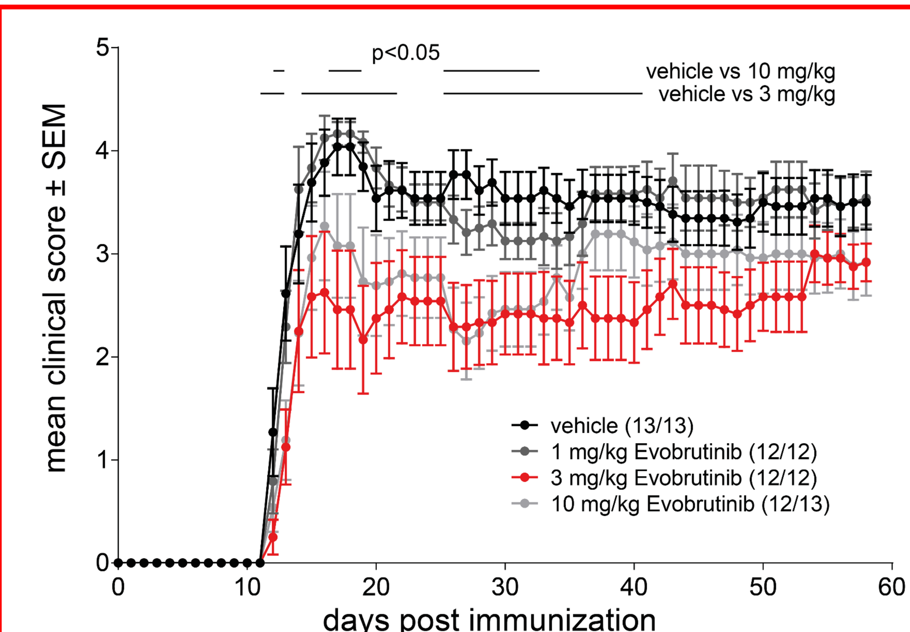
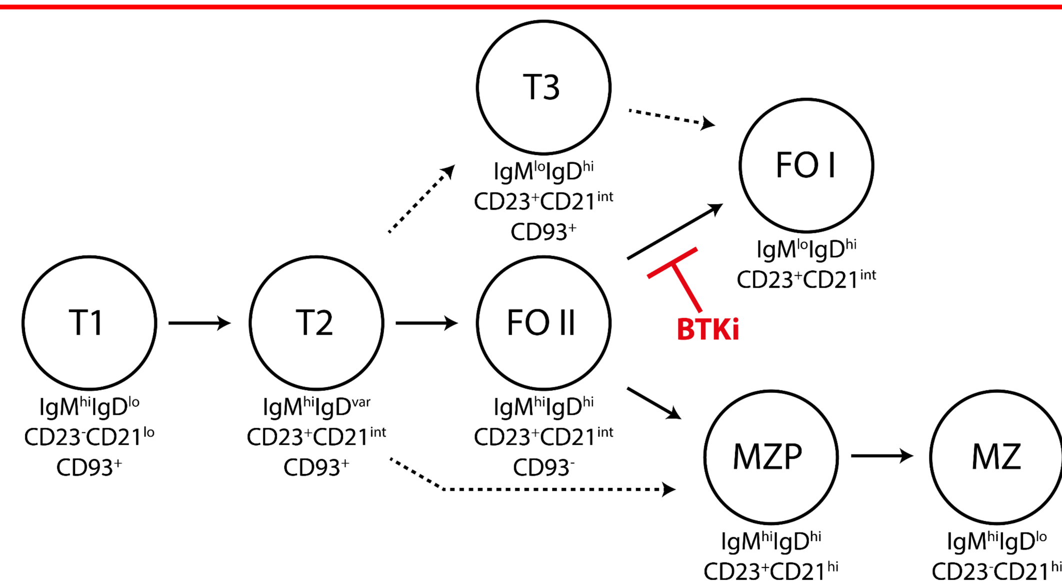


Fig. 1 Prophylactic evobrutinib treatment ameliorates B cell-mediated EAE. Daily, oral treatment started 7 days prior to immunization. Score as follows: 0 = no clinical signs; 1 = tail paralysis; 2 = righting reflex disturbance; 3 = beginning hind limb paresis; 4 = paralysis of both hind limbs; 5 = moribund / death. n=12-13, mean ± SEM; incidence in brackets



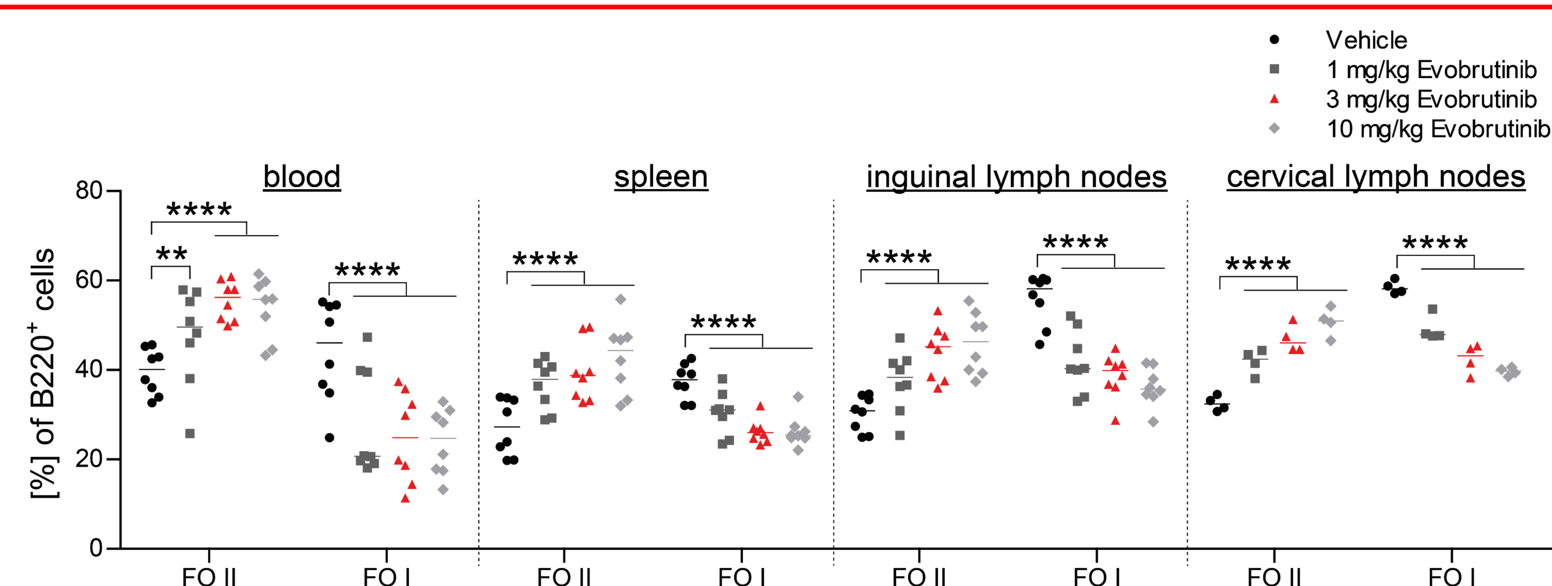


Fig. 2 Evobrutinib inhibits BTK dependent FO II to FO I maturation of B cells. B cells were categorized into transitional (T1-T3), follicular (FO), marginal zone precursor (MZP) and maginal zone (MZ) cells. B cell maturation was assessed in the respective organs 12 days after MOG protein immunization. n=8, mean, ** p<0.01, **** p<0.0001

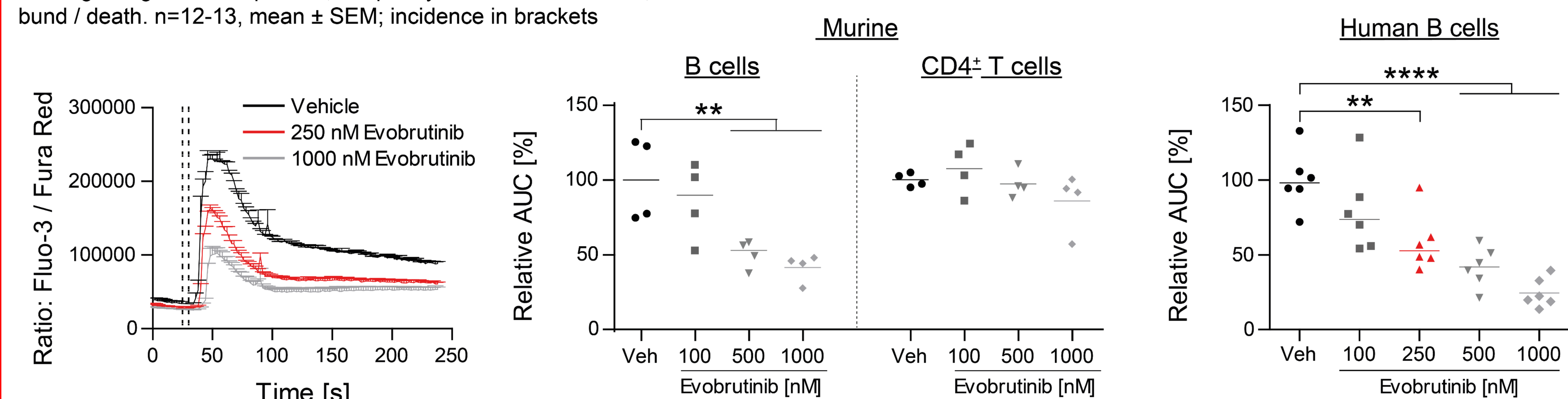


Fig. 3 Intra-cellular calcium mobilization is inhibited by evobrutinib selectively on murine and human B cells. Calcium flux analysis was performed on murine or human B cells using anti-IgM BCR stimulation and on T cells using CD3 / CD28 crosslinking and is displayed as area under the curve (AUC) relative to vehicle control. n=4-6, mean (± SEM), ** p<0.01, **** p<0.0001

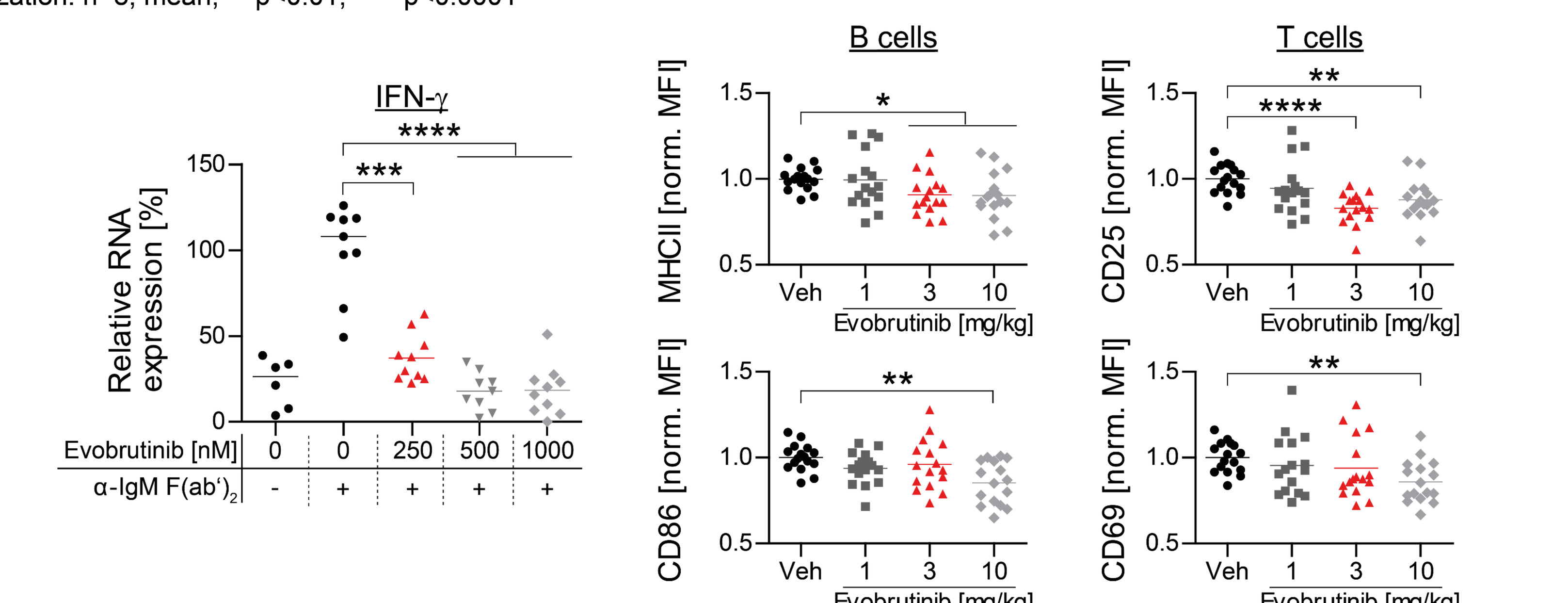


Fig. 4 Evobrutinib inhibits cytokine production of B cells and expression of activation markers on B and T cells. Isolated splenic B cells were treated with the indicated concentrations of evobrutinib and stimulated using αIgM. Cytokine production was analyzed after 3 hours by quantitative PCR. Splenocytes were isolated 12 days after MOG protein immunization and analysed via flow cytometry. n=6-16, mean, * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001

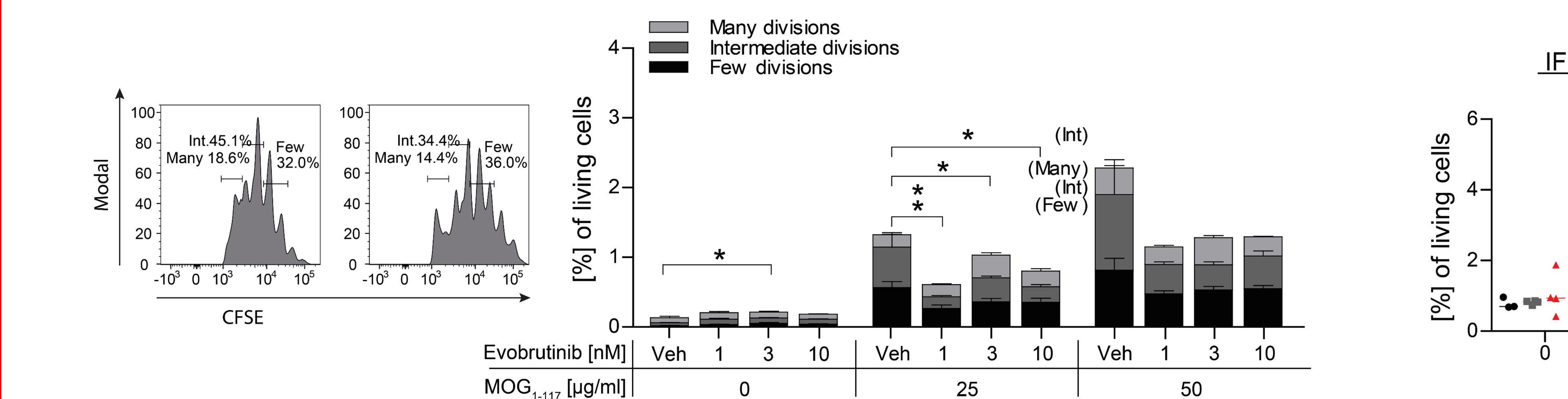


Fig. 5 Evobrutinib inhibits B cell APC function and pro-inflammatory T cell differentiation. Oral treatment of wildtype (WT) mice started 7 days prior to immunization with 75 µg conformational MOG1-117 protein. Splenic B cells from mice 12 days after immunization or WT T cells from 2D2 mice were isolated by magnetic separation. T and B cells were co-cultured for 72h. T cell proliferation was analyzed by CFSE dilution. T cell differentiation was analyzed by intra-cellular flow cytometry for the production of IFN-γ, IL-17 and FoxP3. Mean±SEM, n=4-8 representative data or pooled from at least 2 independent experiments, * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

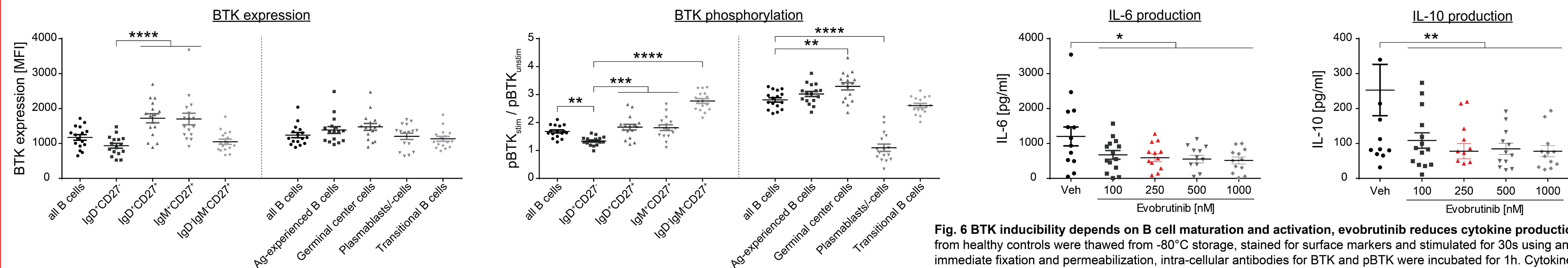


Fig. 6 BTK inducibility depends on B cell maturation and activation, evobrutinib reduces cytokine production. PBMCs from healthy controls were thawed from -80°C storage, stained for surface markers and stimulated for 30s using anti-IgM. After immediate fixation and permeabilization, intra-cellular antibodies for BTK and pBTK were incubated for 1h. Cytokine production was analyzed by ELISA after 3 hours of CpG stimulation. n=16-18, * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

Conclusion and Outlook

Inhibition of BTK reduces the influx of excitatory calcium in B cells upon BCR stimulation and prevents activation and maturation of B cells. This translates into reduced B-cellular cytokine production and antigen-presentation, impairing the development of encephalitogenic T cells. This ultimately reduces CNS infiltration and inflammation, leading to clinical amelioration in a B cell-accentuated EAE model. Taken together with the increased expression of BTK and the enhanced inducibility of BTK phosphorylation in activated and matured human B cells, these findings highlight BTK as a promising new target in inflammatory CNS disease. We are currently further investigating B cell function after BTKi treatment and the potential of evobrutinib in a sequential therapeutic approach after pan B cell depletion to control pathogenic activity of reappearing B cells.

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Sebastian Torke: Nothing to disclose. Roxanne Pretzsch receives a medMS doctoral stipend from the Hertie foundation. Darius Häusler: Nothing to disclose. Roland Grenningloh: Employee of EMD Serono. Ursula Boschert: Employee of MerckSerono. Wolfgang Brück: received honoraria for lectures by Bayer Vital, Biogen, Merck Serono, Teva Pharma, Genzyme, Sanofi-Aventis and Novartis. Member of scientific advisory boards for Teva Pharma, Biogen, Novartis, Celgene, Medday and Genzyme. Receives research support from Teva Pharma, Biogen, Medday, Genzyme and Novartis. Serves on the editorial boards of Neuropathology and Applied Neurobiology, Therapeutic Advances in Neurological Diseases and Multiple Sclerosis International. Martin S. Weber: Editor for PLoS One. Received travel funding and/or speaker honoraria from Biogen-Idec, EMD Serono, Novartis, Roche and Bayer. Receives research support from the National Multiple Sclerosis Society (NMSS; PP1660), the Deutsche Forschungsgemeinschaft (DFG; WE 3547/4-1), from Novartis, EMD Serono, TEVA, Biogen-Idec, Roche and the ProFutura Programm of the Universitätsmedizin Göttingen.