Toxicity screen in purified B cell assay

Version 1.1 (8th April 2024)

Disease area

Multiple sclerosis (MS) is a chronic inflammatory, demyelinating, and neurodegenerative disease in the central nervous system (CNS), around 2.9 million people worldwide are suffering from the disease up to 2023. The typical disease onset is usually between the 3rd and 4th decades of life, with women being affected 2-3 times more often than men. There is no cure for MS, but in the past decades, many drugs developed that can improve long-term outcomes of MS, termed disease modulatory theraps (DMTs), majority of them are immununomodulators, however, they are restricted to relapse remiting MS (RRMS), the majority patients subtype, and considered unresponsive to the secondary progressive MS (SPMS), so far, only one approved drug for PPMS and SPMS. Since development of disability is a late and largely inreversible phenomenon, therefore, it is best to develop a treatment regimen during the earlier relapse remitting phase, in which the inflammation believed to be dominated feature of the disease pathogenesis [1]

Rationale

Historically MS has been considered a T-cell mediated disorder [2], however increasing evidence from past decades suggest that B cells are not only a homogeneous population as antibody producing cells, but also an important player with antibody-independent function, such as antigen presenting and cytokine production. Observational studies using selective B cell depletion therapy (anti-CD20 antibodies) showed highly beneficial effects in limiting new MS disease activity [3, 4] precedes obvious total serum IgG level reduction, but rather decreasing of several proinflammatory mediators such as IL-6 and TNF-α, which indicate that the beneficial effects of B cell depletion therapies attributes to the antibody-independent role of B cells[5].

Aim

To profile purified B cell healthy status upon stimulation in presence of well-defined chemical probes and compounds.

Methods

General protocol: CD19+ B cells purified from Peripheral Blood Mononuclear Cells (PBMCs) are cultured for 2 days with presence of stimulation cocktail and compounds. The effect on cytokines secretion from stimulated B cell upon addition of chemical probes is investigated by multiplex beads array in cell supernatants and cell health status is measured by CellTiter-Glo assay.

Cell culture condition: Cell are cultured in 384 well plate at 100 000 cells/ml, 50µl/well, in duplicates. Culture medium is RPMI supplemented with 10% heat inactivated bovine serum. Chemical probes (at final concentration 1 µM) are preloaded to plate at start of culture and pre-treated for 30 min before stimulation cocktail added. Controls include unstimulated condition (cell in culture medium without added cytokines), stimulated condition with vehicle control only (0,1% DMSO), stimulated condition with the Bruton's tyrosine kinase inhibitor ibrutinib in 0.1% DMSO.

Readout: Flow cytometry is done on freshly isolated B cells to determine the purity and the phenotype of different B cell subsets. Viability of cells is measured at end of culture with CellTiter-Glo assay.

Results

A set of 59 GPCRs antagonists and agonists were cherry picked from the chemogenomic library targeting the GPCRs, the compounds were tested in 6 blood donors including 3 newly diagnosed MS patients without treatment and 3 healthy blood donors. The effect on viability of B cells on day 2 of cell culture in presence of B cell stimulating agents are expressed as fold change normalized to the vehicle control (0,1% DMSO).

The results show that none of the screened compounds compromised cell health status, as defined if more than 20% decrease in cell viability when normalized to the DMSO control.

Raw data excel file is available here.

References

1. Filippi, M., et al., *Multiple sclerosis.* Nat Rev Dis Primers, 2018. **4**(1): p. 43.
2. Dendrou, C.A., L. Fugger, and M.A. Friese, *Immunopathology of multiple sclerosis.* Nat Rev Immunol, 2015. **15**(9): p. 545-58.
3. Bar-Or, A., et al., *Rituximab in relapsing-remitting multiple sclerosis: a 72-week, open-label, phase I trial.* Ann Neurol, 2008. **63**(3): p. 395-400.

4. Techa-Angkoon, P., et al., *Current evidence of rituximab in the treatment of multiple sclerosis.* Mult Scler Relat Disord, 2023. **75**: p. 104729.
5. Li, R., K.R. Patterson, and A. Bar-Or, *Reassessing B cell contributions in multiple sclerosis.* Nat Immunol, 2018. **19**(7): p. 696-707.