Inhibition of Bruton’s Tyrosine Kinase Prevents Inflammatory Macrophage Differentiation: A Potential Role in Multiple Sclerosis
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Objective:
To investigate the effects of Bruton’s tyrosine kinase (BTK) inhibition (using evobrutinib and other BTK tool inhibitors) on differentiation and activation of monocytes and macrophages, which may contribute to multiple sclerosis (MS) disease activity and progression.

Background:
BTK mediates B cell receptor (BCR) and Fc receptor (FcR) signaling in several hematopoietic cells, including macrophages. BTK inhibitors (BTKi) silence B cells, preventing innate immune activation via FcR, suggesting they may be beneficial for treating autoimmune diseases with B cell involvement. Furthermore, BTK has been implicated in mediating signaling of certain cytokine receptors that control macrophage differentiation. The highly-specific oral BTKi evobrutinib inhibited disease development in an experimental autoimmune encephalomyelitis model not amenable to B-cell inhibition, indicating efficacy is mediated by effects beyond the BCR.

Design/Methods:
Peripheral blood monocytes were isolated from healthy volunteers and BTK activation analyzed by Western blot following 30-minute BTKi treatment and subsequent granulocyte-macrophage colony-stimulating factor (GM-CSF) stimulation. GM-CSF-differentiated M1 cell survival was analyzed by flow cytometry following AnnexinV/PI staining. IL-1ß and IL-10 expression was determined by qPCR 48 hours post-GM-CSF stimulation and BTKi treatment. TNF-α levels in cell culture supernatants were measured by ELISA following overnight LPS stimulation and BTKi treatment. M2 macrophage apoptotic cell uptake was analyzed by flow cytometry.

Results:
BTK was activated downstream of the GM-CSF receptor. GM-CSF differentiated M1 cells underwent apoptosis upon BTK inhibition with evobrutinib. In the presence of BTKi, GM-CSF-treated monocytes secreted less TNF-α and expressed less IL-1ß, in addition to upregulating anti-inflammatory gene (e.g. IL-10) expression. Furthermore, BTKi treatment increased M2 macrophage-mediated phagocytosis in vitro.

Conclusions: BTK inhibition hindered M1 macrophage differentiation and skewed monocytes towards an M2 phenotype, while enhancing apoptotic cell uptake by M2 cells. Therefore, BTK inhibition could have additional benefit in the treatment of MS, by targeting both B cells and myeloid cells simultaneously.