Abstract

Regulatory B lymphocytes (Bregs) represent a small heterogeneous subpopulation of B cells which participate in a regulation of immune responses by the antibody-independent mechanisms. The main mechanisms of Breg action is a production of anti-inflammatory cytokines or a direct cell contact through their surface molecules. This study deals with an induction of suppressive Bregs from mouse spleen B cells *in vitro*. We were aiming for a description of an influence of the selected cytokines to the induction of Bregs from lipopolysaccharide (LPS)-stimulated B cells and a determination of the mechanism of Breg action. We also analyzed the ability of inducted Bregs to produce interleukin-10 (IL) and to express genes for Fas ligand (FasL) and programmed death ligand 1 (PD-L1) molecules. We found that only two cytokines, IL-12 and IFN-γ, supported development of Bregs in a population of LPS-stimulated B cells. IFN-γ enhanced production of IL-10 and gene expression of FasL and PD-L1. Furthermore, we analyzed effects of Bregs on macrophages and their following action on T cells. Expression of costimulatory molecules CD80 and CD86, gene expression of IL-1α and the production of IL-6 were tested to determine the effects of macrophages on T cells. Macrophages influenced by Bregs had decreased ability to stimulate proliferation of activated $CD8^+$ T cells. Cocultivation of T cells with the influenced macrophages resulted in a decreased production of proinflammatory cytokine IL-17. Our results showed that IL-12 and IFN-γ enhanced activation of Bregs in a population of LPS-stimulated B cells and Bregs are capable to suppress immune response. A possibility to induce Bregs *in vitro* may have a great impact for their potential use in a clinical setting.

Key words: regulatory B lymphocytes, IL-10 production, cytokines, immunosuppression