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Characterization of the importance of different signaling pathways leading to IL-10 production in human B cells

B cells are central players of the adaptive immune response due to their capability to present antigens, produce antibodies, and secrete cytokines. Besides these positive regulatory roles, the ability of B cells to negatively regulate cellular immune responses and inflammation through their IL-10 production has been described recently. IL-10 is an anti-inflammatory cytokine that is crucial in the down-regulation of immune response by limiting immune cell activation thus protecting self structures from damage. Regulatory potential of IL-10 producing B cells (termed B10 or B_{reg} cells) have been demonstrated in a variety of mouse models of human autoimmune diseases. Adoptive transfer of B_{reg} cells into wild type mice reduces the severity of experimental autoimmune encephalomyelitis, collagen-induced arthritis and systemic lupus erythematosus. Currently, there is no B_{reg} specific phenotypic surface or intracellular marker (such as $FoxP3^+$ for T_{reg} cells), thus this is a functionally defined subset, identified only by their competency to produce and secrete IL-10 following appropriate stimulation. Both human and mice has specific B cell subpopulations which are highly inducible to produce IL-10: $CD1d^{hi}CD5^+$ B cells in the peritoneum, $CD1d^{hi}CD23^-IgM^{hi}CD1d^{hi}$ marginal zone B and $CD1d^{hi}CD23^+IgM^{hi}CD1d^{hi}$ T2 marginal zone precursor B cells in the spleen of mice; and the $CD24^{hi}CD27^+$, $CD24^{hi}CD38^{hi}$ and $CD27^+CD38^{hi}$ subpopulations in human blood were reported, respectively.

Ex vivo amplification and re-administration of this rare IL-10 producing B cell subset may have therapeutic potential in human autoimmune diseases. The magnitude of B_{reg} cell expansion is very important since the number of cells infused during adoptive transfer experiments is critical. Furthermore, since this method will be used for treatment of active disease, time dependency is also a crucial question. Tedder and colleagues reported that expansion of mouse B_{reg} cell *ex vivo* can be

accomplished within 9 days by means of combined CD40L, B lymphocyte stimulator, IL-4 and IL-21 stimulation [1], however the effective conditions for human application is still unknown.

Various agents such as cytokines, TLR ligands, CD40L etc. were reported in the last few year that can induce IL-10 production. Understanding the signaling machinery and the crosstalk between these signaling pathways in human B cells would be helpful to choose the most potent stimulators.

IL-10 is known to boost its own transcription through STAT3 by an autocrine positive feedback loop. In addition, IL-21 and IL-35 also drives B_{reg} development using the same pathway [2]. Kurosaki et al. reported that BCR-induced Ca^{2+} flux resulted in IL-10 production in a PLCγ - Calcineurin - NFAT-dependent way [3]. CREB - another transcription factor - can also positively regulate IL-10 through the PI3K-AKT-GSK3 axis [4]. Besides this, ERK and p38 MAPKs activate CREB directly or indirectly through MSK1 [5]. Finally, confusing reports were published about Lyn and Syk function in IL-10 production [6, 7].

A huge mass of data accumulated in the last few years – most of them on transfected cells, however, upstream signaling pathways leading to the development of human B_{reg} cells are unknown. Therefore, our aim is to follow the activation of these pathways and asses their distinct role in the IL-10 production of primary human B cells.

Peripheral blood mononuclear cells (PBMC) will be isolated from healthy individuals, stimulated with various agents such as anti-Ig, CD40L, CpG, IL-10, IL-21 and IL-35. and measure the IL-10 mRNA levels from sorted B cells after 6h. The activation status of AKT, CREB, ERK, p38, PLCγ and STAT3 will be measured by phospho-specific antibodies on flow cytometer. To assess the importance of these signaling pathways on IL-10 production, IL-10 mRNA levels from AKT-, ERK-, p38-, PLCγ- and STAT3-specific inhibitor-pretreated samples will be compared. We also plan to sort B cells from human PBMC to determine the secreted IL-10 levels from the supernatant of such samples after 48h.

Expanding human B_{reg} cells *ex vivo* can open new horizons in the potential therapeutic applications of this rare B cell subset. We hope that our study will help to find the way to an effective method for the multiplication of the human B_{reg} population. ImmunoTools reagents would be beneficial during our work.

References:

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