ImmunoTools *special* Award 2013

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Regulatory B cells is human

Rheumatoid arthritis (RA) has a worldwide distribution with an estimated prevalence of 0.5-0.75 %. Although the etiology of the disease is still unknown, several risk factors have been identified. Like many autoimmune diseases, RA occurs more frequently in women than in men (3:1 ratio), suggesting a role for sex hormones. Genetic studies have demonstrated that a genetic predisposition resides in the HLA-DR locus. There is also evidence that environmental factors, such as smoking, stress etc. may play a role. RA is characterized by the inflammation in the synovial membrane of diarthrodial joints. Activated leukocytes, neutrophils, CD4 T cells, B cells and monocytes infiltrate the synovial membrane. In addition synovial fibroblasts are involved in the manifestation of the disease. Hyperplasia and chronic inflammation, ultimately lead to the destruction of cartilage and bone. Pain, fatigue and disability in RA can profoundly affect the quality of life.

Recent data strongly suggest that B cells are crucial players in RA. B cells not only provide support to T cells via direct cell-cell contact, but also shape the immune response by producing pro-inflammatory cytokines including IL-6, IFNγ and LTβ.

The relative newcomers in the B cells' world, regulatory B cells (Breg) add a further level to the complexity of regulation autoimmunity. Breg cells have the capacity to control inflammation that may be driven by Th1, Th2 or Th17 cells. This is mediated by IL-10 that directly suppress the differentiation of pathogenic T cells and promotes the development of regulatory T-cells (Treg). IL-10 producing B cells have diverse phenotype (CD19+ CD24hi CD38hi, CD19+ CD24hi CD27+); furthermore, partly different stimuli induce the production of IL-10 in human B cells.

TLR9, a bacterial DNA senor is a potent inducer of IL-10 , however human B cells may also produce IL-4, IL-6, IL-12 and IFNγ that may have an impact on T-cell polarization. It is not known how the production of these cytokines is controlled in vivo during inflammation. Potentially, the suppressive effect of Breg cells should be beneficial in inflammatory autoimmune disease such as RA.

Therefore, the aim of this study is to characterize subpopulation(s) of human peripheral B cells producing IL-10, to test their suppressive function and to find the optimal signals (BCR and/or TLR9 and/or CD40L) that induce human Breg cell differentiation and IL-10 production.

B cells from healthy individuals and RA patients will be purified by negative selection, and then the following subsets will be isolated by flow cytometry-based cell sorting: CD19+ CD27+ memory and CD19+ CD27- naïve B cells, CD19+ CD24hi CD38hi (Breg including immature B cells). Intracellular staining for cytokines (IL-10, TGFβ, TNFα, IL-1, IL-6, IFNγ) will be analyzed by flow cytometer.

One of the most effective biological disease modifying anti-rheumatic drugs (DMARDs) is Rituximab, a human CD20-specific chimeric monoclonal antibody that depletes CD20 bearing B cells. Our aim is to follow reemerging various B cell subsets (naïve, memory, Breg) after rituximab treatment. Other biological therapies (TNF blockers or IL-6 receptor antagonist) may also profoundly affect B cell's function. We are going to monitor B cell subsets in groups of RA patients following biological therapies. The ImmunoTools reagents will be beneficial during this study.