ImmunoTools *special* Award 2023

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PD-1/PD-L1 Interaction in Modulating Th, Tph, and Treg cells Activation: Implications for Immune Regulation in Health and Rheumatoid Arthritis

The proposed study aimed to address three questions. Starting with how regulatory B cells (Bregs) modulate $CD4^+$ T cell subsets‘ activation (Th, Tph, Treg) via PD-1/PD-L1 molecules, then how does the modulation differ among $CD4^+$ T cell subsets and finally, does PD-1/PD-L1 interplay differently modulate $CD4^+$ T cell subsets in healthy controls (HCs) and rheumatoid arthritis (RA) patients. To answer these questions, peripheral blood samples will be collected from HCs and RA patients. Peripheral blood mononuclear cells will be isolated using density gradient method. T and B cells will be negatively isolated using magnetic activating cell sorting, then Th, Tph, and Treg will further be sorted from T cells using fluorescence activated cell sorter by targeting CD4, CXCR5, CD25, CD127, CX3CR1 and CD45RA, the sorted subsets will be validated using monoclonal antibody conjugates against Bcl-6, BLIMP1 and Foxp3 for Th, Tph, and Treg cells respectively. Afterward, we will activate the sorted $CD4^+$ T cell subsets by anti-CD3 + anti-CD28 for two days before co-culturing with $PDL\text{-}1^+$ B cells, and the activation status will be checked by targeting PD-1 marker. B cells will also be pre-stimulated by CpG ODN, and hsCD40L, which induce PD-L1 expression on Breg cells. Finally, we will evaluate cellular interactions by monitoring $CD4^+$ T subsets proliferation, proinflammatory cytokine secretion, and signal transduction in different culture systems prepared from $PD\text{-}1^+$ activated $CD4^+$ T cell subsets and $PD\text{-}L1^+$ Breg cells.

For $CD4^+$ T subsets proliferation assay, Th, Tph, and Treg cells will be uploaded with Carboxyfluorescein succinimidyl ester (CFSE) before stimulation then each subset

will be stimulated as stated above, then co-cultures and triple-cultures will be generated by adding PD-L1$^+$ Breg cells or/and Treg cells to Th and Tph cells monocultures. The proliferation of Th and Tph cells will be evaluated by monitoring CFSE dilution after excluding B cells via anti-CD19 antibody conjugate using flow cytometry.

The proinflammatory cytokine production (IL-21, TNF-α, IFN-γ, IL-17A and IL-6) by Th and Tph cells will be assessed intracellularly from the same culture systems using fluorochrome conjugated antibodies specific for individual cytokines following the intracellular staining protocol.

The expected results would answer the question whether Th, Tph and Treg cells from HCs and RA patients would respond differently to the interaction with Breg cells via the PD-1/PD-L1 crosslinking, and this would provide additional new information for future autoimmune therapeutic approaches.

ImmunoTools antibodies utilization: As mentioned above, CD4$^+$ T cell subsets, including Th, Tph, and Treg cells, will undergo sorting using monoclonal antibody conjugates. As such, the following antibodies from ImmunoTools will be employed for this assay: anti-CD4-PerCP, anti-CD25-PE, and anti-CD45RA-FITC. Subsequently, Th, Tph, and Treg cells will be subjected to stimulation with anti-CD3 and anti-CD28, with the additional use of rh-IL-2 exclusively for the activation of Treg cell cultures. To assess the activation status of Th, Tph, and Treg cells before co-culture formation and in the subsequent proinflammatory cytokine evaluation, anti-CD127-FITC, and anti-PD-1-PE and anti-CD4-PerCP will be employed. In the proinflammatory cytokine analysis, anti-PD-1-PE will be replaced with anti-TNF-α-PE to evaluate functional differences in distinct culture systems. Anti-CD19-APC will be used during proliferation assay to exclude B cells from the analysis in co-culture system. Thus, the ImmunoTools reagents represent a pivotal aspect of this study, given their integral role in every stage of the study.

ImmunoTools *special* AWARD for **Mustafa Mohammed Hamid Talib**
includes 7 reagents

FITC - conjugated anti-human CD45RA, CD127

PE - conjugated anti-human CD25, CD279, TNF-alpha

PerCP - conjugated anti-human CD4

APC - conjugated anti-human CD19

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