ImmunoTools *special* Award 2023

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Targeting PIM1 kinase in T cells in ANCA-associated vasculitis

ANCA-associated vasculitides (AAV) are a group of autoimmune diseases characterized by the inflammation of small blood vessels that predominantly affect the airways, kidneys, and lungs. The pathogenesis of this disease involves the presence of anti-neutrophil cytoplasmic antibodies (ANCAs). The disease is treatable; however, 10% of all patients in remission relapse yearly (1). With the cumulative flares of disease activity, the risk of irreversible organ damage increases. Therefore, novel therapeutic strategies and relapse risk markers are essential for preventing disease relapse.

The pathogenesis of AAV is not fully understood but involves both innate and adaptive immune effector pathways. Aberrations in several T cell subsets, both numerical and functional, have been identified in AAV patients (1,2). However, their significance in AAV pathogenesis remains unclear. Our group has generated single-cell RNA sequencing (scRNA-seq) data of peripheral blood mononuclear cells (PBMCs) from AAV patients with active disease and healthy controls (HCs). Differential expression analysis comparing scRNA-seq datasets from AVV and HC revealed that mRNA expression levels of the PIM1 gene (Pim-1, Proto-Oncogene, Serine/Threonine Kinase) were upregulated in T cells from AVV patients, including sub-populations of naive $CD4^+$ T cells, effector memory $CD4^+$ T cells (Tem), and regulatory T cells (Tregs). Elevated expression levels of PIM1 in $CD4^+$ Tem and Tregs were confirmed in previously generated RNA-seq datasets from bulk-sorted Tem and Tregs of active AAV patients.

Moreover, previous studies have demonstrated that pim-1 plays a key role in human Th1 differentiation, promoting IFN-γ production, while at the same time repressing Th2 differentiation (3). Pim-1 activation has also been shown to inhibit Treg suppressive functions (4). These observations are consistent with aberrations in the T cell compartment reported in AAV patients and suggest that pim-1 inhibition may be a therapeutic option in AAV patients (5).

In this project, we aim to perform *in vitro* studies to observe the kinetics of pim-1 expression and investigate the effects of pim-1 inhibitors on $CD4^+$ T cell activation, proliferation, and differentiation. Briefly, purified $CD4^+$ T cells from AVV patients and HCs will be stimulated with IL-6, IL-15, anti-CD3/28 together with or without pim kinase inhibitors for 3 - 6 days at 37ºC. To assess proliferation, the cells will be stained with cell proliferation dye prior to culturing. After co-culture at the indicated time points, the cells will be collected to determine the kinetics of pim-1 mRNA and protein expression levels using qRT-PCR and western blot respectively. Simultaneously, the cells will be harvested, stained with antibodies for surface markers and intracellular cytokines, and analyzed by flow cytometry.

Therefore, we are submitting our application for the ImmunoTools Special Award, which, if awarded, will enable us to explore our research questions and gain deeper insights into T-cell activity and function in AAV pathogenesis. This will have the potential to uncover new therapeutic targets and markers for disease relapse.

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