ImmunoTools *special* Award 2025

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CD300F IMMUNE RECEPTOR IN REGULATION OF INNATE IMMUNITY

Our group is interested in the role of CD300f in regulating inflammatory signals based on its predominant expression on myeloid lineage cells. CD300f may play an important role in regulating inflammatory signals. Its expression in effector immune cells such as mast cells and macrophages position it as a key immunomodulatory receptor. The natural variants of the receptor can be on the molecular basis of inflammatory diseases and be a useful biomarker. Deciphering their role may be necessary for better predicting, diagnosing, and treating pathologies with an inflammatory profile.

CD300f is a unique receptor as it displays dual activating/inhibitory capacity, interacting with SHP1/SHP2 phosphatases but also with PI3K and Grb2 adaptors (*Alvarez‐Errico et al. 2004, Alvarez-Errico, Sayós and López-Botet 2007*). Interestingly, CD300f amplified the IL-4 or IL-13 activation of macrophages, mast cells, eosinophils, and dendritic cells in culture and also after in vivo lung inflammation, effects that were all importantly dampened in CD300f deficient animals (*Moshkovits et al. 2015*). Though CD300f has been defined as a dual activating/inhibitory receptor necessary for the phagocytosis of apoptotic cells, in vivo studies, suggest that CD300f acts as an inhibitory receptor dampening inflammatory processes in different disease models such as lupus, multiple sclerosis (EAE), and allergy (*Tian et al. 2014*) (*Xi et al. 2010*). The anti-inflammatory effect of CD300f is probably mainly mediated by SHP1/SHP2-dependent inhibition of Toll-like receptor (TLR) signaling by blocking MyD88 and TRIF adaptors (*Kim et al. 2012*). Moreover, it also inhibits mast cell degranulation by SHP1/SHP2 recruitment and inhibition of FcεRI activation (*Alvarez‐Errico et al. 2004*).

Interestingly, CD300f expression and ligand distribution are found in the CNS, and a protective function of this immune receptor has been demonstrated in an excitotoxic acute brain injury (*Peluffo et al. 2012*). Moreover, CD300f modulates macrophage

phenotype, delaying axonal regeneration in a model of peripheral nerve injury (*Peluffo et al. 2015*). Lately, it has been shown that CD300f-deficient microglia have profound metabolic alterations under inflammatory conditions, reducing glycolysis, fatty acid catabolism, mitochondrial oxidative phosphorylation, and autophagy. CD300f is among the most upregulated genes in brain microglia/macrophages after several proinflammatory insults such as intraperitoneal LPS injection or spinal cord injury, and its upregulation has been associated with promyelinating microglia after demyelinating stimuli. (*Lloyd et al. 2019, Evans et al. 2023*) The data suggest that CD300f is essential for maintaining metabolic fitness and reprogramming in microglia and macrophage populations. Microglia is one of the significant pathogenic components in neuroinflammation and is known to respond to proinflammatory mediators released from immune cells such as mast cells (*Skaper, Facci, and Giusti 2014, Skaper, Giusti and Facci 2012*). Mast cells reside in the brain and are an essential source of inflammatory molecules. Mast cells are considered first responders and can initiate and magnify immune responses in the brain (*Skaper et al. 2012*). Mast cell interactions with glial cells and neurons release mediators such as cytokines, proteases, and reactive oxygen species. During neuroinflammation, excessive levels of these mediators can influence neurogenesis, neurodegeneration, and blood-brain barrier (BBB) permeability.

This project is circumscribed in a multidisciplinary project whose main goal is understanding the molecular and cellular basis of major depressive disorder (MDD) linked to CD300f function (*Lago N et al. 2020*). For this purpose, we propose generating different mast cell and macrophage models with CD300f and CD300f natural variants. Mast cells such as RBL cells, LAD2 cells, bone marrow-derived mast cells (BMMCs), and macrophages such as bone-marrow-derived macrophages (BMDMs) and THP1 cells are well-established models. The cell models for CD300f WT/KO receptor BMMCs consist of obtaining differentiated mast cells in vitro from the bone marrow of mice by adding mouse IL-3 for four weeks. GM-CSF is required for four days to induce hematopoietic cell differentiation from bone marrow into macrophages. To detect CD300f stability on our cellular models, we propose to check the apoptosis assay with Annexin V exposition. For the evaluation of the functional effect of CD300f receptor and natural variants in the mast cell model (LAD2 cells) and macrophage model (THP1), we propose to check the release of the cytokine (Interleukin-8, Interleukin-1beta, Interleukin-6, Interleukin-10, TNFalpha) by performing ELISA.

ImmunoTools *special* AWARD for **Lihong Song** includes 6 reagents

FITC - conjugated Annexin V

ELISA: mouse GM-CSF (four reagents),

recombinant mouse cytokines: IL-3

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