Signaling by Programmed Death Ligand 1 (PD-L1) Regulates LEC function

Johnathon Schafer[1,3], Erin D. Lucas[2,4], and Beth A. Jirón Tamburini[1,2,3,4]

University of Colorado School of Medicine: [1]Department of Medicine: Division of Gastroenterology and Hepatology. [2]Department of Immunology and Microbiology. [3]Molecular Biology Graduate Program, [4]Immunology Graduate Program.

Abstract

Programmed Death Ligand 1 (PD-L1) has been studied as a ligand that induces cell death to T cells expressing the receptor, PD-1. Recent data suggest PD-L1 serves other functions beyond that of a ligand. Studies have shown that expression of PD-L1 promotes survival. Consistent with these findings, in vivo studies have demonstrated that loss of PD-L1 results in cellular apoptosis of lymphatic endothelial cells and disrupts dendritic cell trafficking through lymphatics following innate activation. The mechanism by which PD-L1 supports these functions in lymphatic endothelial cells is incompletely understood. Analysis and mass spectrometry of the cytoplasmic domain of PD-L1 identified a conserved serine residue that is phosphorylated in humans and with a high probability of being phosphorylated in mice. To begin to determine if phosphorylation of the cytoplasmic tail of PD-L1 is responsible for any or all of the observed phenotypes demonstrated, we transduced either WT or a phosphomimic of PD-L1 into SVEC4-10 cells, an immortalized lymphatic endothelial cell line. Significantly increasing expression of wildtype PD-L1 in the SVEC4-10 cells made them more resistant to cell death in the presence of TNFa, whereas mutant PD-L1 offered no protection. Wildtype PD-L1 expression also increased SVEC4-10 migration/wound healing in the presence of TNFa. This difference was attributed to differences in F-actin formation in the cells expressing WT PD-L1. Finally, immunoprecipitation of PD-L1 in the SVEC4-10 cells followed by mass spectrometry revealed ZO-2 and SHROOM2 as binding partners to PD-L1. These proteins have been shown to be important for cytoskeletal organization as well as tight junction organization and maintenance. Together these experiments illustrate how PD-L1 may regulate viability and cellular movement. These functions are critical for lymphatic reorganization and dendritic cell trafficking thus understanding how PD-L1 is involved is necessary for a complete understanding of the initiation of the immune response to infection.

Figure 1: PD-L1 Expression on LECs Protects Against Apoptosis

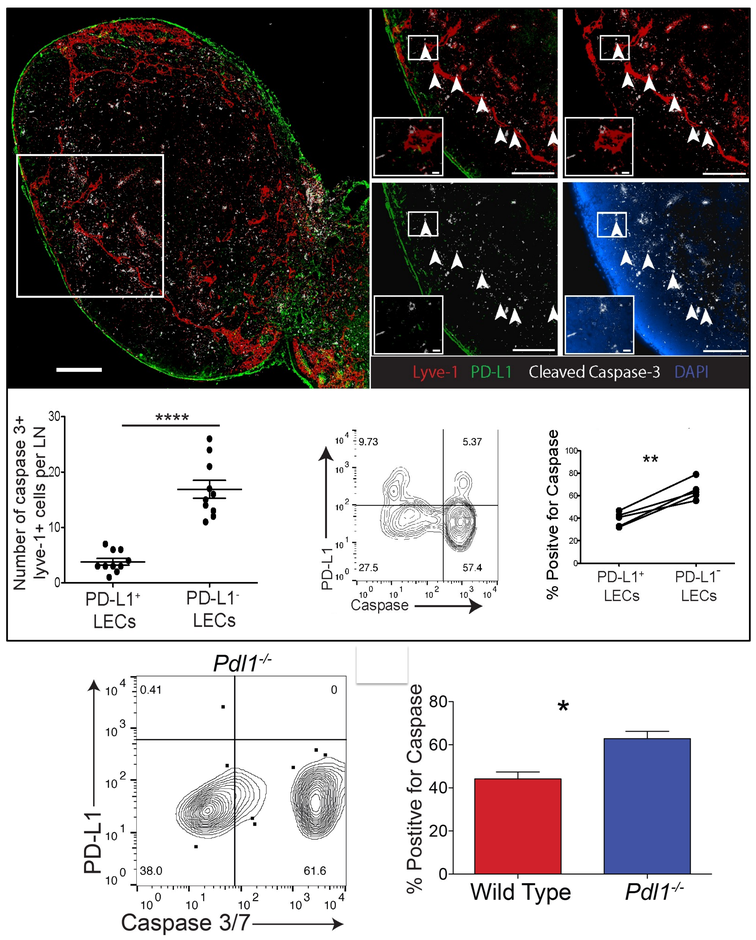


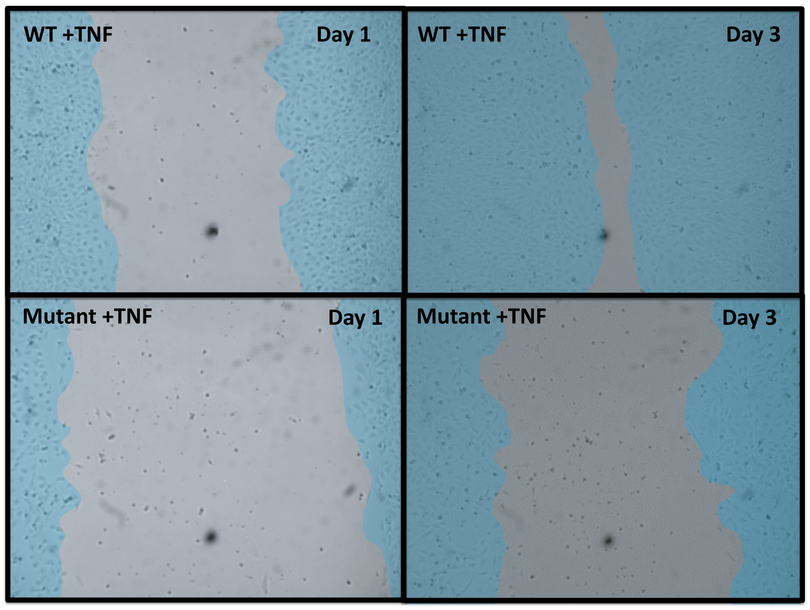
Figure 2: Mutating the cytoplasmic domain of PD-L1 inhibits cell migration

WT

Plasma membrane---RKQVRMLDVEKCGV**EDTSSK**N**RNDTQFE**ET

Cytoplasmic Mutant

Plasma membrane---RKQVRMLDVEKCGV**EDAKDK**N**RNDTQFE**ET



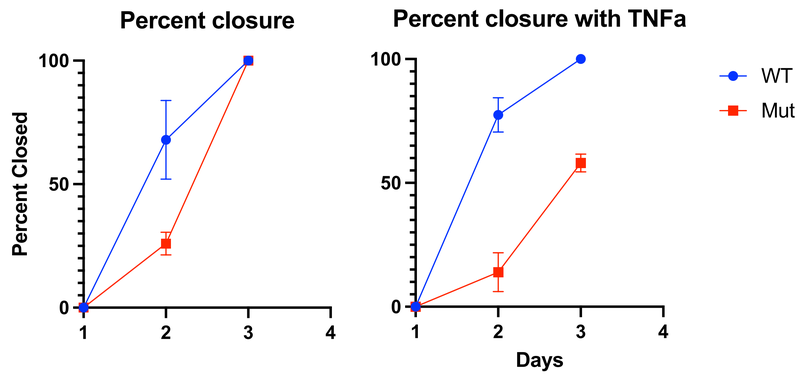


Figure 3: PD-L1 Cytoplasmic Mutant Increases Apoptosis

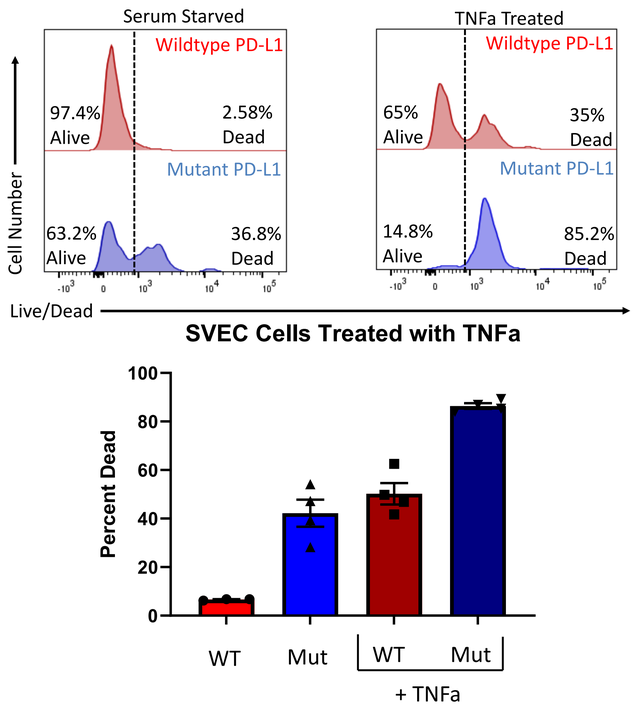


Figure 4: Potential Binding Partners Identified by Co-IP Mass spec Regulate Actin at Cell-Cell Junctions

Accession Number	Negative	Negative	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6
ZO2_MOUSE	0	0	0	7	0	7	14	9
SHRM2_MOUSE	0	0	10	7	7	8	5	0
UBF1_MOUSE	0	0	7	13	3	0	5	16

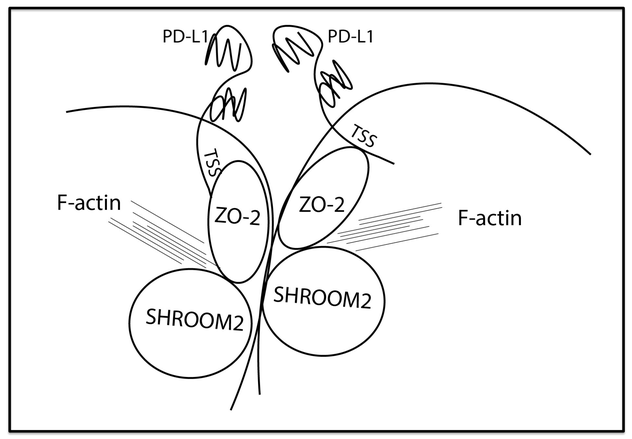
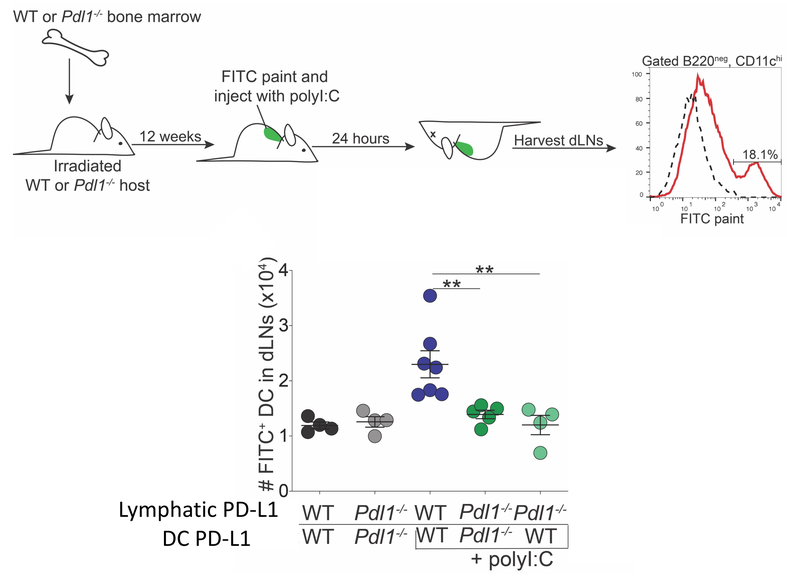
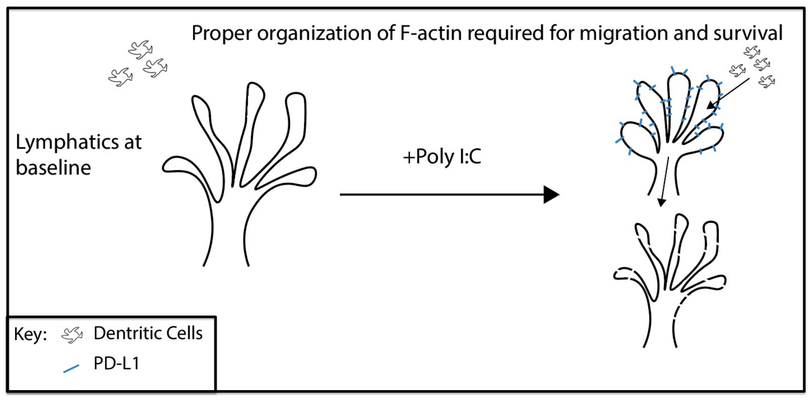


Figure 5: Lack of PD-L1 on Lymphatics Inhibits Dendritic Cell Migration





Conclusions and Next Steps:

Based on the above data, PD-L1 contributes to lymphatic endothelial cell survival and migration during inflammation, a process critical for proper DC migration at the initiation of the immune response. Given that preliminary results indicate SHROOM2 and ZO-2 are potential interacting partners, PD-L1 may perform this function by playing a role in actin dynamics during inflammation. Next steps involve confirming these interactions as well as looking at actin directly to see how the cytoplasmic mutation may affect organization following inflammation.

off lab, Lenz lab, NJH Center for Genes, Environment, and Health/R01AI121209