PD-L1 Signaling Contributes to Early Innate Immune Responses that Limit Systemic Infection with *L. monocytogenes*

Jamie L. Shirley[1,2], Tadg Forward[1,2], and Beth A. Jirón Tamburini[1,2]
[1]University of Colorado Anschutz Medical Campus, School of Medicine, Department of Medicine: Division of Gastroenterology and Hepatology, Aurora, Colorado
[2]University of Colorado Anschutz Medical Campus, School of Medicine: Department of Immunology and Microbiology, Aurora, Colorado

Abstract

Programmed Death 1 (PD-1) interactions with the ligand (PD-L1) are required for an immune checkpoint that minimizes T cell activation to limit tissue damage and prevent autoimmunity. While PD-1 signaling in T cells directly inhibits T-cell receptor signaling, PD-L1 also induces intracellular PD-L1 signaling in the cell bearing the ligand. This PD-L1 signaling has been described in cancer cells where loss of PD-L1 or the cytoplasmic domain of PD-L1 leads to increased susceptibility to T-cell killing and type 1 interferon (T1 IFN). This effect was attributed to increased STAT3 phosphorylation that influenced downstream caspase production. Much less is understood about the significance of reverse PD-L1 signaling within innate immune cells in the context of infection. We recently reported that a cytoplasmic motif within PD-L1 is required for dendritic cell (DC) migration to the draining lymph node. Given that PD-L1 is rapidly upregulated following stimulation, and broadly expressed across multiple innate immune populations, we asked whether PD-L1 reverse signaling may impact other early innate immune cells. To test this, we infected WT and PDL1$^{-/-}$ mice intravenously with *L. monocytogenes* (LM) and evaluated bacterial burden and cytokines associated with STAT3 signaling in each group. We found that the LM burden in the spleen was significantly reduced in PD-L1$^{-/-}$ mice compared to WT controls. In addition, serum IL-6 levels were significantly diminished in PD-L1$^{-/-}$ mice at 1- and 2-days post infection which may suggest a role for PD-L1 in coordinating early inflammatory responses that occurs at the level of innate sensing. Our future goals are aimed at understanding the relationship between PDL1, IL-6, STAT3 and bacterial burden. As T1 IFN signaling by monocytes contributes to increased LM colonization we are also continuing to investigate whether the upregulation of PD-L1 by T1 IFN influences differences in early innate immune responses.

Experimental Design

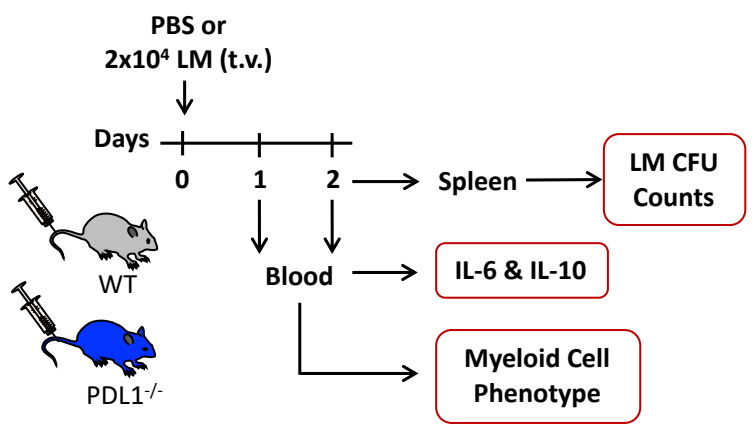


Figure 1. Reduced bacterial burden in the spleen of PDL1$^{-/-}$ mice 2 days post infection

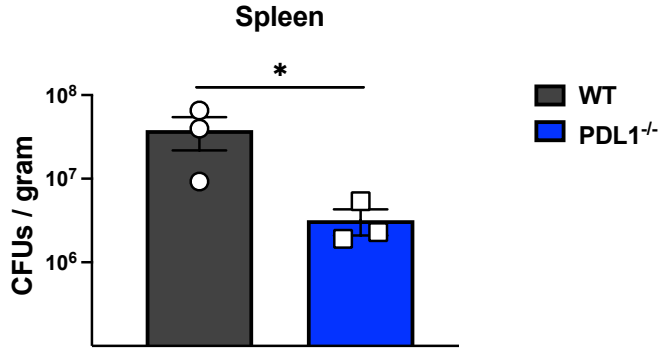


Figure 2. IL-6 Production is impaired in the absence of PDL1 signaling

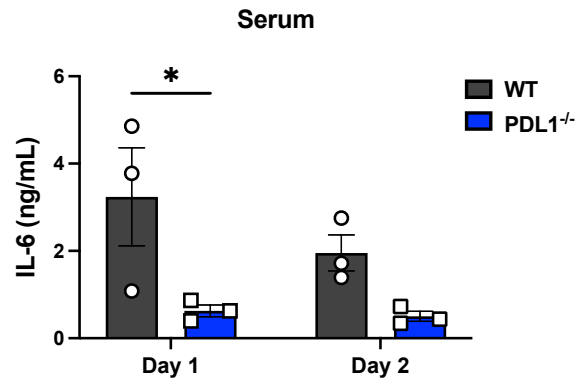
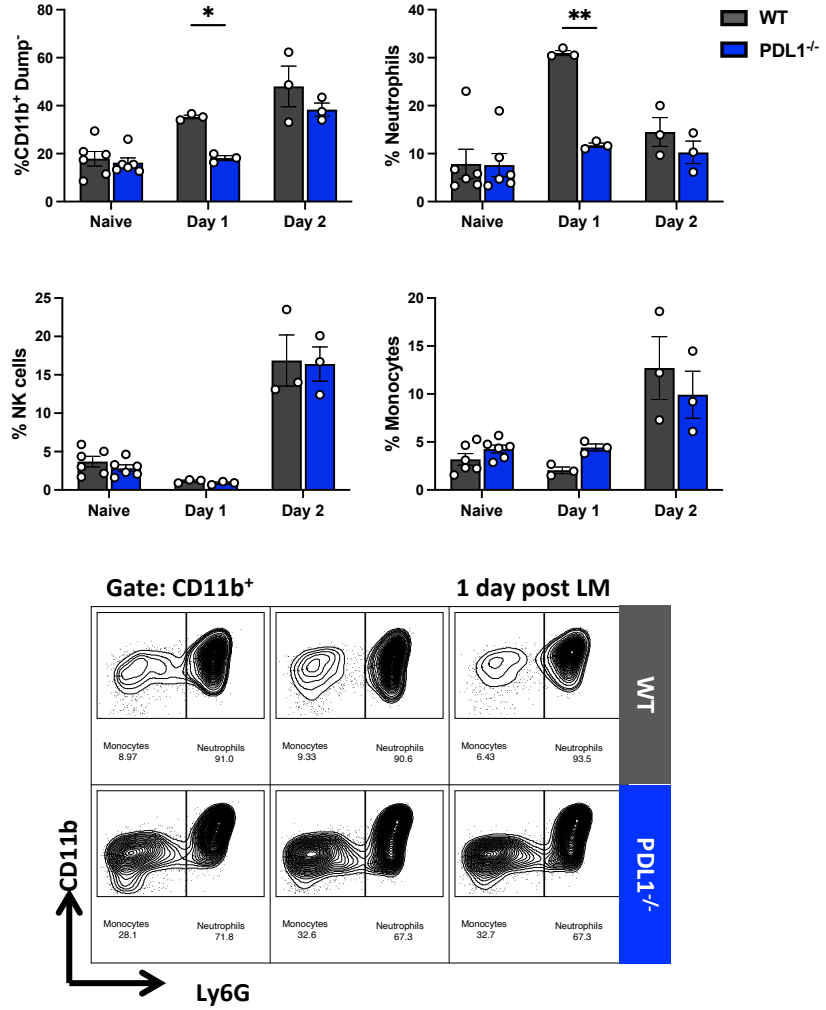


Figure 3. Circulating neutrophils in PDL1$^{-/-}$ mice are not increased 1 day post LM infection



Conclusions

We recently reported that intracellular PDL1 signaling in dendritic cells is required for their migration to the draining LN. Specifically, activation of adaptive responses were impaired when DC migration was required. This finding raised the question of whether PDL1 intracellular signaling may coordinate the activity of other innate immune compartments and impact early innate responses to infection. Here we show that:

- → Loss of PDL1 reduces bacterial burden early after infection suggesting that PDL1 may participate in controlling LM replication and/or spread during systemic infection.
- → IL-6 was increased in the serum following LM infection with WT mice but levels were significantly lower in PDL1$^{-/-}$ mice. This finding may point to an intersection of PDL1 and innate sensing pathways.
- → While the majority of myeloid populations were unaltered between PDL1$^{-/-}$ and WT mice, the percent of circulating neutrophils was significantly lower in the absence of PDL1 one day after LM infection. This difference normalized at 2 days p.i. suggesting that PDL1 may impact neutrophil egress from the bone marrow, or their survival during infection

Future Directions

- → Determine whether other inflammatory cytokines and/or innate immune populations or subsets may be dysregulated in the absence of PDL1
- → Investigate the mechanism that links PDL1 reverse signaling in innate immune cells with innate immune sensing and early control of infection.