Programmed death ligand 1 is neuroprotective during acute viral infection

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Introduction

- Clearance of viral infection is dependent on T cell effector responses.
- In locations such as the nervous system, where cells lack regenerative ability, optimal T cell responses protect but cause minimal collateral damage to the host.
- Recent evidence from infectious hepatitis models suggest a role for programmed death 1 (PD-1) – PD-ligand 1 (PD-L1) pathway in regulation of balance between pathogen clearance and host immunopathology elicited by CD8+ T cells.

Methods

Either 7.8 week old C57/B6 or Balb/c female mice were used in all experiments. Mice were anesthetized and corneas scarified then infected with 10^6 plaque forming units (PFU) HSV-1. On various days post infection (dpi), TG from the acutely infected mice were harvested for frozen section imaging via confocal microscopy or dispersed into single cell suspensions for flow cytometric analyses.

Results

Figure 1. PD-L1 expression on TG neurons is dramatically increased by HSV-1 infection. Representative flow cytometric analyses of TG from naive and mice 8 dpi demonstrate increased neuronal PD-L1 expression as exhibited by increased detection of PD-L1 PE-A in cell population. Gated on CD45, Thy 1.2+, neurons, right of vertical line: naive (neurons, above horizontal line) cells.

Hypothesis

Neuronal PD-L1 expression elicited by ocular HSV-1 infection will significantly decrease the inflammatory response mediated by CD8+ T cells in the TG.

Conclusions

- PD-L1 expression by TG neurons is dramatically upregulated after HSV-1 infection.
- Anti PD-L1 antibody treatment increased CD4+ and CD8+ T cell numbers and increased the number of apoptotic neurons in TG of infected mice.
- These data demonstrate a novel anti-inflammatory, neuroprotective role for neuronal PD-L1 expression during acute viral infection.

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Figure 2. Kinetics of neuronal PD-L1 expression. The percentage of PD-L1 positive neurons is exhibited by flow cytometry data and results from treatment of mice with anti PD-L1 antibody 5 dpi. Difference in mean CD8+ T cell number between control (n=10) and treated mice (n=13) was determined to be significantly different (unpaired t test with equal variance, p=0.001) as was the difference in mean CD4+ T cell number (unpaired t test with equal variance, p=0.05). These results suggest that neuronal PD-L1 expression may dampen the inflammatory response generated by ocular HSV-1 infection.

Figure 3. Neuronal PD-L1 expression is not mediated by CD4+ or CD8+ T cells in the TG of infected mice. TG neurons were analyzed via flow cytometry 7 dpi. All events were collected and gated on live CD45 Thy 1.2- cells. Representative flow from A) naive, B) infected controls, C) infected CD4+ T cell deficient, and D) infected CD4+ and CD8+ T cell deficient demonstrate similar neuronal PD-L1 expression among infected groups, suggesting neuronal PD-L1 expression is not mediated by T cell interactions with infected neurons or T cell-produced inflammatory mediators.

Figure 5. Anti PD-L1 antibody treatment is associated with increased neuronal death in the TG of HSV-1 infected mice. TG neurons (green) of A) naive control and B) HSV-1 infected mice treated with anti PD-L1 antibodies dpi 5 were prepared on whole mounts and visualized using confocal microscopy. Neurons exhibit a prevalence of non-apoptotic neuronal nuclei (blue). Infected TG neurons exhibit increased numbers of apoptotic neuronal nuclei (TUNEL staining, purple) compared to lack of apoptotic nuclei in naive controls, suggesting that PD-L1-mediated dampening of the inflammatory response may be neuroprotective.