Control of pathogenic effector T cell activities in situ by PD-L1 expression on respiratory inflammatory dendritic cells during respiratory syncytial virus infection

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Supplementary Materials

Figure S1. Murine and human T cells express PD-1 at the respiratory tract during RSV infection.

(A). WT Balb/c mice were infected with RSV. PD-1 MFI on CD4⁺ and CD8⁺ T cells at different days post infection are depicted. (B). PBMCs and nasal washes were collected on healthy children or RSV infected children. PD-1 expression on CD4⁺ T cells is depicted. Data are representative of two independent experiments (A).

Figure S2. PD-L2 blockade modestly affects host morbidity. (A). WT Balb/c mice were infected with RSV and treated with control Ab or α-PD-L2. Host morbidity was monitored through weight loss. (B). PD-L1 and PD-L2 expression on lung cells at different days post RSV infection. Data are representative of two independent experiments.
Figure S3. Tfh, germinal center B cell and Ab responses following acute PD-L1 blockade during RSV infection. WT Balb/c mice were infected with RSV and treated with control Ab or α-PD-L1. (A). Tfh and germinal center B cell formation in draining LNs at day 6 post RSV infection. (B and C). RSV-specific IgG in serum (B) and BALF (C) was determined through ELISA at day 6 post RSV infection. Data are representative of two to three independent experiments.
Figure S4. **PD-L1 blockade results in enhanced inflammation and IL-10 production by effector T cells.** WT Balb/c mice were infected with RSV and treated with PBS, control Ab or α-PD-L1. (A). Neutrophils, Eosinophils, CD4+ and CD8+ T cells in the lung at day 9 post infection. (B). % of Foxp3+ Treg cells in lung CD4+ T cells at day 7 post infection. (C). IL-10 levels in the BAL from control Ab or α-PD-L1 treated mice (day 6 and 9 post infection). (D and E). At day 5 post infection, mice were injected with monensin to block the in vivo secretion of cytokines by T cells. (D). IL-10 staining of lung CD4+ and CD8+ T cells without in vitro stimulation. (E) IL-10 staining of lung CD4+ and CD8+ T cells following in vitro PMA and Ionomycin stimulation. Data are representative of two to three independent experiments. *, P< 0.05 as determined by unpaired student t-test.
Figure S5. Inflammatory DCs express high levels of PD-L1 during RSV infection. (A-D). WT Balb/c mice were infected with RSV. (A). Lung inflammatory DC (iDC) gating strategies and Ly6C expression. (B). Kinetics of iNOS expression by inflammatory DCs. (C). % of different cell types in the infected lungs (day 5 post infection). (D). Inflammatory DC cell numbers in the lung following RSV infection. (E). PD-L1 expression (MFI) on lung inflammatory DCs at different days post infection. (F). WT Balb/c mice were infected with RSV and treated with control Ab or α-IL-10R blocking Ab. PD-L1 expression on inflammatory DCs. Data are representative of two to three independent experiments.
Figure S6. IRF1 expression on lung inflammatory DCs is dependent on type I and II IFNs. WT and IFNAR1-deficient mice were infected with RSV and treated with α-IFN-γ as indicated. IRF1 message in lung inflammatory DCs at day 5 post infection. Data are representative of two independent experiments.