

MYELOID TLR4 SIGNALING DRIVES CYTOKINE-MEDIATED CD4⁺ T-CELL CYTOTOXICITY AND REDUCES PANCREATIC TUMOR BURDEN

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Background: Pancreatic ductal adenocarcinoma (PDAC) remains profoundly resistant to immunotherapy. Building on our observation that TLR4-agonism reduces PDAC growth in murine models, we sought to define how myeloid TLR4 signaling links innate immune activation to cytotoxic CD4⁺ T-cell function and tumor control. **Methods:** KPC PDAC cells were implanted subcutaneously in C57BL/6 wild-type (WT), global TLR4 knockout (TLR4-KO) mice, and myeloid-specific TLR4-deficient *Tlr4^{fl/fl}LysM-Cre* (TLR4-ΔMφ) mice. LPS (1mg/kg, *i.p.*) was administered on day 10. Serum cytokines, splenic macrophage cytokine transcripts, and splenic/intratumoral immune populations were assessed. CD4⁺ T-cell cytotoxicity was measured by *ex-vivo* calcein-release assay. Cytokine dependence was tested by TNF-α/IFN-γ neutralization. Synthetic TLR4-agonist with a favorable toxicity profile (BECC44) was evaluated for anti-tumor efficacy.

Results: LPS induced a rapid cytokine surge, including TNF-α, IFN-γ, within 6 hours, returning to baseline by 24-48 hours. Splenic macrophages showed concomitant upregulation of cytokine transcripts at 6 hours. CD4⁺ T cells acquired a *granzyme-B*⁺ cytotoxic phenotype, and splenic CD4⁺ T cells from LPS-treated WT mice demonstrated increased *ex-vivo* tumor-cell killing. In TLR4-ΔMφ mice, LPS-induced cytokine upregulation, CD4⁺ T-cell cytotoxicity, and tumor reduction were markedly attenuated. Neutralization of TNF-α/IFN-γ abrogated LPS-mediated tumor reduction. BECC44 demonstrated reduced systemic toxicity as expected. Interestingly, it also exhibited significant anti-tumor efficacy.

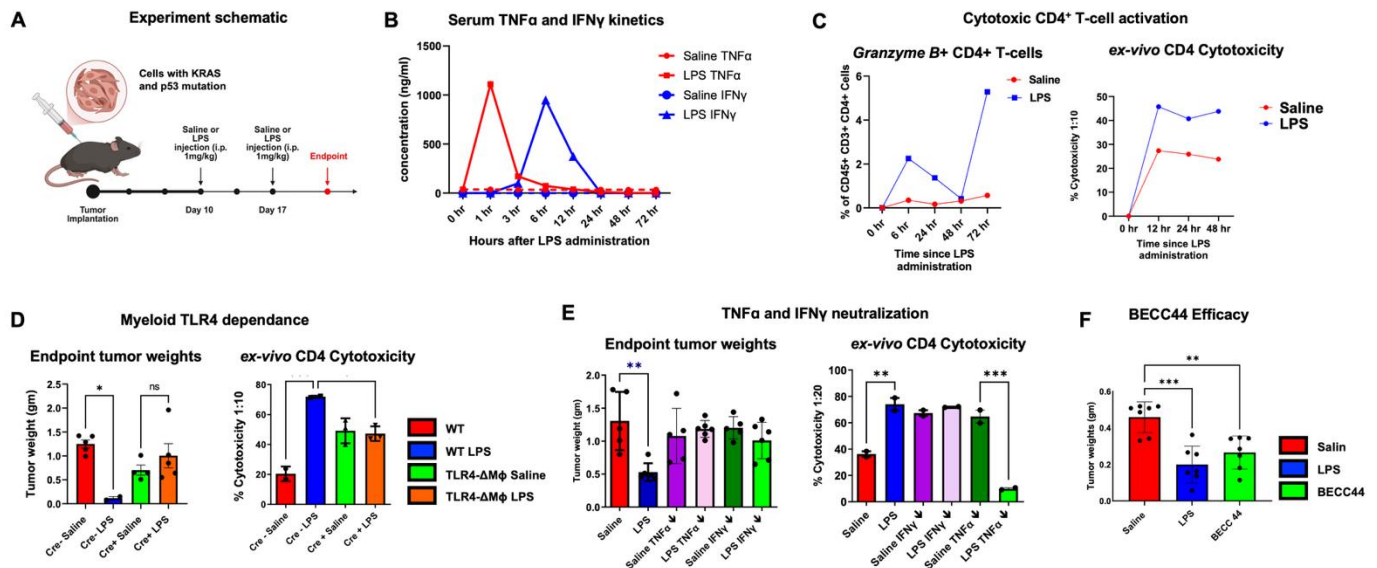


Figure 1. Myeloid TLR4 signaling links early cytokine release to cytotoxic CD4⁺ T-cell activation and pancreatic tumor control. (A) Experimental schematic. KPC pancreatic cancer cells were implanted subcutaneously, followed by TLR4 agonist treatment and tumor assessment (B) LPS induced an early serum cytokine response, with rapid induction of TNF-α and IFN-γ. (C) LPS promoted cytotoxic CD4⁺ T-cell activation, shown by increased Granzyme B⁺ CD4⁺ T cells and enhanced *ex vivo* CD4⁺ T-cell cytotoxicity. (D) In myeloid-specific TLR4-deficient mice, LPS-mediated tumor reduction and CD4⁺ T-cell cytotoxicity were attenuated, supporting a requirement for myeloid TLR4 signaling. (E) TNF-α and IFN-γ neutralization reduced LPS-mediated tumor control and abrogated CD4⁺ T-cell cytotoxicity, identifying these cytokines as functional mediators of the anti-tumor response. (F) BECC44, a synthetic TLR4 agonist, demonstrated preliminary anti-tumor efficacy. Data are shown as mean ± SEM; individual points represent replicates where shown. *p<0.05, **p<0.01, ***p<0.001.

Conclusion: Myeloid TLR4 signaling links early TNF-α/IFN-γ release to cytotoxic CD4⁺ T-cell function and PDAC tumor reduction. BECC44 provides *proof-of-concept* that synthetic TLR4 agonists may retain anti-tumor efficacy while minimizing systemic toxicity, supporting TLR4 agonism as a potential strategy to reprogram “cold” PDAC microenvironment towards a cytotoxic anti-tumor state.