Delivery of TLR7 agonists by Deep-Primed™ T cells induces immune activation and improves anti-tumor activity in mice while circumventing systemic toxicity

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Total number of authors: 19

Publication date: 2019

Document Version
Publisher's PDF, also known as Version of record

Link back to DTU Orbit

Citation (APA):
Delivered TLR7 agonists by Deep-Primed™ T cells induces immune activation and improves anti-tumor activities in mice while circumventing systemic toxicity

Presented at the Society for Immunotherapy of Cancer (SITC) 34th Annual Meeting November 6-10, 2019 National Harbor, MD

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Introduction
TLR7 agonists boost immune responses in the tumor microenvironment (TME), primarily through dendritic cell (DC) engagement, enhancement of antigen presentation and T cell co-stimulation. However, multiple TLR agonists have displayed unfavorable PK/PD profiles and considerable toxicities upon systemic administration. To overcome these limitations, we designed a T cell mediated delivery system for TLR7 agonists that targets the TME and the lymphatic system to maximize efficacy while avoiding a T cell mediated delivery system for TLR7 agonists that targets the unfavorable PK/PD profiles and considerable toxicities upon stimulation. However, multiple TLR agonists have displayed TLR7 agonist and several liposomal formulations were screened for

Materials and experimental design
TLR7 agonists and several liposomal formulations were screened for optimal T cell targeting and release, as measured by HPLC. Formulations with desired characteristics were further characterized in mice with Pemb1+ melanoma xenografts to determine Deep TLR Primed T cell efficacy and immunological effects.

Key findings
- Torque’s Deep TLR Primed T cells release a potent small molecule agonist of TLR7 over an extended period of time
- Deep TLR Primed T cells mediate superior tumor growth inhibition in the B16 melanoma syngeneic mouse model
- Deep TLR-mediated agonist delivery increases T cell expansion and extends host survival
- The cytokine profile suggests reduced exhaustion of Deep TLR Primed T cells vs. controls
- Deep TLR Primed T cells caused no significant weight loss. Transient plasma levels of pro-inflammatory cytokines (TNFα and IL-6 not shown) remained below 5% of known toxic levels (Sorenson et al. 2014, Board et al. 2009, Tateda et al. 1996). This suggests that our Deep TLR Primed T cell therapy has the potential to be efficacious and well tolerated.

1. Deep TLR Primed T cells contain TLR7 agonist loaded liposomes and display slow payload release over time

2. Deep TLR Priming increases T cell expansion in vivo and reduces PD-1 expression

3. TLR7 agonist delivery enriches pDCs in draining LNs as well as endogenous CD8 T cells and MDSCs in tumors

4. Deep TLR Primed T cells promote tumor growth inhibition and extend host survival

5. Deep TLR shows low potential toxicity

Figure 1. Deep TLR Primed T cells release a potent TLR7 agonist over an extended period of time. (A) Torque TLR7 agonist A displays a high potency in inducing CD8+ expansion in syngeneic presenting cells in vivo. Each agonist was preincubated in antigen-presenting G5 cells (10^9) and injected i.p. into 10^7 T cells per syngenic mouse. The total daily cells were then divided and injected i.v. into the mice. TLR7 agonist B displays low potency in inducing CD8+ expansion in syngeneic presenting cells in vivo. Each agonist was preincubated in antigen-presenting G5 cells (10^9) and injected i.p. into 10^7 T cells per syngenic mouse. The total daily cells were then divided and injected i.v. into the mice. (B) TLR7 agonist C displays low potency in inducing CD8+ expansion in syngeneic presenting cells in vivo. Each agonist was preincubated in antigen-presenting G5 cells (10^9) and injected i.p. into 10^7 T cells per syngenic mouse. The total daily cells were then divided and injected i.v. into the mice. (C) TLR7 agonist D displays a high potency in inducing CD8+ expansion in syngeneic presenting cells in vivo. Each agonist was preincubated in antigen-presenting G5 cells (10^9) and injected i.p. into 10^7 T cells per syngenic mouse. The total daily cells were then divided and injected i.v. into the mice. (D) TLR7 agonist E displays a high potency in inducing CD8+ expansion in syngeneic presenting cells in vivo. Each agonist was preincubated in antigen-presenting G5 cells (10^9) and injected i.p. into 10^7 T cells per syngenic mouse. The total daily cells were then divided and injected i.v. into the mice.

Figure 2. Deep TLR Primed T cells display accelerated expansion and reduced PD-1 in tumor-bearing, lymphopenic mice. (A) After Deep TLR Primed or control PMEL T cells were i.c. injected into B16F10 tumor bearing syngenic G5 mice, (B) PMEL T cell control at the indicated time points was assessed by flow cytometry. Data are shown as fold change over the PMEL T cell control group. Expansion curves were also observed in tumor-free sham mice (not shown). (C) Percentage of PD-1 expressing T cells in peripheral blood and tumors. P-values for comparisons between the indicated groups were calculated using Student’s t-test.

Figure 3. Deep TLR Primed T cells release a potent TLR7 agonist over an extended period of time. (A) Torque TLR7 agonist A displays a high potency in inducing CD8+ expansion in syngeneic presenting cells in vivo. Each agonist was preincubated in antigen-presenting G5 cells (10^9) and injected i.p. into 10^7 T cells per syngenic mouse. The total daily cells were then divided and injected i.v. into the mice. TLR7 agonist B displays low potency in inducing CD8+ expansion in syngeneic presenting cells in vivo. Each agonist was preincubated in antigen-presenting G5 cells (10^9) and injected i.p. into 10^7 T cells per syngenic mouse. The total daily cells were then divided and injected i.v. into the mice. TLR7 agonist C displays low potency in inducing CD8+ expansion in syngeneic presenting cells in vivo. Each agonist was preincubated in antigen-presenting G5 cells (10^9) and injected i.p. into 10^7 T cells per syngenic mouse. The total daily cells were then divided and injected i.v. into the mice. (B) TLR7 agonist D displays a high potency in inducing CD8+ expansion in syngeneic presenting cells in vivo. Each agonist was preincubated in antigen-presenting G5 cells (10^9) and injected i.p. into 10^7 T cells per syngenic mouse. The total daily cells were then divided and injected i.v. into the mice. (C) TLR7 agonist E displays a high potency in inducing CD8+ expansion in syngeneic presenting cells in vivo. Each agonist was preincubated in antigen-presenting G5 cells (10^9) and injected i.p. into 10^7 T cells per syngenic mouse. The total daily cells were then divided and injected i.v. into the mice.

Figure 4. Deep TLR Primed T cells mediate superior tumor growth inhibition in the B16 melanoma syngeneic mouse model. Experimental setup as in Fig. 3. 1x10^7 Tumor growth inhibition in a bilateral open delivery of Deep TLR Primed PMEL T cells compared to all other groups. The curves show tumor growth kinetics in mice with tumor start volumes of ~200 mm^3 in both tumor sites. (A) PMEL T cells mediate superior tumor growth inhibition in the B16 melanoma syngeneic mouse model.

Figure 5. ACT of Deep TLR Primed T cells exhibits low potential toxicity. Deep TLR Primed as a potent TME TLR7 agonist. Treatment with soluble TLR7 agonist. PD-1 downregulation suggests reduced exhaustion of Deep TLR Primed T cells vs. controls. Deep TLR-mediated agonist delivery increases pDCs in draining lymph nodes and endogenous CD8 T cells in tumors, consistent with the known effects of TLR7 agonists in vivo (Mouries et al. 2008). Deep TLR-mediated agonist delivery increased MDSC content in the TME which may be beneficial given TLR7 agonists are known to convert MDSCs into functional APCs (Spinelli et al. 2016).

**Deep TLR Primed T cells**

**Deep TLR Agonist**

**Deep TLR Primed T cell**

**PMEL T cells**

**PMEL T cells + systemic TLR7 agonist**

**Deep TLR Primed T cells**

**Buffer**

**PMEL T cells**

**PMEL T cells + systemic TLR7 agonist**

**Deep TLR Primed T cells**

**PMEL T cells**

**PMEL T cells + systemic TLR7 agonist**

**Deep TLR Primed T cells**

**Buffer**

**PMEL T cells**

**PMEL T cells + systemic TLR7 agonist**

**Deep TLR Primed T cells**