Deep TLR Primed™ T cells induce potent anti-tumor activity without systemic toxicity

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Introduction

In a mouse melanoma model. The agonists work primarily through two mechanisms: antigen presenting cell (APC) engagement and enhancement followed by T cell co-stimulation. However, multiple TLR agonists, including TLR7/8 agonists, have displayed considerable toxicities upon systemic administration. To circumvent this problem, we developed a T cell mediated delivery system of TLR7 agonists that can target the TME and lymphoid organs to maximize efficacy while avoiding systemic toxicities. Torque’s Deep Primed™ T cell technology enhances T cell function by tethering immune modulators to the T cell before adoptive cell transfer. Primed™ T cell technology enhances T cell function by tethering immune modulators to the T cell before adoptive cell transfer (ACT) and by using Torque’s multi-targeted T cell (MTC) platform that primes the T cells against multiple tumor antigens. Herein, we screened several liposomal formulations containing two different TLR agonists for both in vitro agonist loading and release in mouse and human T cells followed by in vivo testing in a mouse melanoma model.

Abstract

TLR7 agonists have been shown to augment immune responses in the tumor microenvironment (TME). The agonists work primarily through two mechanisms: antigen presenting cell (APC) engagement and enhancement followed by T cell co-stimulation. However, multiple TLR agonists, including TLR7/8 agonists, have displayed considerable toxicities upon systemic administration. To circumvent this problem, we developed a T cell mediated delivery system of TLR7 agonists that can target the TME and lymphoid organs to maximize efficacy while avoiding systemic toxicities. Torque’s Deep Primed™ T cell technology enhances T cell function by tethering immune modulators to the T cell before adoptive cell transfer (ACT) and by using Torque’s multi-targeted T cell (MTC) platform that primes the T cells against multiple tumor antigens. Herein, we screened several liposomal formulations containing two different TLR agonists for both in vitro agonist loading and release in mouse and human T cells followed by in vivo testing in a mouse melanoma model.

Results

1. TLR agonists 1 and 2 are specific for TLR7

Figure 1. (A) HEK cells expressing human TLR7 or TLR8 were cultured in media without selection agent for at least 1 generation. After adding different concentrations of TLR agonist 1, TLR agonist 2, or the Dermastim™ formulation, media was collected for 24 h TLR agonist 1 reporter gene analysis. (B) HEK cells expressing human TLR7 or TLR8 were cultured in media without selection agent for at least 1 generation. After adding different concentrations of TLR agonist 1, TLR agonist 2, or the Dermastim™ formulation, media was collected for 24 h TLR agonist 2 reporter gene analysis.

2. Optimal liposome formulation maximizes agonist loading and extends drug release

Figure 2. (A) Human T cells were loaded and PMEL T cells with liposomes. After 1 h incubation, the liposomes were collected and released into media containing 10 ng/mL IFN-α. (B) Human T cells were activated and loaded with liposomes. After 1 h incubation, the liposomes were collected and released into media containing 10 ng/mL IFN-α. (C) Drug release from PMEL T cells loaded with liposomes. After 1 h incubation, the liposomes were collected and released into media containing 10 ng/mL IFN-α.

3. Deep TLR loaded T cells retain viability and extend TLR agonist release

Figure 3. (A) Apoptosis levels from Deep TLR Primed™ T cells in vitro. TLR agonists were applied to Torque’s Deep Priming process, cells were loaded with Deep TLR, and then cultured. (B) Human T cells were activated and loaded with liposomes. After 1 h incubation, the liposomes were collected and released into media containing 10 ng/mL IFN-α. (C) Deep TLR Primed™ T cells were loaded with PMEL cells and cultured under the media environment. TLR agonist (10 nM) was measured using HBSS after 1 h of culture and protein precipitation at each concentration of agonist. (D) TLR agonist retained within cells and released into the media was assessed by HPLC to measure twice weekly along with weight.

4. Deep TLR Primed™ T cells increase cell expansion and tumor control in vivo

Figure 4. (A) PMEL T cells were loaded with Deep TLR agonists and then PMELs were loaded with liposomes. After 1 h incubation, the liposomes were collected and released into media containing 10 ng/mL IFN-α. (B) Drug release from PMEL T cells loaded with liposomes. After 1 h incubation, the liposomes were collected and released into media containing 10 ng/mL IFN-α. (C) Drug release from PMEL T cells loaded with liposomes. After 1 h incubation, the liposomes were collected and released into media containing 10 ng/mL IFN-α.

Conclusions

• Torque’s Deep TLR Primed™ T cells released a potent small molecule agonist of TLR7 over an extended period of time.
• Two TLR7-specific agonists capable of liposome encapsulation were identified.
• Formulation optimization enabled high concentrations of two different TLR7 agonists to be loaded on both mouse and human T cells with extended release.
• The optimal liposomal formulation enabled encapsulation of high concentrations of TLR7 agonist loaded onto MTCs with minimal effect on viability and proliferative capacity.
• Deep TLR Primed™ T cells remain viable and release TLR agonist slowly over 10 days.
• Deep TLR Primed™ T cell expansion exceeds that of CD8 T cells alone or co-administered with systemic TLR7 agonist.
• ACT with Deep TLR Primed™ T cells provides a novel avenue to leverage the immune stimulating potential of TLR agonists for superior anti-tumor efficacy while avoiding systemic exposure and toxicities - key current bottlenecks to successful TLR therapy.
• In the future, agonist delivery via Deep-Primed™ tumor antigen-specific autologous T cells could target a wide variety of tumors and their distant metastases, enabling a new immunotherapeutic strategy.

References

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