Our studies show its unique modulation of the STING pathway as a potential mechanism for the anti-viral activity of CD8+ T-cells by inducing STING dimerization: Implications for enhanced vaccine applications

INTRODUCTION

- PV-10, a formulation of rose bengal sodium (RBS), has garnered attention for its anti-tumor cytotoxicity and safety profile in several types of cancer.[1-4]
- In murine models, PV-10 administration led to regression of lesions and metastases through CD8+ T-cell activity, increased interferon-gamma (IFN-γ), and infiltration of dendritic cells (DCs).[5-6]
- Our previous studies show PV-10 activates stimulator of interferon (IFN) genes (STING), demonstrating its potential as a vaccine adjuvant in PV-10-mediated systemic anti-tumor immune responses.[7]
- In this study, we show PV-10 activates the central innate immunity pathway involving STING and enhances CD8+ T-cell responses to antigenic peptide-based vaccination.

METHODS

- PV-10 (10% solution of RBS in 0.9% saline) was provided by Provectus Biopharmaceuticals, Inc.
- STING dimerization was assessed in THP-1 human leukemia monocytic cells by immunoblot and immunoprecipitation-mass spectrometry analysis.
- Conditioned media from THP-1 cells treated with PV-10 was collected for analysis by 42-ply cytokine/chemokine array.
- CD8+ T-cells and DCs were isolated from healthy donor peripheral blood mononuclear cells (PBMCs) and cultured in the presence of cytokines, as described previously.[8]
- CD8+ T-cells were primed by DCs pulsed with synthetic peptides representing hepatitis B virus (HBV) surface antigen (HBsAg).[9]
- CD8+ T-cell responses were examined upon stimulation with HBsAg-positive PLC/PRF/5 hepatoma cells by IFN-γ ELISpot assay.

RESULTS

- PV-10 treatment induces STING activation

Figure 1. PV-10 induces STING dimerization. (A) Leukocytes from THP-1 cells treated with 100 µM PV-10 for 4-24 hours were immunoblotted for monomeric (25 kDa) and dimerized (50 kDa) STING, indicating non-canonical activation of STING. Immunoblot for total and phosphorylated IFN-α showed canonical activation of the STING pathway in GCAMP-treated cells. β-Actin was used as a loading control, shown previously.[10] (B) STING was immunoprecipitated from THP-1 cells treated with PV-10 and the 70 kDa band was excised from the gel (area indicated by the red box) for mass spectrometric identification of bound proteins (upper panel). The top protein identified, including STING and other associated proteins, are listed in order of rank (lower panel).

- PV-10 upregulates cytokines/chemokines

Figure 2. PV-10 treatment upregulates pro-inflammatory cytokines and chemokines. THP-1 cells were treated with 100 µM PV-10 for 0-48 hours and conditioned media was collected for cytokine/chemokine analysis, compared to control cells treated with PBS (PB-PV-10) (including previously presented data for IFN-γ, IL-2, IL-6, IL-10, MCP-1, MIP-3-β). PV-10 increases IFN-γ secretion by HBsAg-treated CD8+ T-cells

Figure 3. PV-10 increases IFN-γ secretion by HBsAg-primed CD8+ T-cells stimulated by HBsAg-PLC/PRF/5 cells. CD8+ T-cells treated with PBS or 1 µM PV-10 were primed with peptides (HBV-1, HBV-2, HBV-3) representing hepatitis B virus (HBV) surface antigen (HBsAg) and stimulated with HBsAg-positive PLC/PRF/5 hepatoma cells (A) or HBsAg-negative B12 cells (B). The mean relative IFN-γ-secreting spot forming cells (SPFC) normalized to no peptide controls was quantified by ELISpot assay (left panels) and representative images are shown (right panels). (A) As controls, CD8+ T-cells were stimulated with PBS and compared to media, T-cell, or cancer cells (PLC/PRF/5 or B12) alone by IFN-γ ELISpot assay. Recombinant IFN-γ was used to confirm antibody target specificity.

CONCLUSIONS

- This report demonstrates, for the first time, the ability of PV-10 to function as an effective adjuvant to enhance T-cell responses, including anti-viral and anti-cancer vaccines.
- Our studies show its unique modulation of the STING pathway as a potential mechanism for this activity.
- Future clinical studies should investigate PV-10 in combination with targeted immunotherapies.

ACKNOWLEDGEMENTS

This research was funded in part by Provectus Biopharmaceuticals, Inc.

REFERENCES


Presented at the SITC’s 38th Annual Meeting, November 1-5, 2023