1125 IN VITRO CHARACTERIZATION OF CELLULAR RESPONSES ELICITED BY ENDOSOMAL TLR AGONISTS ENCAPSULATED IN Qβ VIRUS-LIKE PARTICLES

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Background In most cancer patients with solid tumors, pre-existing dendritic cell (DC) activity is limited which impedes the ability of various immunotherapy agents to trigger an anti-tumor immune response. Toll-like receptor (TLR) agonists have received significant attention as an immunotherapeutic agent for cancer therapy due to their ability to activate DCs and promote T cell activation. Specifically, TLR7, TLR8, and TLR9 have been studied in several clinical and preclinical studies, primarily because these receptors are expressed in different DC subsets. Here we have tested and compared immune cell activation and pro-inflammatory cytokine production stimulated by TLR7, TLR9, and a dual TLR7/8 agonist when encapsulated in Qβ virus-like particles (VLPs).

Methods Immune cell activation in response to TLR7, TLR9, or a dual TLR7/8 agonist was evaluated using peripheral blood mononuclear cells (PBMCs) and PBMC/tumor cell co-cultures using human papillomavirus (HPV)-positive (HPV+) and HPV-negative (HPV-) head and neck squamous cell carcinoma (HNSCC) cell lines. Monocyte, NK cell, T cell, and DC activation were analyzed by flow cytometry. Proinflammatory cytokine (IFN-γ and TNF-α) accumulation was measured by ELISA and intracellular cytokine staining (ICS) was used to measure cytokine expression by immune cells responding to TLR agonism. Antibody-mediated neutralizing of cytokines was used to assess their role in TLR agonist-mediated T cell and NK cell activation. Finally, whole PBMC were fractionated to identify direct and indirect activation of T and NK cells by TLR7/8 agonists.

Results Results showed that all three TLR agonists activated pDCs and monocytes. However, combined activation of TLR7/8 was most effective at NK and T cell activation and effector cytokine (IFN-γ and TNF-α) production. IFN-γ release from immune cells was increased in the presence of HPV+ HNSCC cells compared to HPV- cells. ICS showed that NK cells were the major source of IFN-γ whereas monocyte and pDCs were the main sources of TNF-α release. Neutralizing antibodies for IFN-γ and TNF-α showed little suppressive effects on CD4+ T, CD8+ T, and NK cell activation. Immune cell depletion experiments showed that antigen-presenting cell (APC) depletion completely abrogated TLR7/8 mediated CD4+ and CD8+ T cell activation suggesting that the observed T cell activation may be mediated by crosstalk between APC and T cells.

Conclusions Altogether, delivery of TLR7/8/9 agonists through Qβ virus-like particles may potentially induce a robust anti-tumor immune response and is worthy of further investigation in *in vivo* cancer models as a novel immunotherapeutic strategy.

http://dx.doi.org/10.1136/jitc-2023-SITC2023.1125