ACTIVATION OF CD8+ T CELLS IN THE PRESENCE OF MULTIPLE TLR AGONISTS AFFECTS THE EXPRESSION OF T-CELL CHECKPOINT RECEPTORS VIA IL-12 AND TYPE-1 INTERFERON

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Background T-cell checkpoint receptors are expressed when T-cell are activated, and activation of these receptors can impair the function of T-cells and their anti-tumor efficacy.¹ We previously found that T-cells activated with cognate antigen increase the expression of PD-1, while this can be attenuated by the presence of specific Toll-like receptor (TLR) agonists.² This effect was mediated by IL-12 secretion from professional antigen presenting cells and resulted in CD8+ T cells with greater anti-tumor activity. In the current report, we sought to determine whether combination of TLR agonists can further affect the expression of T-cell checkpoint receptors and improve T-cell anti-tumor immunity.

Methods OT-1 CD8+ T cells were stimulated with peptide (SIINFEKL) and dendritic cells (DC) in the presence of two different TLR agonists. The cells were collected and evaluated for the expression of T-cell checkpoint receptors (PD-1, CTLA-4, CD160, CD244, LAG-3, TIM-3, TIGIT and VISTA) by flow cytometry, and for transcriptional changes by RNA-seq. Purified DC were stimulated with TLR combinations and evaluated for cytokine release by ELISA. The anti-tumor efficacy of vaccination using peptide and TLR agonist combinations was evaluated in EG7-OVA tumor-bearing mice.

Results Activation of CD8+ T cells in the presence of specific TLR ligands resulted in decreases in expression of PD-1 and/or CD160. These changes in T-cell checkpoint receptor expression were modestly affected when TLR ligands were used in combination, and notably with combinations of TLR1/2, TLR3, and TLR9 agonists. Immunization of tumor-bearing mice, co-administered with combinations of these agonists, showed greater anti-tumor effects. However, while the effect of TLR1/2 and/or TLR9 was abrogated in IL12KO mice, TLR3 demonstrated anti-tumor activity when co-administered with peptide vaccine. RNA sequencing of TLR-conditioned CD8+ T-cells revealed IL-12 pathway activation, and IFN γ pathway activation following TLR3 stimulation. Stimulation of DC with TLR3 agonist, alone or in combination with other TLR agonists, resulted in increased IL-12 and IFN γ secretion. Co-incubation of OT-1 splenocytes with rIL12 and/or rIFN γ during peptide activation led to reduced expression of PD-1, and this could be reversed with antibodies blocking IL12R or IFNAR-1.

Conclusions Multiple TLR agonists can modulate the expression of T-cell checkpoint receptors, notably PD-1, by upregulating the secretion of IL-12 and IFN γ . These data provide the mechanistic rationale for choosing optimal combinations of TLR ligands to use as adjuvants to improve the efficacy of anti-tumor vaccines.

REFERENCES

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