Background Only a fraction of cancer patients responds to current antibody-based immune checkpoint inhibitors.1 Our lab has identified vasoactive intestinal peptide-receptor (VIP-R) signaling as a targetable immune checkpoint pathway in cancer. VIP is a small neuropeptide with known immunosuppressive effects on T cells, in particular, CD4+ T cells.2–5 However, little is known about VIP-R signaling in CD8+ T cells. To define mechanisms by which VIP limits T cell activation and function, we studied the regulation of VIP and VIP receptors (VIP-R) in T cells following their activation in vitro and in mouse models of cancer.

Methods T cells from healthy human donors and murine spleenocytes were activated using anti-CD3 coated plates. Western blots measured intracellular pre-pro-VIP, along with its cognate receptors; VPAC1 and VPAC2. Purified cultures of CD4+ and CD8+ T cells were used to interrogate the protein expression on specific T cell subsets. Activation and chemokine receptor expression was assessed by flow cytometry to evaluate T cell response to VIP-R antagonists in vitro and in tumor-bearing mice engrafted with pancreatic cancer cell lines.

Results Both murine and human T cells upregulate pre-pro-VIP following TCR stimulation with similar kinetics of VIP receptors between species. VIP expression is upregulated in vivo following treatment of tumor-bearing mice with anti-PD1 MoAb. VIP expression is temporally correlated with the upregulation of other co-inhibitory molecules. VPAC1 expression modestly increased in activated T cells while VPAC2 expression decreased. A non-canonical high molecular weight (HMW) form of VPAC2-related protein robustly and transiently increase in activated T cells while VPAC2 expression decreased. A non-canonical high molecular weight (HMW) form of VPAC2-related protein robustly and transiently increase in activated T cells. Expression of HMW form of VPAC2 is only detected in activated CD4+ T cells. Of note, activated CD4+ but not CD8+ T cells upregulate pre-pro-VIP. Pharmacological inhibition of VIP-R signaling significantly increased CD69+, OX40+, Lag3+, and PD1+ expression in CD4+ subsets compared to activated T cells without VIP-R antagonists (p < 0.05). In contrast, CD8+ T cells upregulate VPAC1 but not VPAC2 receptor following activation. VIP-R antagonist treatment of activated CD8+ T cells significantly decreased CXCR4+ expression (p < 0.05). CXCR3 and CXCR5 expression were not affected by VIP-R antagonist treatment.

Conclusions VIP-R signaling is a novel immune autocrine and paracrine checkpoint pathway in activated CD4+ T cells. Activated CD4+ and CD8+ T cells demonstrate different kinetics of VPAC1 and VPAC2 expression, suggesting different immune-regulatory responses to VIP-R antagonists. Understanding VIP-R signaling induced during T cell activation can lead to specific drugs that target VIP-R pathways to enhance cancer immunotherapy.

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