PD-1 INHIBITS BYSTANDER T CELL ACTIVATION AND PROTECTS FROM ACTIVATION INDUCED CELL DEATH

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Background Immune checkpoint inhibition (ICI) targeting PD-1/PDL-1 is being increasingly applied and for longer periods of time. Aside from rapid response to antigen stimulation, memory T cells can also be directly activated and perform effector functions via cytokine signaling alone in the presence of high concentrations of inflammatory or immunostimulatory cytokines due to expression of CD132/CD122 receptor complexes.1-3 These ‘bystander-activated’ T cells can therefore amplify T cell effector responses particularly in aged individuals where a higher proportion of memory T cells exists.4 While the role of PD-1/PDL-1 on antigen-specific T cell responses has been extensively characterized, its role in bystander T cell responses is less clear.

Methods We examined the role of the PD-1/PD-L1 pathway during bystander activation using multiple mouse and human model systems. T cells from mice treated with high-dose (HD) rhIL-2 were evaluated for bystander activation using flow cytometry for NKG2D, CD69, granzyme B, CD25, Ki67, and PD-1 expression. Mouse T cells from control or TCR-transgenic OT-1 mice (which are specific for ovalbumin and thus not antigen-experienced) were stimulated in vitro with rhIL-2 or anti-CD3/28 to model bystander versus TCR-stimulated signaling. Activation, proliferative, and apoptotic responses (via annexin V staining) were assessed at various time-points. Effects of PD-1 blockade or loss was also assessed. We compared these results gating on PD1+ and PD1- T cell populations and subsequently repeated the same procedure using human T cells isolated from human PBMCs. We then assessed human T cells isolated from patients undergoing HD rhIL2 treatment for cancer, gating on PD1+ and PD-1- activated T populations.

Results Significantly reduced activation and proliferative responses were observed by activated PD-1+ bystander T cells compared to the PD-1- populations in both the mouse and human T cells following HD IL2 treatment in vitro or in vivo. PD-1- bystander-activated T cells also had increased apoptosis via activation induced cell death (AICD). Concurrent blockade or absence of PD-1 signaling in the mouse models resulted in greater activation responses comparable to PD-1- cells, but this also resulted in increased AICD and cell loss.

Conclusions The PD-1/PD-L1 pathway also inhibits antigen-nonspecific bystander-activated memory T cell responses and protects cells from AICD. While blockade of this pathway can result in increased bystander activation and effector functions, it also leads to increased AICD and T cell loss. These findings imply possible consequences of continuous PD-1 blockade application on the maintenance of the finite memory T-cell pool.

REFERENCES

Ethics Approval All studies and protocols complied with ethical regulations and humane endpoints and were approved by University of California Davis (UCD) IACUC. Mice were housed in AAALAC-accredited animal facilities at UCD under specific-pathogen-free conditions.

For human blood samples, signed informed consent was obtained before enrollment. The study was approved by the Providence Health System Regional Institutional Review Board, Oregon.