Molecular and systemic mechanisms of PD-1 function

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PD-1 is an inhibitory receptor expressed on activated T-cells. SHP-2 tyrosine phosphatase interacts with PD-1 and is critical for PD-1-mediated inhibition. The cytoplasmic tail of PD-1 contains an immunoreceptor tyrosine-based inhibitory motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). SHP-2 is a key mediator of PD-1 inhibitory function and has been reported to interact with either ITIM or ITSM of PD-1. We sought to identify the precise motif of PD-1 that is mandatory for SHP-2 interaction and PD-1 inhibitory function. Using GST-SHP-2 fusion protein we determined that PD-1 interacted with SHP-2 after PD-1 ligation with simultaneous TCR/CD3-mediated activation. This interaction required phosphorylation of the ITSM and was abrogated when the ITSM tyrosine Y248 was mutated to phenylalanine. In contrast, when the ITIM tyrosine Y223 was mutated, interaction of PD-1 with SHP-2 remained unaffected. Based on these findings, we hypothesized that phosphorylation of Y248 might be indicative of PD-1-mediated inhibitory signaling. We generated an antibody specific for Y248 and examined expression and function of PD-1pY248^{+} T cells. In peripheral blood from healthy humans, PD-1pY248^{+} cells were detected within the CD4^{+} but mostly CD8^{+} T cell populations, mainly in central memory and effector memory subsets and in much lower extent in terminally differentiated effectors. Although PD-1^{high} did not correlate with altered ability of CD8 T cells to produce effector cytokines, PD-1pY248^{+} expression correlated with impaired production of IFN-γ and TNF-α in response to TCR/CD3+CD28-mediated stimulation. Thus, PD-1pY248 might serve as a biomarker indicative of PD-1 mediated inhibitory signaling in patients with chronic infections and cancer. Systemic effects of PD-1pY248^{+} T cells in health and disease will be discussed.