The Immune Checkpoint Blockers PD-1, LAG-3 and TIGIT Are Associated With HIV Persistence During ART

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Background: The persistence of HIV in a small pool of long-lived latently infected resting CD4 T cells is a major barrier to viral eradication. Interfering with the immunological mechanisms that maintain latency in these cells may lead to novel therapeutic strategies to cure HIV infection. Several immune checkpoint blockers (ICBs) have been shown to actively reduce T cell activation, proliferation and cytokine production in CD4 T cells. We hypothesized that expression of one or several of these ICBs contribute to HIV persistence by continuously and actively promoting HIV latency in infected CD4 T cells.

Methodology: Expression of PD-1, CTLA-4, LAG-3, TIGIT, TIM-3, BTLA, 2B4 and CD160 were measured by flow cytometry on PBMCs from 48 subjects on ART for >3 years with HIV viral load inferior to 50 cop./mL and a CD4 count > 350 cells/μL. The frequencies of CD4 T cells harboring integrated HIV DNA, total HIV DNA, 2-LTR circles and cell associated unspliced (CA-US) HIV RNA were determined by qPCR. Integrated HIV DNA was also measured in sorted memory CD4 T cell subsets expressing some of these ICBs. More specifically, the impact of PD-1 engagement on HIV latency was evaluated in CD4 T cells isolated from virally suppressed subjects using beads coated with anti-CD3, anti-CD28, PD-L1 or the appropriate control.

Results: Absolute CD4 T cell counts were negatively correlated with the expression of PD-1, LAG-3 and TIGIT in CD4 T cells (r=-0.53 p<0.0001, r=-0.51 p=0.0002, r=-0.40 p=0.005, respectively). Interestingly, the frequency of CD4 T cells harboring integrated HIV DNA was positively correlated with the expression of these markers (r=0.29 p=0.06, r=0.31 p=0.04 and r=0.46 p=0.002, for PD-1, LAG-3 and TIGIT, respectively). With the exception of TIGIT with 2-LTR circles (r=0.39 p=0.009), no statistically significant associations were found between these markers and total HIV DNA, 2-LTR circles or CA-US HIV RNA. Memory CD4 T cells expressing high levels of PD-1 or LAG-3 were enriched for HIV integrated DNA when compared to their negative counterparts. Engagement of PD-1 with its ligand PD-L1 inhibited viral production induced by TCR stimulation in latently infected cells (mean=94.8 % of inhibition), suggesting a functional role for this receptor in the maintenance of HIV latency.

Conclusions: We identified PD-1, LAG-3 and TIGIT as markers associated with incomplete CD4 T cell restoration and HIV persistence during ART. Our data further demonstrate that PD-1 and LAG-3 identify cells carrying integrated HIV DNA in virally suppressed subjects. Collectively, these data suggest that a limited number of specific ICBs may actively promote HIV persistence during ART. Interfering with PD-1 and LAG-3 signaling may disrupt HIV latency and pave the way for the development of novel curative strategies.