PD-1 Identifies Latently HIV-infected Non-proliferating and Proliferating CD4+ T-cells

Renee M. van der Sluis1,3, Nitaasha A. Kumar1,3, Vanessa A. Evans1,3, Rafick-Pierre Sékaly4, Rémi Fromentin4, Nicolas Chomont4, Paul U. Cameron1,3 and Sharon R. Lewin1,3

Introduction

CD4+ T-cells from HIV-infected individuals on antiretroviral therapy (ART) expressing the Immune Checkpoint (IC) marker of T-cell activation PD1, are preferentially infected2. Characterizing the role of PD1 and other IC in HIV persistence during ART may identify new potential targets for eliminating latently infected T cells. We have previously reported that myeloid [mDC] and monocytes induce integrated latency in resting non-proliferating2 and proliferating4 CD4+ T-cells and propose that this model can mimic interactions that occur in lymphoid tissue in vivo.

Hypothesis and Aims

We hypothesise that DC-T-cell interactions in vitro will induce expression of IC markers and this will alter the establishment and maintenance of latent infection in proliferating and non-proliferating T-cells. In this study we aimed to:

1. Define the expression of IC markers following co-culture and HIV infection of antigen presenting cells (APC) with resting T-cells
2. Determine whether latent infection is enriched in proliferating and non-proliferating T cells expressing IC markers

Methods

We performed flow cytometry on day 3 post-infection with CCR5-tropic eGFP reporter virus. Expression of IC ligands PD-L1/PD-L2 (ligands for PD-1), Galectin 9 (possible ligand for Tim3), HVEM (ligand for B and T cell antagonist (BTLA) and poliovirus receptor), PVV/poliovirus receptor like 2; PVLV2 (ligands for T cell immunoreceptor with Ig and ITIM domains; TIGIT) on mDC and monocytes were measured on all infected cells. Data points represent the mean of 3-5 donors ±SEM.

Results 1. Expression of IC ligands are similar following co-culture with mDC or monocytes

Results 2. Differential expression of IC markers on proliferating and non-proliferating CD4+ T cells co-cultured with monocytes

Results 3. HIV latency is enriched in PD-1+ non-proliferating and proliferating CD4+ T cells

Implications

- Up-regulation of Immune Checkpoint markers and their ligands may facilitate the maintenance of latent infection of CD4+ T-cells
- Disrupting the function of PD-1 could potentially be exploited to inhibit replenishment of the reservoir and/or reverse latency

Conclusions

1. Myeloid DC and monocytes showed comparable IC Ligand expression levels
2. IC markers are differently expressed on proliferating and non-proliferating T cells following co-culture with monocytes
3. HIV latency is enriched in non-proliferating cells expressing high levels of PD-1, Tim-3, CTLA-4 or BTLA but not LAG-3 or TIGIT
4. HIV latency is enriched in proliferating CD4+ T cells expressing high levels of PD-1

References:
1. Chomont et al. Nat Med 2010; 4
2. Evans et al. PLoS Pathog 2012; 4
3. Kumar et al. AIDS 2011; 5
4. Kumar et al. AIDS 2014

Figure 1. Resting CD4+ T-cells were cultured alone or with either syncytium mDC or monocytes. Cells were infected with CCR5-tropic eGFP reporter virus. Expression of IC ligands PD-L1/PD-L2 (ligands for PD-1), Galectin 9 (possible ligand for Tim3), HVEM (ligand for BTLA) and poliovirus receptor, PVV/poliovirus receptor like 2; PVLV2 (ligands for T cell immunoreceptor with Ig and ITIM domains; TIGIT) on mDC and monocytes were measured on all infected cells. Data points represent the mean of 3-5 donors ±SEM.