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

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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 infected[1,2]. 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 (m)DC[3] and monocytes[4] induce integrated latency in resting non-proliferating[3] and proliferating[5] CD4+ T-cells and propose that this model can mimic interactions that occur in lymphoid tissue in vivo.

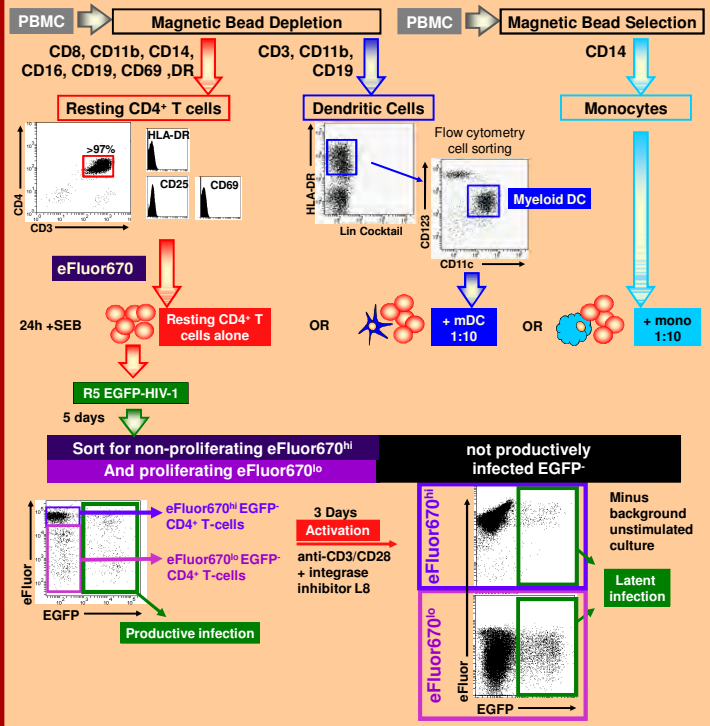
[1]Chomont *et al.* Nat Med 2010; [2]Fromentin *et al.* CROI 2014; [3]Evans *et al.* PLoS Pathogens 2013; [4]Kumar *et al.* AIDS 2014; [5]Kumar *et al.* AIDS 2014;

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



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

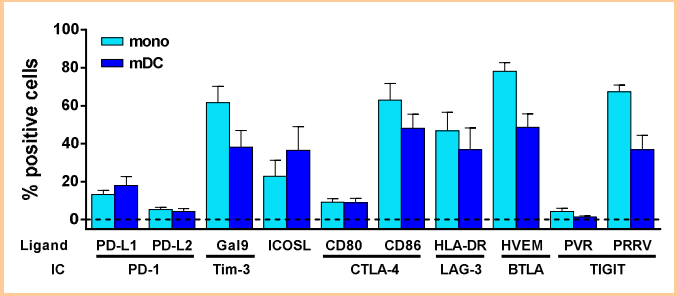


Figure 1. Resting CD4+ T cells were cultured alone or with either syngeneic mDC ( ) or monocytes ( ). Cells were infected with CCR5-tropic eGFP-reporter virus. Expression of IC ligands PD-L1/PD-L2 (ligands for PD-1), Galectin 9 (possible ligand for Tim3) CD80/CD86 (ligand for CTLA-4), HLA-DR (ligand for LAG-3), herpes virus entry mediator; HVEM (ligand for B and T cell attenuator (BTLA)) and poliovirus receptor; PVR/poliovirus receptor-like 2; PVRL2 (ligands for T cell immunoreceptor with Ig and ITIM domains; TIGIT) on mDC and monocytes were measured on d1 post infection. Data points represent the mean of 3-5 donors ±SEM.

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

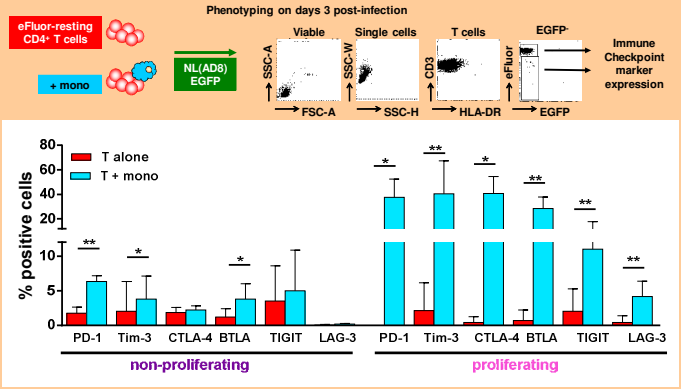


Figure 2. Resting CD4+ T cells were cultured alone ( ) or with syngeneic monocytes ( ). Expression of Immune Checkpoint markers on resting CD4+ T cells was determined by flow cytometry on day 3 post-infection with CCR5-tropic eGFP-reporter virus. Data points represent mean of 3-9 donors ±SEM.

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**

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

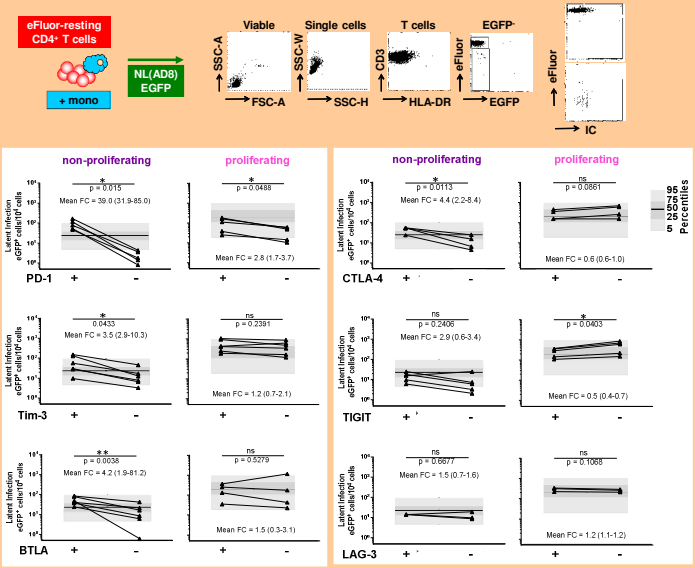
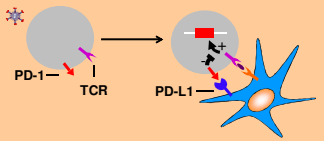


Figure 3. Resting CD4+ T cells were cultured with syngeneic monocytes and infected with CCR5-tropic eGFP-reporter virus. At day 5 post-infection cells were selected for CD3+, HLA-DR- and GFP- expression. Non-proliferating (eFluor^{HI}) and proliferating (eFluor^{LO}) cells were further sorted on the basis of IC expression into high (+) and low (-) expressing populations. Sorted cells were activated with anti-CD3/CD28 in the presence of the integrase inhibitor L8/Raltegravir for 3 days. Percentiles are calculated using the number of latently infected cells in all sorting experiments selecting for proliferating and non-proliferating cells (n=29), p values determined by student t test.

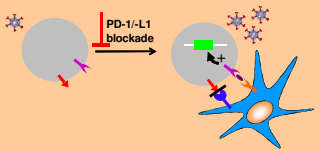
Implications

- Up regulation of Immune Checkpoint markers and their ligands may facilitate the maintenance of latent infection of CD4+ T cells



→ Active suppression of T cell activation and viral replication

- Disrupting the function of PD-1 could potentially be exploited to inhibit replenishment of the reservoir and/or reverse latency



Cell death
→ virus production
→ immune clearance