

Percentage and Relative Levels of Expression of PD-1 and PD-L1 in Treated HIV-1+ Individuals as a Marker of Disease Status and Therapy Efficacy

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ABSTRACT

Background: Ligation of the programmed death (PD)-1 molecule by its ligands PD-L1 and PD-L2 can lead to anergy and exhaustion of virus-specific CD4 and CD8 T cells which may result in an inhibition of function. In this cross-sectional study we assessed the expression of PD-1 and its associated molecule PD-L1 in a cohort of treated chronically HIV-1-infected individuals, as compared to levels observed in uninfected healthy individuals. **Methods:** Freshly separated PBMC from 22 chronically HIV-1-infected patients on effective HAART and 10 seronegative controls were assessed for percentage and mean fluorescence intensity (MFI) of PD-1 and PD-L1 on T cells. Further analysis of PD-L1 on the total PBMC population was performed. Drugs used in the HAART regimens of the patients were recorded. Statistical analysis of variance (ANOVA) and Mann Whitney U tests were performed where $p < 0.05$ was the cut off for significance. **Results:** Patients plasma HIV-1 RNA load was below detection limit (50 copies/ml) and median CD4 T-cell count was 547 cells/ μ l of blood (range 152-1576). The percentage of PD-L1 expressing cells was slightly elevated in HIV-1-infected patients compared to uninfected controls, however this difference did not reach statistical significance ($p=0.7144$). The percentage of T cells expressing PD-1 seen in patients on boosted-PI regimen ($n=12$) was slightly lower to that obtained for NNRTI-treated patients ($n=10$), ($p=0.0789$). MFI for PD-L1 was increased in HIV-1 infected patients on both PBMC ($p=0.0005$) and gated T cells ($p=0.005$). **Conclusions:** No statistically significant differences in the percentage of cells expressing either PD-1 or PD-L1 were observed between the infected and uninfected cohort. The higher relative MFI of PD-L1 observed in HIV-1-infected individuals compared to uninfected controls may be a signature of a persistent anergic state. The small differences in the numbers of PD-1 expressing cells observed between the two drug regimens may reflect a more potent action of the boosted-PI treatment. Previous reports show that untreated viremic patients have higher expression of PD-1, our data suggest that the observed similarities in percentage expression between controls and HIV-1+ treated patients are indicative of normalisation of PD-1 expression after initiation of HAART, however PD-L1 MFI levels remain higher in HIV-1+ patients.

BACKGROUND

HIV-1 chronic infection is characterised by anergic or exhausted HIV-1-specific CD8 T cells. Such cells are able to produce antiviral cytokines but lack cytolytic function and therefore are unable to mount an effective immune response against HIV-1. T-cell anergy is caused by a number of factors including T-cell receptor (TcR) ligation in the presence of impaired costimulation and cell death induced by apoptosis [1]. Highly active antiretroviral therapy (HAART) can partially reverse anergy but a complete reversal of anergy allowing effective control of HIV-1 is not achieved. One molecule that seems to be involved in promoting and regulating anergy is CD279 or Programmed Death 1 (PD-1), a member of the CD28 family involved in signal transduction in combination with T cell receptor signalling. PD-1 is expressed on all lymphoid cells and was first cloned from cells highly susceptible to apoptosis. PD-1 ligand (PD-L1) is mainly expressed on professional and non professional antigen presenting cells (APC) and is linked to a negative regulation of T-cell proliferation, activation and cytokine production [2, 3].

In recent studies the possible role of the PD-1/PD-L1 receptor signalling pathway in exhaustion of HIV-1-specific CD8+ T cells has been explored. PD-1 was found to be up-regulated on HIV-1-specific CD8+ T cells in treatment naive patients, and its expression correlated with the impaired function of these cells, as well as being a predictor of disease progression [4, 5]. These correlations were also observed when PD-1 expression was measured on CD4+ T cells. The blockade of the PD-1/PD-L1 pathway rescued functionality of some CD8+ T cells *in vitro*, including proliferation and cytokine production [4, 5]. *In vivo* in a mouse model of lymphocytic choriomeningitis virus infection, blockade led to virus specific CD8 T-cell responses being greatly enhanced, leading to a reduced viral load [4-6].

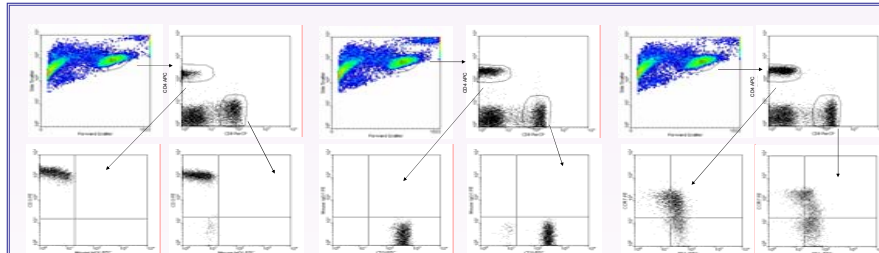


Figure 1: Representation of gating strategy for flow cytometric analysis of PD-1. Positivity is set using isotype control antibodies.

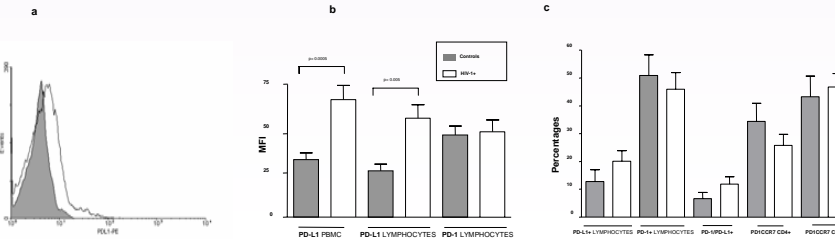


Figure 2: Representative histogram showing MFI of PD-L1 of stained PBMC (a) from HIV-1+ subject compared to an uninfected control. Graph b shows cumulative data for PD-L1 MFI on the total PBMC ($p=0.0005$) and on the gated lymphocytes ($p=0.005$); and PD-1 in uninfected controls and HIV-1+ patients. The right hand panel (c) shows the percentages of cells positive for PD-1, PD-L1, in CCR7+, CD4+ or CD8+ lymphocyte populations respectively.

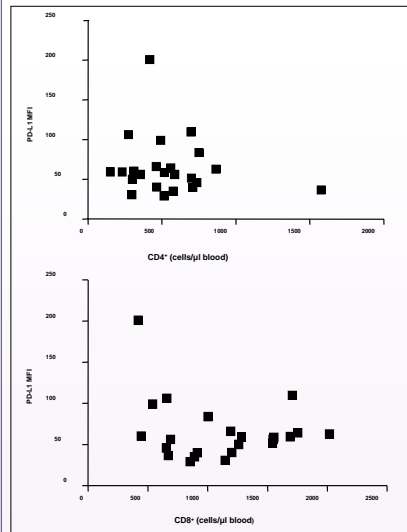


Figure 3: PD-L1 MFI and percentages showing lack of correlation with CD4 and CD8 cells counts

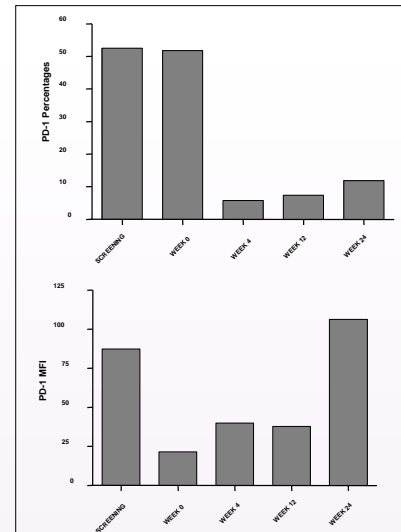


Figure 4: Example of time course of PD-1 expression on the whole PBMC population in a patient after Pneumovax vaccination

METHODS

In this study 22 HIV-1+ individuals on HAART were tested. The drug regimen, CD4 and CD8 T cell count and plasma viral load of the patients was recorded. In addition 10 uninfected controls were tested. Peripheral blood mononuclear cells (PBMC) were separated from whole blood by density centrifugation. PBMCs were stained with monoclonal antibodies to CD3, CD4, CD8, PD-1, PD-L1 and CCR7, and analysed by flow cytometry. Lymphocytes were identified based on their forward and side scatter characteristics. The total PBMC population, CD3+, CD4+, and CD8+ populations were assessed for both percentage expression and mean fluorescence intensity (MFI) of PD-1 and PD-L1 and CCR7 (Fig 1).

RESULTS & DISCUSSION

In this study we measured levels of PD-1 and its ligand PD-L1 in HIV-1 chronically infected individuals receiving HAART, both Non Nucleoside Reverse Transcriptase Inhibitor (NNRTI) and boosted Protease Inhibitor (PI) regimens. Patients had undetectable viral load (< 50 copies/ml plasma) and median CD4 T-cell count of 547 cell/ μ l of blood (range 152-1576).

The histogram in Fig 2a shows a representative example of the increase of MFI for PD-L1 which was remarkably increased in the HIV-1-infected group both on the total PBMCs ($p=0.0005$, Fig 2b) and on gated CD3+ T cells ($p=0.005$, Fig 2b) in accordance with findings by other groups. We found that the percentages of CD4+ and CD8+ T cells expressing PD-L1 were slightly raised in the HIV-1-infected group regardless of treatment regimen when compared to uninfected controls ($p=0.7144$, Fig 2c). No difference between infected and uninfected individuals was noted in PD-1 expression on either CD4+ or CD8+ T cells, also no difference was observed in CCR7 levels (Fig 2c). A slight reduction in expression in patients taking boosted PI compared with patients taking NNRTI was observed. No correlation between CD4 and CD8 numbers and PD-L1 was found (Fig 3). A longitudinal study was also performed in which patients received Pneumovax vaccine and were followed for 24 weeks (an example is shown in Fig 4).

Our results show no difference in PD-1 expression between the seronegative and treated seropositive groups. This is likely to be due to the suppressive action of HAART and the lack of persistent HIV-1 antigenic stimulus which is a consequence of effective HAART [4]. Expression of PD-L1 appears not to be affected by HAART and remains elevated on PBMC from HIV-1 treated patients possibly contributing to the lack of complete recovery from anergy after initiation of HAART. This data suggests that PD-L1 modulation could facilitate reversal of anergy, and also indicates that the ligand rather than the receptor may be a more relevant target for novel therapeutic interventions. However further studies are necessary to investigate the physiological role of PD-1/PD-L1 signalling and possible adverse effects that may occur by disrupting this receptor-ligand pathway.

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