Research Letter

Soluble Plasma PD-1 and Tim-3 in Primary HIV Infection

Eva ZILBER[1*], Genevieve E MARTIN[1*], Christian B WILLBERG[1,2], Julie FOX[3], Nneka NWOKOLO[4], Sarah FIDLER[5,6], John FRATER[1,2,7], on behalf of the CHERUB Investigators

[1]University of Oxford, Nuffield Department of Medicine, Oxford, United Kingdom
[2]National Institute of Health Research Biomedical Research Centre, Oxford, United Kingdom
[3]Guys and St Thomas' NHS Trust, Department of Genitourinary Medicine and Infectious Disease, London, United Kingdom
[4]Chelsea and Westminster Hospital, London, United Kingdom
[5]Imperial College, Division of Medicine, Wright Fleming Institute, London, United Kingdom
[6]Imperial College National Institute of Health Research Biomedical Research Centre, London, United Kingdom
[7]Oxford Martin School, Oxford, United Kingdom

* these authors contributed equally

Corresponding authors:

John Frater and Genevieve Martin, Nuffield Department of Medicine, Peter Medawar Building for Pathogen Research, John Radcliffe Hospital, Oxford, UK. Email: john.frater@ndm.ox.ac.uk; genevieve.martin@ndm.ox.ac.uk

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Summary

Soluble forms of the co-inhibitory receptors PD-1 and Tim-3 exist, but their relationship with T cell surface expression remains unclear. When measured by ELISA in plasma, sPD-1 and sTim-3 were elevated during PHI, decreased on ART to levels found in controls, and correlated with cell surface expression. We conclude that sPD-1 and sTim-3 are easy to measure biomarkers of immune exhaustion which potentially eliminate the need for flow cytometry.

Main text

Antiretroviral therapy (ART) dramatically improves the life expectancy of people living with HIV [1, 2], and prevents onward viral transmission [3, 4]. However, in some treated individuals the co-inhibitory receptors PD-1 and Tim-3 [5] are persistently elevated in primary and chronic HIV infection, and are associated with T cell exhaustion [6-9]. This T cell dysfunction is increasingly recognised as a limitation to curative strategies such as “kick and kill” which will rely on an effector immune response. Furthermore, PD-1 expression during primary HIV infection (PHI) correlates with viral reservoir size and predicts time to viral rebound after treatment interruption [10]. Importantly, the success of cancer immunotherapy to target T cell exhaustion [11] has prompted investigation into whether these pathways can be targeted as part of an HIV cure strategy [12].

Both PD-1 and Tim-3 also have soluble forms which can interact with these signalling pathways. Soluble PD-1 (sPD-1) is an alternative splice form of PD-1 [13], while soluble Tim-3 (sTim-3) is produced when Tim-3 is cleaved from the cell surface by the sheddase ADAM10 [14]. sTim-3 is increased during acute, early, and chronic untreated HIV infection, and correlates with plasma viral load (pVL) [14]. Recently, sPD-1 was shown to be increased in viraemic patients when compared to both healthy controls and patients on long-term ART [15]. The relationship between plasma levels and T cell expression of these markers is unknown. Here, we measured plasma sPD-1 and sTim-3 pre-treatment and one year after ART initiation in PHI. We investigated the relationship of soluble co-inhibitory receptor plasma concentration with key clinical parameters and with the respective receptor surface expression on CD4+ and CD8+ T cells.

48 participants from the HEATHER cohort were selected based on sample and data availability. All were male, with the median time from estimated seroconversion to ART initiation of 46 [IQR 28–70] days. The median baseline pVL was 5.50 [IQR 4.49–6.55] log_{10} HIV RNA copies/ml, median CD4 cell count 526 [IQR 405.2–667.8] cells/mm^3. 10 healthy controls (HCs) (REC 16/YH/0247) were all male, with median age 34.5 [IQR 30.5-42.5] years. sPD-1 and sTim-3 in plasma was measured using Human PD-1 (PDCD1) ELISA kit (Thermo Fisher Scientific, Waltham, MA USA) and Quantikine ELISA Human TIM-3 Immunoassay kit (R&D Systems, Minneapolis, MN USA) in accordance with manufacturers' instructions. T cell PD-1 and Tim-3 expression was measured using flow cytometry. Cell surface staining was with LiveDead Near IR, Tim-3 PE, PD-1 PE-eFluor610, CD3 BV570, CD4 BV605, CD8 BV650 as detailed elsewhere [16]. Data were acquired on an LSR II (BD) and analysed using FlowJo Version 10.0.8r1 (Treestar).

HIV DNA was quantified following a protocol described in detail elsewhere [17]. All samples were run in triplicate at two dilutions, and assay standards were run in duplicate.

Groups were compared using Kruskal-Wallis tests with pairwise comparison using Dunn's test. Linear mixed-effects models were fitted with data transformations as appropriate. Analysis was performed with GraphPad Prism (version 7.0b) and R (version 3.4.0) with MASS (version 7.3-47), nlme (version 3.1-131), and corrplot (version 0.84) packages.

We initially aimed to determine if soluble PD-1 and Tim-3 expression was perturbed in PHI and was associated with their respective surface co-inhibitory receptor expression.

PD-1 and Tim-3 expression was quantified in samples from the 48 HIV+ participants and 10 HCs. sPD-1 and sTim-3 were significantly elevated in PHI when compared to HCs. Following 1 year of ART, both decreased to levels not different from those found in HCs (Figure 1A,B). This demonstrates elevation of sPD-1 during PHI for the first time, and confirms previous findings for sTim-3 [14]. Soluble forms of the co-inhibitory receptors behaved similarly to surface forms: PD-1 and Tim-3 expression was increased on both CD4+ and CD8+ T cells in PHI compared to HCs, and decreased significantly with ART (Figure 1C-F). Furthermore, soluble co-inhibitory receptor expression correlated with respective cell surface expression (Figure 1G,H).

Considering the known relationship between T cell exhaustion, HIV clinical parameters and reservoir size, we investigated the relationship of soluble co-inhibitory receptors with pVL, CD4 T cell count, CD4/CD8 ratio (all at baseline), and reservoir size after one year on ART. sPD-1 correlated significantly with all four, and pVL and CD4 count were more strongly correlated with this soluble form than surface PD-1 expression on CD4 or CD8 T cells (Figure 1G).

To investigate the nature of these relationships further, we used linear mixed effects (LME) modelling to determine which variables were independently associated with sPD-1 levels. The use of LME models allowed for consideration of longitudinal sampling of HIV-infected participants. The optimal model included the time-point, CD4 and CD8 PD-1%, baseline pVL and CD4/CD8 ratio (Supplementary Table 1).

Importantly, there were significant relationships between sPD-1 levels and pVL and CD4/CD8 ratio, and their interaction (Supplementary Table 1). As expected, there was a relationship between CD4+ PD-1% expression and sPD-1 concentration, although this was not statistically significant (p=0.059) when these measures of clinical progression were also considered. Overall, sPD-1 expression is linked to CD4 T cell expression of this marker, and measures of disease progression during PHI. Further investigation of sPD-1 as an easily obtainable and clinically relevant measure of T cell functionality during PHI is warranted.

Similarly to Tim-3 expression on CD8 T cells, sTim-3 correlated with pVL, CD4/CD8 ratio, and reservoir size (Figure 1H). In the optimal LME model, sTim-3 was associated with the time-point and CD8 Tim-3% only. Therefore, sTim-3 might be a good surrogate for surface Tim-3 expression.

In conclusion, sPD-1 and sTim-3 are associated with their respective surface receptor expression and with key clinical parameters. Importantly, they are cheap and easy to measure and do not require viable fresh or cryopreserved cells, or the access to flow cytometry facilities. These soluble markers could therefore be useful adjuncts for studying immune exhaustion and potential immunotherapy development, and may have value for screening or stratification within clinical trials.

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AUTHORSHIP CONTRIBUTIONS

The experiments were conceived and designed by GEM, EZ, CBW, and JFr. Experiments were performed by GEM and EZ. Data were analyzed by GEM and EZ. Design and recruitment of the trials was performed by JFr, NN, JF and SF. The paper was written by GEM, EZ, JFr and CBW with input from all authors.

DISCLOSURE OF CONFLICTS OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Figure Legend

Figure 1. Soluble PD-1 and Tim-3 expression are perturbed in PHI and are associated with respective surface co-inhibitory receptor expression and clinical parameters. (A-B), soluble PD-1 and Tim-3 concentration in plasma. (B-F) Co-inhibitory receptor expression on CD4+ and CD8+ T cells. All three groups were compared using Kruskall-Wallis tests (overall $p<0.001$ for all tests), with subsequent pairwise comparisons performed using Dunn's test. *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$, ****: $p<0.0001$. (G-H) Corrplots showing Spearman's correlations between pairs of variables at baseline where the colour and size of circle corresponds to the correlation coefficient between these two variables; only significant ($p<0.05$) correlations shown.