



Dual Function of the Inhibitory Co-receptor PD-1 as a Marker for Activated and Functionally Impaired T Cells in SIV-infected Rhesus Macaques

Nadine C. Salisch ^{1,3}, Amany S. Awad ¹, Gordon Freeman ⁴, David T. Evans ¹, and R. Paul Johnson, MD ^{1,2}

¹ New England Primate Research Center, Harvard Medical School, One Pine Hill Drive, Southborough, MA, 01772. ² Infectious Disease Unit, Massachusetts General Hospital, 149 13th Street, Charlestown, MA 02129. ³ Institute for Clinical and Molecular Virology University Hospital, Friedrich-Alexander University Erlangen-Nuremberg, Schlossgarten 4, D-91054 Erlangen, Germany. ⁴ Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA, 02115

Introduction

In the setting of HIV/SIV infection, PD-1 expression on antigen-specific T cells has been reported to correlate with markers of disease progression, to elevate susceptibility to apoptosis, to inhibit proliferative capacity, and to negatively impact on net cytokine release, leading to the conclusion that PD-1 is a marker of T cell exhaustion. However, PD-1 is also upregulated in the setting of acute viral infection not characterized by T cell exhaustion, and the extent to which PD-1 represents a specific marker for dysfunctional T cells or an activation marker is still controversial.

Methods

Using polychromatic flow cytometry, we characterized PD-1⁺ SIV-specific CD8 T cells in macaques infected with wild-type SIVmac239, SIVmac251, or attenuated SIVmac239Δ3 or SIVmac239Δnef. SIV-specific cells were identified using Gag CM9 or Tat SL8 MHC-class I tetramers and were analyzed for proliferation (Ki-67), activation (HLA-DR), and expression of effector molecules (perforin, granzyme B) or CD28. Viral loads were determined by quantitative real-time PCR.

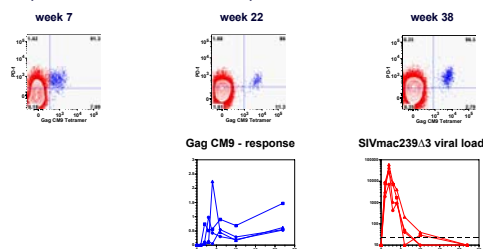
Conclusions

In contrast to recent reports, our data suggest that PD-1 serves a dual function in chronic retroviral infection.

- PD-1 is expressed on antigen-specific CD8 T cells in well-controlled infection with attenuated SIVmac239Δ3.
- In wild-type SIV infection, PD-1 is upregulated on activated, proliferating cells that express effector molecules.
- CD28, a co-stimulatory receptor, is progressively downregulated on PD-1⁺ SIV-specific CD8 T cells during the course of wt SIVmac239 infection.

PD-1 expression should be evaluated in the context of other markers to specifically identify dysfunctional cells.

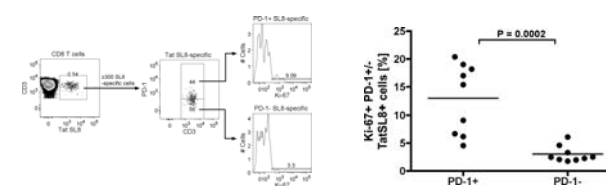
Gag CM9 – specific CD8 T cells in SIVmac239Δ3 infected animals retain high levels of PD-1 expression after clearance of plasma viremia



Upper panels: PD-1 expression on Gag CM9 – specific CD8 T cells of one representative rhesus macaque 7, 22, and 38 weeks post-infection with attenuated SIVmac239Δ3. Graphs show CD8 T cells from peripheral blood.

Lower graphs: Gag CM9 – specific CD8 T cell responses in 4 Mamu-A*01⁺ rhesus macaques until 22 weeks post-infection with SIVmac239Δ3, as identified by binding of Mamu-A*01:CM9 tetrameric complexes. The right panel shows viral load of SIVmac239Δ3 in the plasma of the same animals over the same period of time.

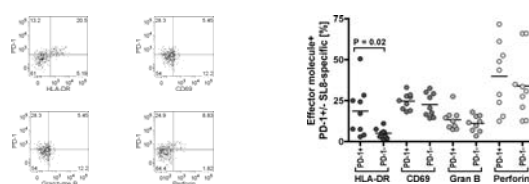
PD-1 positive Tat SL8–specific T cells show high levels of Ki-67 expression, a marker for ongoing proliferation



Left: Gating strategy employed to identify Ki-67 expression on PD-1⁺ and PD-1⁻ Tat SL8-specific CD8 T cells. PBMC were stained with antibodies against CD3, CD8, CD4, PD-1, and Ki-67, and Tat SL8-specific CD8 T cells were identified by tetramer staining. ≥ 300 SL8-specific CD8 T cells were collected, gated into PD-1⁺ and PD-1⁻ cells, and then analyzed for the expression of Ki-67.

Right: Difference in the percentage of Ki-67 expression on PD-1⁺ versus PD-1⁻ Tat SL8-specific cells in SIV-infected rhesus macaques (n = 9).

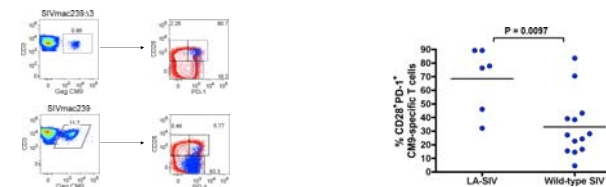
PD-1 positive Tat SL8–specific T cells show an activated phenotype and express similar levels of cytotoxic molecules as PD-1 negative SL8-specific cells.



Left: Representative flow cytometry dot plot graphs showing the expression of the activation markers HLA-DR and CD69, and the effector molecules granzyme B and perforin on Tat SL-8 specific cells. ≥ 300 Tat SL8-specific cells were collected, gated into PD-1⁺ and PD-1⁻ subsets and analyzed for the expression of HLA-DR, CD69, granzyme B, and perforin.

Right: Percentage of activation and effector molecule-expressing cells in PD-1⁺ and PD-1⁻ Tat SL8-specific cells in SIV infected rhesus macaques (n = 9).

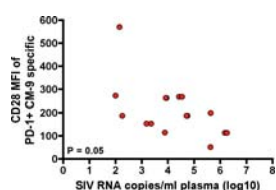
Loss of CD28 expression as a potential co-defining characteristic of functionally impaired SIV-specific T cells



Left: Expression levels of PD-1 and CD28 on Gag CM9-specific CD8 T cells in two representative animals infected with attenuated SIVmac239Δ3 (upper) or wild-type SIVmac239 (lower), respectively. Left panels show cells gated on CD3⁺CD8⁺CD4⁻ lymphocytes, right panels an overlay of Gag CM9-specific cells with total CD8⁺ T cells.

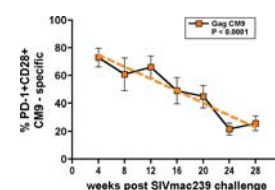
Right: Percentage of CD28⁺PD-1⁺ Gag CM9-specific CD8 T cells in animals infected with attenuated SIVmac239Δ3 or SIVmac239Δnef (n = 6, 'LA-SIV', left) and animals infected with wild-type SIVmac239 or SIVmac251 (n = 13, 'Wild-type SIV', right).

CD28 expression inversely correlates with viral load



Expression levels of CD28 on PD-1⁺ Gag CM9-specific CD8 T cells as a function of plasma viral loads in animals chronically infected with wild-type SIVmac239 or SIVmac251 (n = 16).

Progressive loss of CD28 expression during the course of wild type SIVmac239 infection



Longitudinal analysis of the percentage of PD-1⁺CD28⁺ Gag CM9-specific CD8 T cells in Mamu-A*01⁺ animals infected with SIVmac239 between 4 and 28 weeks post infection (n = 5).