INHIBITORY RECEPTOR EXPRESSION CORRELATES WITH HIV PERSISTENCE AND IMMUNE RESPONSES

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BACKGROUND

- In chronic viral infections, increased expression of inhibitory receptors (IR) or checkpoint inhibitors is associated with a hierarchical loss of T cell effector function which then lead to virus persistence.
- We have previously shown that frequencies of T cells with high PD-1 expression were associated with less HIV-specific T cell responses. Other studies have shown that CD4+ T cells expressing certain IR combinations are enriched for HIV DNA.
- In a cohort of persons with HIV (PWH), with suppressed plasma viremia on ART fort at least 4 years, we evaluated which specific IR combinations were associated with HIV persistence and HIV-specific immune responses.

METHODS



A5321 Cohort

- Cryopreserved PBMC from the HIV(+) participants obtained at year 4 on ART were used for the assays.
- IFNγ ELISPOT was done to evaluate specific immune responses to HIV-specific peptides as well as CMV and EBV.
- Associations were evaluated between the frequencies of T cells expressing various IR combinations and
  - Different measures of HIV persistence
  - ELISPOT responses to different peptides

1) Total HIV DNA
2) Cell-associated RNA
3) Single copy assay
4) IPDA

1) Soluble biomarkers of inflammation and anti-inflammation
2) T cell activation and cycling
3) HIV-specific immune responses
4) Expression of checkpoint inhibitors

STUDY POPULATION DEMOGRAPHICS (N=95)

Age at initiation of ART, median (Q1, Q3), years	39 (31, 47)
Age at A5321 entry, median (Q1, Q3) years	48 (38, 53)
Sex at birth – female	20 (21%)
Race/Ethnicity:	
White Non-Hispanic	49 (52%)
Black Non-Hispanic	18 (19%)
Hispanic (Regardless of Race)	26 (27%)
American Indian, Alaskan Native	2 (2%)
Pre-ART plasma HIV-1 RNA, median (Q1-Q3), log10 copies/mL	4.6 (4.3-5.0)
A5321 entry plasma HIV-1 RNA, copies/mL	<40 (99%)
Pre-ART CD4+ T-cell count, median (Q1-Q3), cells/mm^3	290 (159, 384)
A5321 entry CD4+ T-cell count, median (Q1-Q3), cells/mm^3	665 (505, 839)
Years on therapy at A5321 entry, median (Q1-Q3)	6.8 (4.4, 7.6)

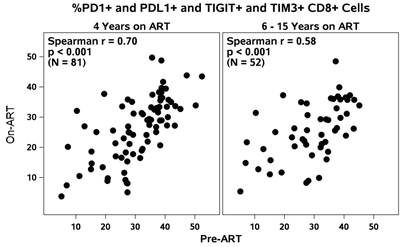
The frequency of T cells expressing multiple inhibitory receptors is associated with greater persistence of HIV-infected cells, as measured by total HIV DNA, and stronger IFNγ ELISPOT responses to HIV peptides.

MULTIPLE IR EXPRESSION AND IMMUNE RESPONSES

We evaluated 126 combinations of inhibitory receptors. Of those, only the frequencies of CD8+ T cell expressing specific IR combinations listed in the table below showed significant correlations with HIV-specific immune responses.

IR Combination	Gag		Env		Pol		Nef/Tat/Rev	
	Spearman	P value	Spearman	P value	Spearman	P value	Spearman	P value
%CD8 PD1+ TIGIT+ TIM3+	0.11	0.36	0.02	0.89	0.23	0.046	0.18	0.14
%CD8 PD1+ TIGIT+ TIM3+ PDL1+	0.10	0.40	0.01	0.94	0.24	0.043	0.17	0.14
%CD8 PD1+ PDL1+ TIGIT+	0.12	0.30	0.00	0.98	0.24	0.040	0.16	0.17
%CD8 TIGIT+ TIM3+	0.17	0.14	0.06	0.62	0.26	0.029	0.26	0.029
%CD8 PDL1+ TIGIT+ TIM3+	0.15	0.21	0.04	0.71	0.26	0.025	0.23	0.046
%CD8 PD1+ TIGIT+	0.12	0.30	0.00	0.99	0.23	0.05	0.15	0.21
%CD8 PDL1+ TIGIT+	0.17	0.14	0.04	0.72	0.27	0.021	0.23	0.05
%CD8 TIGIT	0.19	0.11	0.07	0.58	0.24	0.040	0.23	0.05

Fig 1



- Frequencies of CD8+ T cell expressing combinations of PD1, PDL1, TIGIT, and TIM3 were positively correlated with IFNγ ELISPOT responses to Pol, and Nef/Tat/Rev.
- No correlations were observed with Gag, Env, Vif/Vpr/Vpu, CMV, and EBV-specific responses.
- Pre-ART frequencies of CD8+ T cells expressing all 4 IR correlated with frequencies on ART (Figure 1).
- No correlations were observed with frequencies of CD4+ T cells expressing multiple IR.

MULTIPLE IR EXPRESSION AND HIV PERSISTENCE

IR Combination	CA-RNA		Total HIV DNA		iSCA		IPDA	
	N=71		N=89		N=87		N=42	
	Spearman	P value	Spearman	P value	Spearman	P value	Spearman	P value
%CD4 PD1+ TIM3+	0.11	0.35	0.26	0.013	0.20	0.06	0.24	0.13
%CD4 PD1+ PDL1+ TIM3+	0.11	0.38	0.26	0.014	0.20	0.06	0.23	0.14
%CD4 TIM3+	0.21	0.08	0.31	0.004	0.07	0.49	0.21	0.18
%CD8 PD1+ TIM3+	0.13	0.29	0.21	0.045	0.06	0.56	0.22	0.17

Fig 2a

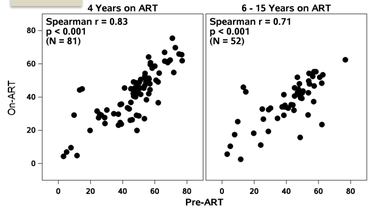
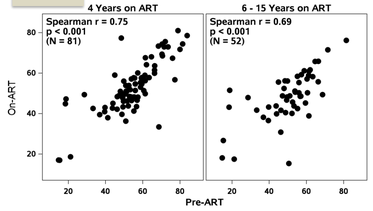


Fig 2b



- Of the 126 IR combinations evaluated, only the frequencies of T cells expressing PD1, TIM3, and PDL1 positively correlated with total HIV-1 DNA levels at year 4 on ART.
- Frequencies of CD4+ TIM3+ T cells also positively correlated with total HIV-1 DNA.
- We also observed a modest association between levels of residual viremia as measured by iSCA and the frequencies of CD4+ T cells expressing the 3 IR.
- Frequencies of T cells expressing PD1 and TIM3 pre-ART strongly correlated with frequencies on ART (r=0.69-0.83; p<0.001; Figures 2a and 2b).

CONCLUSIONS & IMPLICATIONS

- After long-term suppressive ART, increased frequencies of T cells expressing multiple inhibitory receptors (specifically PD1, PDL1, and TIM3) are associated with higher levels of total HIV-1 DNA.
- Frequencies of CD8+ T cells expressing multiple inhibitor receptors positively correlated with IFNγ ELISPOT responses to Pol and Nef/Tat/Rev.
- These findings suggest that persistent antigen expression from infected cells can stimulate both IFNγ response and expression of inhibitory receptors with a net effect of failure to clear infected cells.
- The therapeutic strategy of blocking single inhibitory receptors in HIV may not be enough to cause significant immunologic and virologic response.

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