2B4+ PD1+ NAÏVE AND MEMORY CD4+ T CELLS ARE ASSOCIATED WITH RESIDUAL VIREMIA ON ART

Cynthia R Klamar[1], Feiyu Hong[1], John Bui[1], Anthony R Cillo[1], Arcadio Agudelo-Hernandez[1], Deborah K McMahon[1], Charles R Rinaldo[2], John W Mellors[1], Bernard J Macatangay*[1]

[1]University of Pittsburgh School of Medicine, [2]University of Pittsburgh Graduate School of Public Health

CROI 2015 Conference on Retroviruses and Opportunistic Infections

POSTER #389 LB

BACKGROUND

- In HIV infection, T cell expression of inhibitory receptors such as PD1, has been associated with functional impairment as well as abnormal distribution of naïve and memory T cell subsets.
- Multiple inhibitory receptors have also been reported to be markers of HIV-1 cellular reservoirs in individuals who are on antiretroviral therapy.
- We evaluated 63 different combinations of inhibitory receptor (IR: PD1, CTLA4, TIM3, 2B4, CD160, and LAG3) expression, in naïve (T_N), central memory (T_{CM}) and effector memory (T_{EM}) CD4+ T cells of viremic [VIR] and ART-suppressed HIV-1(+) individuals [ART].
- We determined whether co-expression of multiple IRs on the different CD4+ T cell subsets is associated with residual viremia, and levels of cell-associated RNA/DNA in PBMC.

METHODS & DEMOGRAPHICS

HIV-1(+) VIREMIC [VIR]
N=10
Median Age= 49
Median CD4+ Count= 240 cells/mm³
Median HIV-1 RNA= 10500 cps/mL

HIV-1(+) ART-SUPPRESSED [ART]
N=30
Median Age= 51
Median CD4+ Count= 642 cells/mm³
Median years suppressed= 7.4 yrs

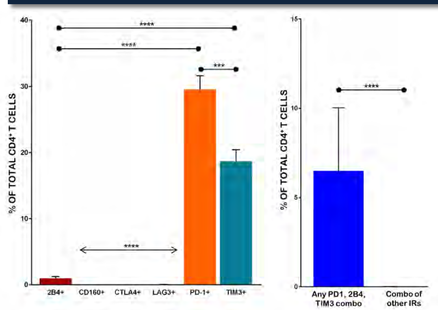
PCR
- Residual plasma HIV-1 RNA was quantified by single copy assay with 2-step RT qPCR assay targeting integrase region of *pol*
- Total HIV-1 DNA and unspliced HIV-1 RNA

Flow cytometry
- Live/Dead, CD3, CD4, CD8
- CD45RA, CCR7
- PD1, CTLA4, TIM3, 2B4, LAG3, CD160

Analysis
- FlowJo was used to evaluate expression of 6 IRs; Boolean gating and SPICE were used to evaluate 57 combinations of IR co-expression

Statistical Analyses
- Comparison between groups using Mann Whitney
- Spearman correlations
- GraphPad Prism 6

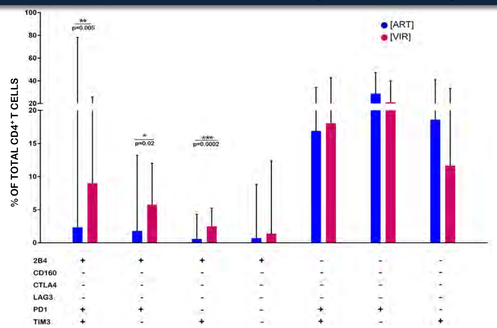
SINGLE & COMBINATION IR EXPRESSION ON TOTAL CD4 [ART]



- Among ART-suppressed individuals, the frequencies of CD4+ T cells expressing PD1, TIM3, or 2B4 alone, were significantly higher than those expressing CD160, CTLA4, and LAG3 (left; mean + SEM; p<0.0001).
- The frequency of CD4+ T cells expressing PD1 alone was highest.
- The frequencies of CD4+ T cells expressing any combination of PD1, TIM3, and 2B4 (PD1/TIM3/2B4+, PD1/TIM3+, 2B4/PD1+, and 2B4/TIM3+) were significantly higher than those expressing any combination involving the other IRs, even if the combination included 2B4, PD1, and/or TIM3. (right; p<0.0001)

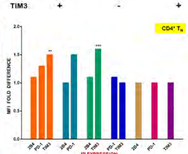
ACKNOWLEDGMENTS: This study is supported by internal funding from the University of Pittsburgh Department of Medicine and in part by Leidos Biomedical Research, Inc., contract 12XS547, through the National Cancer Institute. The University of Pittsburgh Clinical and Translational Science Institute is supported by the National Institutes of Health through grant numbers UL1RR024153 and UL1TR000005. The authors would also like to thank Patricia Peters, Renee Weinman, Sara Mattiucci, Lisa Klevens, and Jamie Ideluca of the Pitt Treatment and Evaluation Unit, Luann Borowski, Aarika Yates, and Angela Anthony of the University of Pittsburgh ACTG Immunology Specialty Lab, **and especially, the volunteers from the Pittsburgh AIDS Center for Treatment Clinic.**

2B4, PD1, & TIM3 EXPRESSION ON TOTAL CD4 [ART vs VIR]

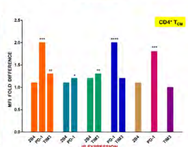


- The graph on the right shows a comparison of 2B4, PD1, and TIM3 single and co-expression (combo) on total CD4+ T cells in the ART and VIR groups.
- The frequencies of total CD4+ T cells co-expressing all 3 IRs as well as those co-expressing 2B4/PD1 and 2B4/TIM3 are significantly higher in viremics.

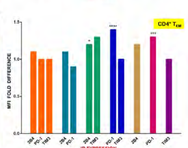
IR EXPRESSION	CD4 T_N [ART] 2B4	PD1	TIM3	CD4 T_N [VIR] 2B4	PD1	TIM3
2B4+ PD1+ TIM3+	488	375	359	530	478	530
2B4+ PD1+	441	416	-	447	635	-
2B4+ TIM3+	427	-	256	478	-	400
PD1+ TIM3+	-	191	294	-	219	299
2B4+	368	-	-	386	-	-
PD1+	-	208	-	-	205	-
TIM3+	-	-	210	-	-	214



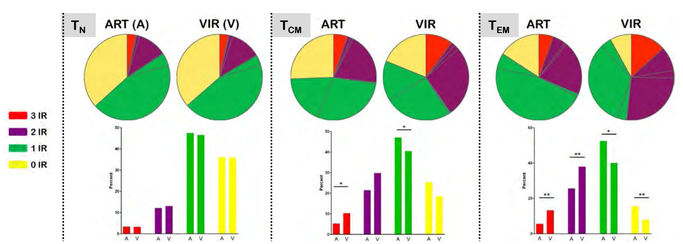
IR EXPRESSION	CD4 T_{CM} [ART] 2B4	PD1	TIM3	CD4 T_{CM} [VIR] 2B4	PD1	TIM3
2B4+ PD1+ TIM3+	421	561	294	476	1095	374
2B4+ PD1+	359	577	-	386	703	-
2B4+ TIM3+	396	-	227	474	-	297
PD1+ TIM3+	-	402	269	-	796	318
2B4+	348	-	-	372	-	-
PD1+	-	467	-	-	855	-
TIM3+	-	-	214	-	-	223



IR EXPRESSION	CD4 T_{EM} [ART] 2B4	PD1	TIM3	CD4 T_{EM} [VIR] 2B4	PD1	TIM3
2B4+ PD1+ TIM3+	424	863	267	465	823	254
2B4+ PD1+	412	854	-	440	761	-
2B4+ TIM3+	404	-	203	498	-	263
PD1+ TIM3+	-	684	282	-	926	273
2B4+	384	-	-	477	-	-
PD1+	-	713	-	-	934	-
TIM3+	-	-	228	-	-	222

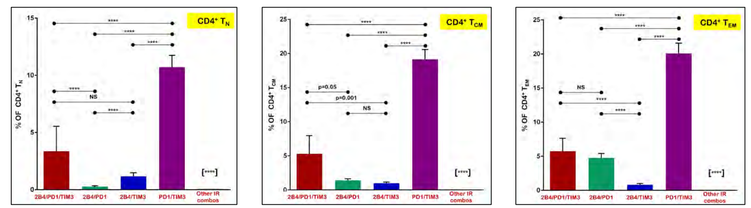


- The tables show the mean fluorescence intensities (MFI) of 2B4, PD1, and TIM3 when co-expressed with each other or when expressed by themselves on T_N, T_{CM}, and T_{EM} in the ART and VIR groups.
- The bar graphs show the fold-difference (y-axis) in MFI of VIR relative to ART for 2B4, PD1, and TIM3 on each CD4+ T cell subset. The x-axis shows the different combinations (color-coded) of IR expression.
- In T_N, TIM3 expression when co-expressed with 2B4 and with 2B4 and PD1, was significantly higher in VIR.
- In T_{CM}, PD1 expression, when expressed singly or when co-expressed with the other two IRs, was significantly higher in the VIR group.
- The increased PD1 expression in T_{EM} was also seen when expressed alone or with TIM3 in the VIR group.



- Pie charts show the proportion of T_N, T_{CM}, and T_{EM} expressing 0-3 IRs in ART and VIR. The bar graphs compare the frequencies of each CD4+ T cell subset in ART and VIR expressing 0-3 IRs.
- There is no difference in the frequencies of single IR and multiple IR co-expression between ART and VIR in T_N. There is a higher frequency of multiple IR co-expression in T_{EM} of VIR compared to T_{EM} of ART.

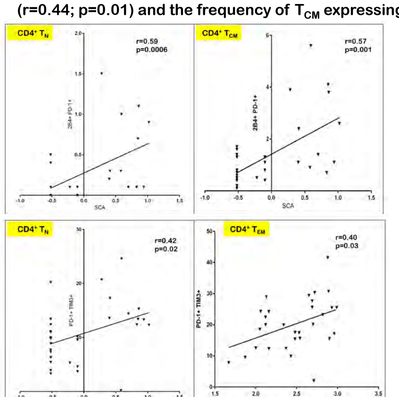
2B4, PD1, & TIM3 CO-EXPRESSION ON T_N, T_{CM}, & T_{EM} [ART]



- Bar graphs show a comparison of the frequencies of T_N, T_{CM}, and T_{EM} (y-axis) expressing the different combinations of 2B4, PD1, and TIM3 co-expression (x-axis). The frequencies of CD4+ T cell subsets expressing other IR combos (whether or not they included 2B4, PD1, and/or TIM3) were <0.1% and significantly lower (p<0.0001) than the combos that exclusively included these 3 IRs.
- The frequencies of each CD4+ T cell subset co-expressing PD1 and TIM3 were significantly higher than the other combinations, comprising up to a mean of 20.1% in T_{EM}.

IR CO-EXPRESSION, RESIDUAL VIREMIA, & HIV-1 RNA/DNA [ART]

- In total CD4+ T cells, single and multiple co-expression of 2B4, PD1, or TIM3 did not correlate with residual viremia or cell-associated RNA/DNA. Data not shown.
- Levels of residual viremia modestly correlated with the frequency of T_N expressing PD-1 alone (r=0.44; p=0.01) and the frequency of T_{CM} expressing 2B4 alone (r=0.42; p=0.02). Data not shown.



- Highly significant correlations were observed between the residual viremia and the frequency of T_N co-expressing 2B4 and PD1 as well as the frequency of T_{CM} co-expressing these 2 IRs.
- 2B4 and PD1 co-expression in all CD4+ T cell subsets did not correlate with cell-associated HIV-1 RNA.
- Although the frequencies of CD4+ T cell subsets co-expressing PD1 and TIM3 are highest, only modest correlations were observed between PD1+ TIM3+ T_N frequency and residual viremia.
- PD1+ TIM3+ T_{EM} frequency also had a modest correlation with cell-associated HIV-1 DNA.
- No correlations were observed with cell-associated HIV-1 RNA.

CONCLUSIONS

- HIV-1-infected, viremic individuals have greater frequencies of memory CD4+ T cells co-expressing multiple IRs, specifically 2B4, PD1, and TIM3 than ART-suppressed individuals. Viremic individuals also have higher expression of IRs in both naïve and memory CD4+ T cells.
- Despite viral suppression on ART, a substantial fraction of naïve and memory CD4+ T cells co-express different combinations of 2B4, PD1, and TIM3, with the specific combination of PD1 and TIM3 accounting for a fifth of memory CD4+ T cells.
- The frequency of naïve and central memory CD4+ T cells co-expressing 2B4 and PD1 is strongly associated with the levels of residual viremia but not cell-associated HIV-1 DNA or RNA.
- These results suggest that targeting more than one IR is needed to maximize effects on HIV-1 persistence.