PTEN OVEREXPRESSION IN ANTIGEN-SPECIFIC B CELLS FROM HIV-INFECTED INDIVIDUALS ON ART

Lesley R. de Armas[1], Suresh Pallikkuth[1], Li Pan[1], Stefano Rinaldi[1], Nicola Cotugno[2], Sarah F. Andrews[3], Rajendra Pahwa[1], Adrian B. McDermott[3], Paolo Palma[2], Savita Pahwa[1]

[1]University of Miami, Miami, FL, USA, [2]Bambino Gesú Children's Hospital, Rome, Italy, [3]Vaccine Research Center, NIAID, Bethesda, MD, USA

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Abstract

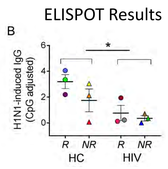
Memory B cells (MBC) respond to secondary antigen challenge to protect against infection and to boost immunity following vaccinations. Despite effective treatment, chronic HIV infection disturbs MBCs by reducing numbers and altering functionality due to hyper-activation and increased apoptosis leading to suboptimal antibody responses against common infectious agents. We used single cell gene expression analysis to evaluate antigen-specific memory B cells in peripheral blood of virally-suppressed HIV-infected individuals and healthy controls stratified by serum H1N1 antibody response 3 weeks post-administration of the seasonal trivalent inactivated influenza vaccine. We used a fluorescent probe to isolate influenza H1N1-specific B cells and a multiplexed and targeted RT-PCR approach to measure expression levels of 96 genes involved in B cell activation and function. Gene profiling revealed a 4-gene predictive signature containing the phosphoinositide-3 kinase (PI3K) inhibitor, PTEN, for identifying antigen-specific MBC from HIV-infected individuals compared to healthy controls. Gene co-expression analysis showed that in addition to overexpression of PTEN, there was increased co-expression of type I interferon-associated genes with PTEN on single cell level in HIV compared to controls. This study highlights the persistent defects in MBC from HIV-infected individuals and points to the PI3K signaling pathway as a target for potential immune intervention.

Study Objectives

- To evaluate transcriptional variation that can influence antigen-specific memory B cell functionality in the context of vaccine-induced antibody responses and chronic HIV infection.
- To identify novel correlates of functional memory B cell responses.

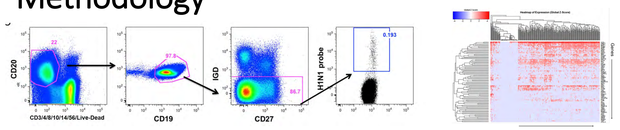
Study Participants

Gender	HIV status	Age (yrs.)	H1N1 Serum Titer			Response Status
			T0	T2	T2/T0	
Male	Negative	62	10	10	1	Non-Responder
Male	Negative	66	20	40	2	Non-Responder
Female	Negative	67	80	80	1	Non-Responder
Male	Negative	60	40	160	4	Responder
Female	Negative	61	40	640	16	Responder
Female	Negative	66	40	320	8	Responder
Male	Positive	67	20	40	2	Non-Responder
Male	Positive	66	40	80	2	Non-Responder
Male	Positive	62	40	80	2	Non-Responder
Female	Positive	62	10	160	16	Responder
Male	Positive	60	10	160	16	Responder
Male	Positive	76	20	1280	64	Responder



HIV-infected, ART-treated individuals with virus suppression (HIV RNA < 40 copies/ml) for at least 1 year prior to enrollment and HIV-uninfected healthy controls (HC) were recruited from University of Miami, Jackson Memorial, and VA Hospitals in Miami, FL. Serum Ab titers to H1N1 influenza strains were evaluated separately using an HAI assay. HAI titers are expressed as the reciprocal of the highest serum dilution at which hemagglutination was prevented.

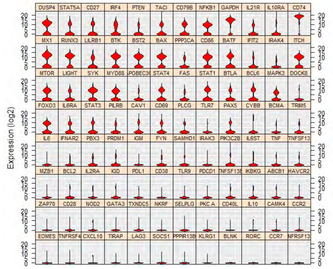
Methodology



Cryopreserved PBMC samples (T2) were thawed and rested before staining with monoclonal antibodies. The HA probe specific for H1N1 was generated and conjugated for flow cytometry (Whittle, *et al*. **2014** *J Virol*). For cell sorting, H1N1+ cells were sorted into a 96-well plate containing 9ul CellsDirect (Thermofisher) RT-PCR amplification buffer and reagents (Cotugno, *et al*. **2017** *Front Immunol*). Resulting cDNA was loaded onto BioMark IFC 96x96 chip (Fluidigm). A total of 360 H1N1-specific B cells were sorted from 12 participants (range from a single individual 4-59).

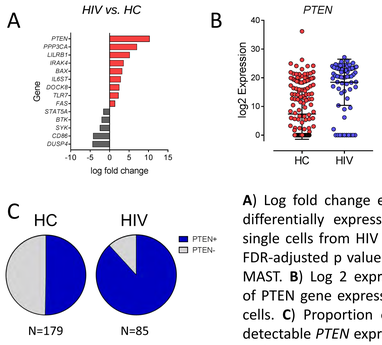
Results

I. Single cell gene expression data from H1N1-specific B cells



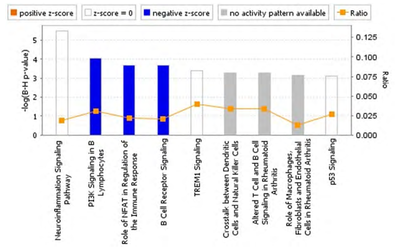
Violin plot showing the distribution of expression for each gene from 96-gene panel in all cells (n=360). Initial analysis showed a bimodal distribution of expression for most genes. Raw data underwent 'cellular detection rate' (CDR) filtering to remove outlier samples and genes based on dataset distribution. Overall 96 cells and 36 genes were filtered out following quality control sanitation steps and differential gene expression analysis was subsequently performed to contrast transcriptional profiles of H1N1-specific B cells between HC and HIV-infected participants.

II. Differential Gene Expression: HIV vs. HC



A) Log fold change expression of differentially expressed genes in single cells from HIV and HC with FDR-adjusted p value < 0.05 using MAST. **B**) Log 2 expression levels of PTEN gene expression in single cells. **C**) Proportion of cells with detectable *PTEN* expression in HIV and HC.

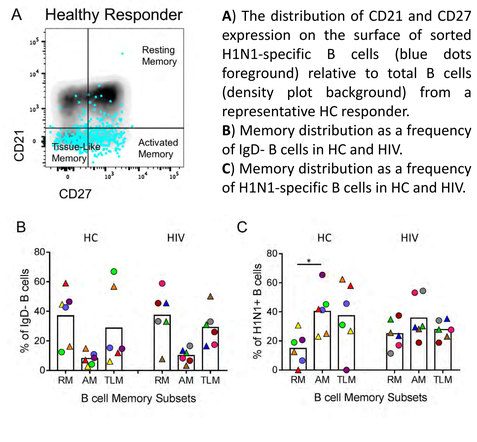
III. Pathway Analysis: HIV vs. HC



Pathway analysis using Ingenuity Pathway Analysis software shows top results using the 14 differentially expressed genes between HIV and HC.

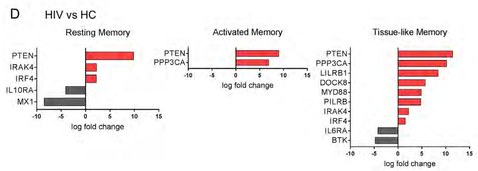
Results (contd.)

IV. Memory B cell subset distribution in H1N1-specific B cells.



A) The distribution of CD21 and CD27 expression on the surface of sorted H1N1-specific B cells (blue dots foreground) relative to total B cells (density plot background) from a representative HC responder.
B) Memory distribution as a frequency of IgD- B cells in HC and HIV.
C) Memory distribution as a frequency of H1N1-specific B cells in HC and HIV.

V. Memory B cell subset gene expression for H1N1-specific B cells.



Log fold change expression of differentially expressed genes in single cells from HIV relative to HC in each memory B cell subset using MAST analysis (FDR-adjusted p value < 0.05).

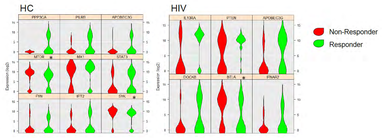
VI. *PTEN* is co-expressed with IFN-inducible genes in H1N1-specific cells from HIV

Target Gene	Query Gene	Pearson Coefficient	P value	Pearson Coefficient	P value
		Healthy Control		HIV	
PTEN	*APOBEC3G*	**0.18**	**0.02**	**0.37**	**0.001**
PTEN	*IGM*	**0.45**	**<0.0001**	**0.32**	**0.004**
PTEN	*IRAK4*	**0.52**	**<0.0001**	**0.34**	**0.002**
PTEN	*STAT5A*	**0.34**	**<0.0001**	**0.27**	**0.02**
PTEN	*BTK*	**0.21**	**0.009**	0.13	0.26
PTEN	*PPP3CA*	**0.21**	**0.01**	0.01	0.92
PTEN	*TLR7*	**0.27**	**0.001**	0.08	0.48
PTEN	*BST2*	0.03	0.67	**0.26**	**0.02**
PTEN	*DOCK8*	-0.04	0.65	**0.34**	**0.003**
PTEN	*IFNAR2*	0.13	0.12	**0.25**	**0.03**
PTEN	*IL10RA*	0.07	0.41	**0.42**	**0.0001**
PTEN	*NFKB1*	0.05	0.56	**0.24**	**0.04**

Co-expression of *PTEN* was determined by correlation analysis with all other genes in the panel. These results indicate that PTEN associated signaling pathways are perturbed in B cells from HIV-infected individuals despite viral suppression with cART.

Results (contd.)

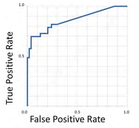
VII. Gene signatures associated with Influenza vaccine response.



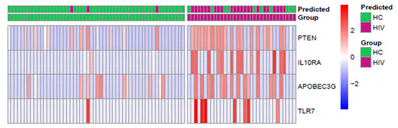
Differential gene expression was evaluated in vaccine responders and non-responders in HIV and HC separately and is shown in violin plots with p<0.05. All genes were higher in responders unless the gene name is followed by *.

VIII. PTEN drives a predictive signature able to discriminate between H1N1-specific Memory B cells from HIV and HC

To select key features from 33 genes for classifying 2 groups (HC and HIV), log2 transformed data was fit to a multinomial logistic regression model via LASSO penalty using R package "glmnet". 10-fold cross-validation (CV) was applied and the value of regularization parameter lambda giving minimum mean CV error (LamdaMin) was determined.



ROC curve for two-class neural network showing prediction accuracy of 0.864. Single cell gene expression data for 33 genes was split 2:1 for train: test.



Z score heat map (scaled by gene) showing 'test' set of single cells with prediction and actual grouping based on 4-gene signature.

Conclusions

- Single cell gene expression data (Fluidigm BioMark targeted-approach) revealed overexpression of *PTEN* in H1N1-specific memory B cells from HIV-infected individuals and identified a predictive signature of 4 genes including *PTEN*, *IL10RA*, *APOBEC3G*, and *TLR7*.
- Our study provides a framework for analysis of antigen-specific cells using single cell gene expression analysis, provides insight into persistent defects in B cell-mediated immunity in the context of treated HIV infection, and introduces potential targets of intervention to improve vaccine responses.
- See full publication: de Armas *et al*, **2019** *Scientific Reports*

Acknowledgements

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