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IgM Memory B Cell Heterogeneity in Immune Responses to Pneumococcal Vaccination in HIV-positive and Healthy Individuals

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Abstract

Background: Both normal aging and HIV infection impact B cell functionality and lead to activation of resting B cells, memory cell depletion and altered gene expression. As a result, HIV+ individuals and the elderly fail to demonstrate robust and durable immune responses against pneumococcal polysaccharides. Herein, we assessed altered B cell function in high risk groups by utilizing single cell technology.

Methods: HIV-positive individuals with CD4+T cell counts >200 on Antiretroviral Therapy (ART) and HIV-negative individuals age groups 21-40 and 50-65 received pneumococcal vaccination. Serum IgG and IgM PPS-specific antibodies were measured pre- and post-immunization using ELISA method. Evaluation of B cells was performed using flow cytometry and single cell RT-PCR.

Results: IgM memory B cells are important players in responding to pneumococcal antigens and are present in reduced quantities in HIV+ and aging HIV- individuals. Single cell analysis of IgM memory B cells demonstrated heterogeneity and identified two unique subpopulations. One of the subpopulations represents B cells with higher expression of TACI and BAFF-R and is more likely to dominate in T-cell independent immune responses. IgD+IgM+memory B cells were present in equal proportions in both subpopulations.

Conclusion: Pneumococcal vaccine responses in HIV+ and aging HIV- individuals are multifactorial and largely depend on the abundance and phenotypic characteristics of IgM memory B cells.

Keywords: HIV • B cells • Single cell • Pneumococcal vaccine

Introduction

Streptococcus pneumoniae is a human pathogen and a significant cause of morbidity and mortality in both children and adults worldwide [1]. Pneumococcal vaccinations are routinely recommended as a preventative measure for children, the elderly, and those who are immune-compromised including HIV+ individuals [2]. These vaccines protect against the most common serotypes associated with invasive pneumococcal disease and have been shown to be effective in children and young adults [3-5]. However, they fail to induce potent and durable immune responses in the elderly and HIV+ individuals [4,6-8]. The goal of this study is to elucidate underlying causes of inability to develop robust immune responses to pneumococcal vaccination in these high risk populations.

Serum titers and functionality of anti-pneumococcal antibodies following vaccination have been actively studied, however the underlying basis of decreased immune responsiveness in the elderly and HIV+ remains to be elucidated [9-12]. A critical component of the immune responses against pneumococcal antigens is the B cell compartment, therefore a better understanding of B cell perturbations that occur with age and/or after acquiring immune deficiencies is required to understand the mechanisms of impaired vaccine responses. It has been suggested that IgM memory B cells are indispensable for the production of anti-polysaccharide antibodies [13-16]. In support of this concept, it has been demonstrated that individuals with diminished or absent IgM memory B cells including persons with asplenia, whether congenital or acquired, patients with Common Variable Immunodeficiency (CVID) and infants under the age of two, all share the same clinical features: increased susceptibility to infections caused by encapsulated bacteria such as *S. pneumoniae* and impaired response to polysaccharide vaccines [17,18].

We developed and validated a PPS-specific staining method that allowed detection of PPS-specific B cells and identification of the phenotype of B cells responding to Pneumococcal Polysaccharide Vaccine-23 (PPV-23)/ Pneumovax-23) in healthy young adults. We confirmed that the predominant population of polysaccharide specific B cells indeed expresses the IgM memory phenotype (IgM+CD27+) as previously postulated [17,19]. However, it remains to be elucidated how specific cellular states within IgM memory B cells impact functionality and polysaccharide-specific immunity.

In this study, we investigated the effect of HIV infection and/or aging on the immune responses to pneumococcal vaccination with vaccination regimen that includes two types of pneumococcal vaccine Prevnar-13 and Pneumovax-23 in young and aging HIV- vs. well-controlled HIV+ persons on long term ART with CD4 counts above 200 and undetectable HIV viral loads.

Materials and Methods

Design and study population

Study volunteers were recruited at the University of Toledo Medical Center or at the Medical University of South Carolina (MUSC) over the course of six years from 2013-2019. The study was monitored and approved by the

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Institutional Review Board (IRB) at the University of Toledo and the Institutional Review Board at MUSC. Written informed consent was obtained from all subjects (Table 1).

Exclusion criteria included: active infection (except HIV), PPV<5 years prior, pregnancy, history of autoimmune disease, cancer, organ transplantation, splenectomy, end stage kidney or liver diseases, immunoglobulin therapy, bleeding disorders. Volunteers were questioned about any prior hospitalizations consistent with pneumococcal infection. Eligibility criteria for all HIV+ individuals were defined as undetectable viral loads (≤400 copies/mL), CD4 >200 cells/μL and ART for at least 1 year. HIV+ cohorts had a history of PPV vaccination at least 5 years prior to study enrolment. Volunteers received a single dose of PCV13 followed by a single dose of PPV23 8 weeks later (PCV/PPV); HIV- individual in 21-40 age-group followed a different vaccination protocol based on ACIP recommendations; they received a single dose of PPV23 and had no history of previous immunization with pneumococcal vaccine.

ELISA

Serum samples were used to measure pneumococcal capsular polysaccharide-specific IgG and IgM responses pre- to 1-month post-immunization with PPV23 and pre-to 90 days post-immunization with PCV13/PPV23. Serotype-specific IgG and IgM levels were detected by Enzyme-Linked Immunosorbent Assay (ELISA) in accordance with the training manual published by WHO as previously described [20]. The serotype-specific antibodies bound to the ELISA plate were developed using o-phenylenediamine substrate and read at 490 nm on a microplate reader. Linear regression fits were used to determine the antibody concentrations, and these were reported as μg/mL.

Flow cytometry and cell sorting

Peripheral Blood Mononuclear Cells (PBMCs) were collected from HIV+ and HIV- study volunteers on day 0 (pre-immunization) and day 7 (post-immunization with PPV23). Lymphocytes were separated using Ficoll Hypaque method and stained with fluorochrome-conjugated monoclonal antibodies using the following anti-human antigens:CD19 (APC-Cy), CD27 (PerCP-Cy5.5), and IgM (APC); for post-immunization samples the cells were also labeled with fluorescently conjugated pneumococcal PPS14 (5-DTAF) or PPS23F (5-DTAF) for analysis of polysaccharide specific B cells as previously described [19]. Additionally, cells were stained with LIVE/DEAD Blue fluorescent reactive dye in order to exclude dead cells from the analysis. IgM memory B cells (CD19+CD27+IgM+) were sorted using Beckman MoFLo Astrious sorter based on the gating strategy described in (Supplementary Figure 1). Analysis was performed using FlowJo software.

Single cell assays

IgM memory B cells (IgM+CD27+) (n=1621) were used for pre-immunization single cell RT PCR analysis were isolated and sorted from 17 donors: 4 young HIV- individuals, 5 aging HIV- individuals, 5 young HIV+ individuals, 3 aging HIV+ individuals. Cells (n=263) used for post-immunization single cell RT PCR were isolated from 4 donors: 1 young HIV-, 1 aging HIV+, 1 young HIV+, and 1 aging HIV+.

cDNA: Single cell gene expression experiments were performed using Fluidigm 96.96 qPCR Dynamic array Integrated Fluidic Circuits (IFCs) and HD Biomark Instrument on sorted IgM memory B cells (CD19+CD27+IgM+). C1 Single Cell Auto Prep System was used to capture single cells and perform targeted pre-amplification utilizing C1 Integrated Fluidic Circuits (IFCs). Cell lysing, reverse transcription, pre-amplification and harvesting the amplified products was performed according to Fluidigm's recommended protocol (PN 100-4904 L1).

Gene expression analysis: A PCR master mix containing both EvaGreen and ROX was used; primers were designed using D3 assay design specific for genes involved in TI-2 responses (BAFF-R, TACI, BCMA, CD80, CD86, CD40, CD21, TLR9, AICD, TCF3), apoptosis (Bcl-2, Bcl-XL, Mcl-1, BAX, BAD, BIM, FAS, BIK, TNSF15, Siva1), cytokines and receptors (IL-1β, IL-4, IL-6, IL-7, IL-8, IL-10, IFNGR, IFN-γ, TNF-α, TLR1, TLR2, TLR4, CCR2, CXCL8), transcription factors (IRF4, BACH2, PRDM1, XBP1, Bcl6, Pax-5, IKZF3, SPIB, FOXO1), lymphocyte signaling (CBLB, FCRL2, CD1D, CD200, LAIR1, CD72, RFTN1, LCP2, GCSAM, NTE5, CD39, MS4A1, ENTPD1), metabolism (HKII, PFKP, ENO1, PGAM, LDHA, TYMS, CPT1, GPT2). Single cell qPCR was performed according to Fluidigm's recommended protocol (PN 100-9792 B1).

Statistical analysis

ELISA and flow cytometry: Different groups were compared using an unpaired t test. P-values<0.05 were considered statistically significant.

Single cell RT PCR: The main goals in this analysis were to (1) compare the proportion of each cell type by HIV status and age group and (2) to analyze at gene expression between HIV/Age groups within each cell type and compare within group between cell types. The data included 1621 cells from 17 unique donors for pre-immunization analysis and 268 cells from 4 unique donors for post-immunization analysis. The association between cell type with HIV status and age group was evaluated using a Generalized Estimating Equation (GEE) approach using cell type as the dependent variable, assuming a binary distribution and a logit link. The model included fixed effects for age group, HIV status, and the interaction between age group and HIV status as well as a random subject effect to account for clustering within patients.

We also examined differences in gene expression for the 65 genes considered in the RT-qPCR analysis between HIV and age groups within the 2 cell populations as well as differences within group between the two cell populations. Data were cleaned prior to analysis using the Fluidigm SC work flow described in the Fluidigm Singular Analysis Toolset User Guide. Cleaning

Table 1. Baseline characteristics of study participants.

Variables	HIV-infected		HIV-uninfected	
	Young (12)	Aging (21)	Young (58)	Aging (15)
Demographics				
Mean age (range)	31 (24-40)	54 (50-65)	27 (21-42)	56 (52-65)
Male (%)	9 (75)	16 (76)	39 (66)	7 (46)
African American	6 (50)	10 (48)	16 (29)	1 (7)
Caucasian	6 (50)	11 (52)	42 (71)	14 (93)
Clinical History				
Receiving ART[3] 1 year (%)	12 (100)	21 (100)	N/A	N/A
Nadir CD4 count (cells/mL) Mean	395	280	N/A	N/A
(range)	(222-748)	(200-938)		
Laboratory Data at Enrollment				
CD4 T cell count (cells/mL)	608	689	N/A	N/A
Mean (range)	(375-1299)	(331-1298)		
HIV viral load (copies/mL) £40 (%)	12 (100)	21 (100)	N/A	N/A

the data included replacement of values the reported "No Call" or "Fail" with 0, identifying outliers, and calculation of Log2Ex by subtracting the observed Ct from 24- if this value was negative the Log2Ex was reported as 0 and otherwise it was reported as 24-Ct (Gene).

The association between gene expression with HIV status, age group, and cell type was evaluated using a series of linear regression model. Differences between the groups were evaluated based on contrasts from the linear model and differences considered in this analysis included all pairwise comparisons by combinations of HIV status and Age within cell type (12 comparisons) and pairwise comparisons by cell type within group (4 comparisons). The p-values were adjusted using the Tukey's Honestly significant difference approach which is consistent with the Fluidigm SC R package.

Results

Reduced Serum anti-PPS antibody levels to serotype 14 and 23F in HIV+ individuals and the elderly

Pre- to post- vaccination serum IgM and IgG levels increased significantly in all groups for both serotype 14 and 23F, with the exception of the young HIV+ group, which did not demonstrate a significant increase in serum IgG antibody titer to serotype 23F. Young HIV- individuals had significantly higher levels of IgG and IgM serum antibody levels to serotypes 14 and 23F post-vaccination and were considered vaccine responders as compared to aging HIV- and both young and aging HIV+ groups (Figure 1). There were no differences in the immune response between young HIV+ and aging HIV+ groups. Serum IgM levels to serotype 14 and serum IgG levels to serotype 23F were significantly higher in the aging HIV- group compared to the aging HIV+ group (p=0.0009 and p=0.01 respectively).

The phenotype of PPS-specific B cells

The analysis of post-immunization PPS-specific B cells in young and aging HIV+ individuals revealed there are no significant differences between young and aging HIV+ individuals and there is no age-related shift in the phenotype of PPS-specific B cells in HIV+ (Figure 2A).

In HIV+ adults over the age of 50, the predominant response to pneumococcal vaccination more closely resembled the response of healthy young adults, expressing the IgM+CD27+ phenotype, albeit at significantly lower levels (Figure 2B). It is important to note, that both aging HIV- and aging HIV+ individuals demonstrated significantly lower numbers of B cells (all phenotypes) compared to healthy young adults (not shown).

Single cell gene expression profiling revealed heterogeneity within human IgM memory B cell population

Hierarchical clustering analysis of gene expression showed that there is a segregation of the pre-immunization IgM memory B cell population into two subpopulations. These two subpopulations are distinct and present in all four study groups (young HIV- and HIV+ as well as aging HIV- and HIV+) (Figure 3A). The frequency of subpopulation 1 was 56% in young HIV-, 39% in young HIV+, 27% in aging HIV-, and 67% in aging HIV+. The absolute number of IgM memory B cells was significantly lower ($p<0.05$) in aging HIV+ individuals as compared to healthy young adults (Figure 3B).

Gene expression results subpopulation 1 vs. subpopulation 2: For this analysis, we focused on genes involved in T cell independent immune responses which included CD40, TLR9, BCMA, CD86, AICDA, CD21, CD80, TACI, TCF3, and BAFF-R (Figure 3D). We found significant differences in gene expression levels in members of BAFF/APRIL family between subpopulation 1 and subpopulation 2. Levels of BAFF-R were significantly higher in subpopulation 1 in young HIV- ($p<0.0001$), aging HIV- ($p<0.0001$), young HIV+ ($p<0.0001$), and aging HIV+ ($p<0.0001$) as compared to subpopulation 2. Similarly, TACI levels were significantly higher in subpopulation 1 in all groups examined (young HIV- $p<0.0001$, young HIV+ $p<0.0001$, aging HIV- $p<0.0001$, aging HIV+ $p<0.0001$). BCMA was significantly higher in young HIV- ($p<0.0001$), aging HIV- ($p=0.008$) and HIV+ ($p=0.001$) in subpopulation 1 as compared to subpopulation 2. We also assessed expression levels of co-stimulatory molecules CD80, CD86, and CD40. We found that levels of CD80 were higher in subpopulation 1 as compared to subpopulation 2 in aging HIV- ($p<0.0001$), young HIV+ ($p<0.0001$), and aging HIV+ (0.0001) but not in young HIV-. There were no differences in expression of CD86 and CD40 between subpopulation 1 and subpopulation 2 in young and aging HIV+ as well as aging HIV- group. However, we found significant differences in young HIV-

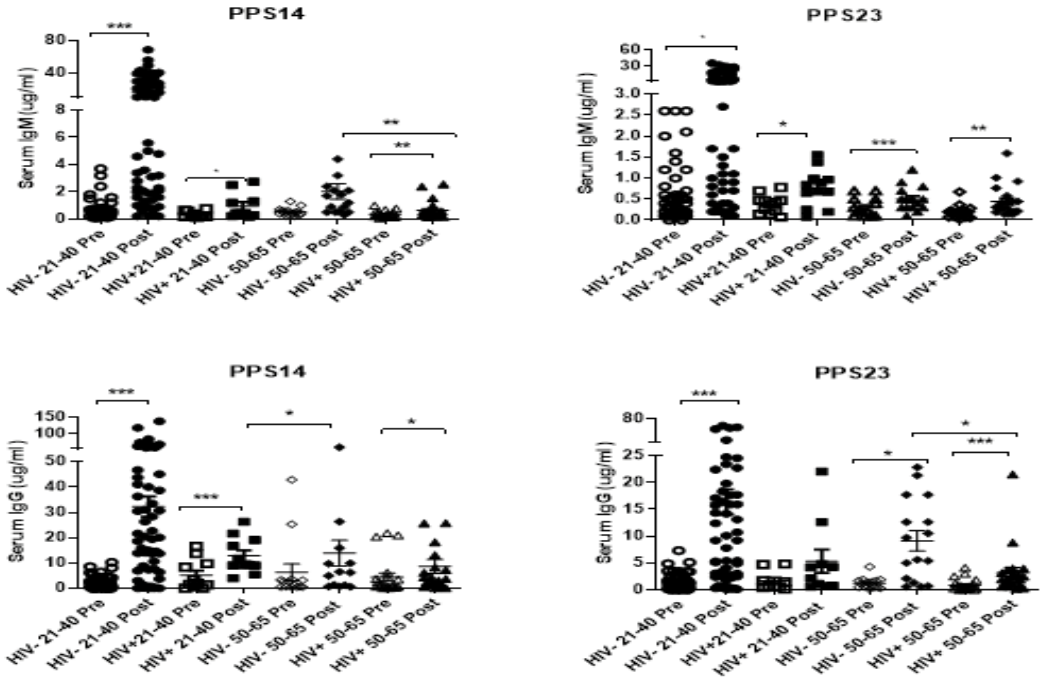

Figure 1. Polysaccharide-specific serum antibody levels to serotypes 14 and 23F. Serum IgM and IgG antibody levels were measured in young and aging HIV+ individuals and age-matched HIV- negative individuals pre- and post-immunization with pneumococcal vaccination. Changes in serum antibody levels in a single group were compared using paired t-test and changes between the groups were compared using Wilcoxon sign rank t-test. P-values <0.05 were considered significant. *p <0.05, ** <0.01, ***p <0.0001.

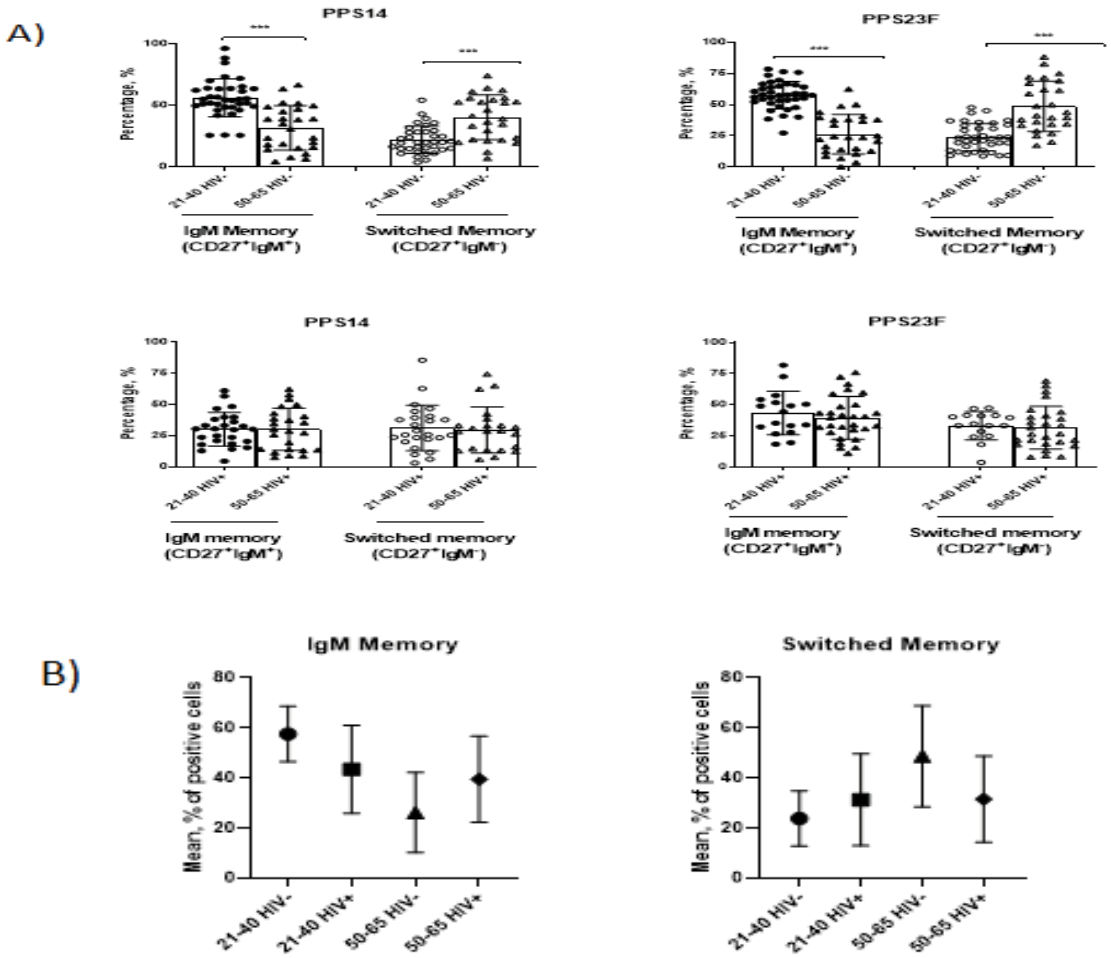

Figure 2. Phenotypic characteristic of PPS-specific B cells in young and aging HIV- and HIV+ individuals. A) The phenotype of PPS-specific B cells changes with age in healthy individuals but not in HIV+ individuals. The phenotypes of B lymphocytes responding to pneumococcal vaccination were determined by flow cytometry. B) Mean of post-immunization PPS-specific IgM memory and switched B cells phenotype in young adults, aging HIV-, and aging HIV+. P-values <0.05 were considered significant. *p <0.05, ** <0.01, ***p <0.0001.

group; levels of CD86 and CD40 were significantly lower in subpopulation 1 as compared to subpopulation 2 (p<0.0001, p<0.0001 respectively). TCF3 was significantly higher in Subpopulation 1 versus Subpopulation 2 in all groups. There was no difference detected in TLR9 or AICDA between subpopulations or groups while AICDA expression between subpopulations differed only in aging HIV+. Overall, subpopulation 1 was characterized by higher BAFF-R, TACI, BCMA, and CD80 compared to subpopulation 2 (Figure 3).

Similarly, our initial analysis of polysaccharide specific IgM memory B cells demonstrated segregation into two subpopulations that was observed in IgM memory B cells analyzed pre-immunization in all study groups. (Supplementary Figure 3). The levels of expression of BAFF-R were significantly higher in subpopulation 1 as compared to subpopulation 2 in young HIV- (p<0.0001), aging HIV- (p<0.0001), young HIV+ (p<0.0001); the levels of BAFF-R in subpopulation 1 appeared to be higher in aging HIV+ group, however these differences did not reach statistical significance. TACI levels were also significantly higher in subpopulation 1 in young HIV- group (p<0.0001) and aging HIV- group (p<0.0001) as compared to subpopulation 2. Levels of AICDA were significantly higher in young HIV- individuals as compared to aging HIV- (p <0.0001) and aging HIV+ (p<0.0001) (Supplementary Figure 3D).

Discussion

In this study, we identified the phenotype of polysaccharide specific B cells in aging HIV+ and aging HIV- groups and characterized genetic signatures of IgM memory B cells pre- and post-immunization on a single cell level. Our findings revealed that in healthy individuals there is an age-related shift in the phenotype of PPS-specific B cells from IgM memory in young adults to switched memory in aging persons. In contrast, PPS-specific B cells isolated from young and aging HIV+ individuals did not demonstrate a similar age-related shift in B cell phenotype. Interestingly, aging HIV+ individuals phenotypically resembled young HIV- individuals and displayed predominant PPS-specific IgM memory B cell phenotype albeit at much lower numbers both in percentage and absolute numbers (Figure 2).

Despite the fact that young HIV- and both HIV+ groups shared a seemingly similar phenotype of PPS-specific cells, the ability to generate anti-PPS antibodies in response to pneumococcal vaccination differed markedly in HIV+ persons. We hypothesized those functional variances in IgM memory B cells may be driven by heterogeneity present within the IgM memory population leading to skewed gene distribution and potentially resulting in diminished vaccine responses in HIV+ individuals. We approached this hypothesis by utilizing a single cell RT PCR method which allowed us to perform unbiased characterization of IgM memory B cells pre- and post-immunization [21]. Unlike traditional approaches, using bulk cell analysis, single cell analysis allows to dissect immune responses to a greater extent. This becomes apparently important in the case of phenotypically similar but functionally different cells such as IgM memory B cells in aging HIV+ individuals where a fraction of the cell population can be potentially dysfunctional or dysregulated and bulk analysis fails to recognize these changes [22]. Furthermore, recent studies have shown that immune cell types that are traditionally defined by a specific set of markers, can in fact, consist of different subtypes that share overlapping signatures [23-25]. Using a single cell approach, we were able to delineate the heterogeneity of human IgM memory B cells by investigating 65 genes, mainly involved in the regulation of B cell function and differentiation (Figure 3).

Analysis of IgM memory B cells obtained pre-immunization revealed

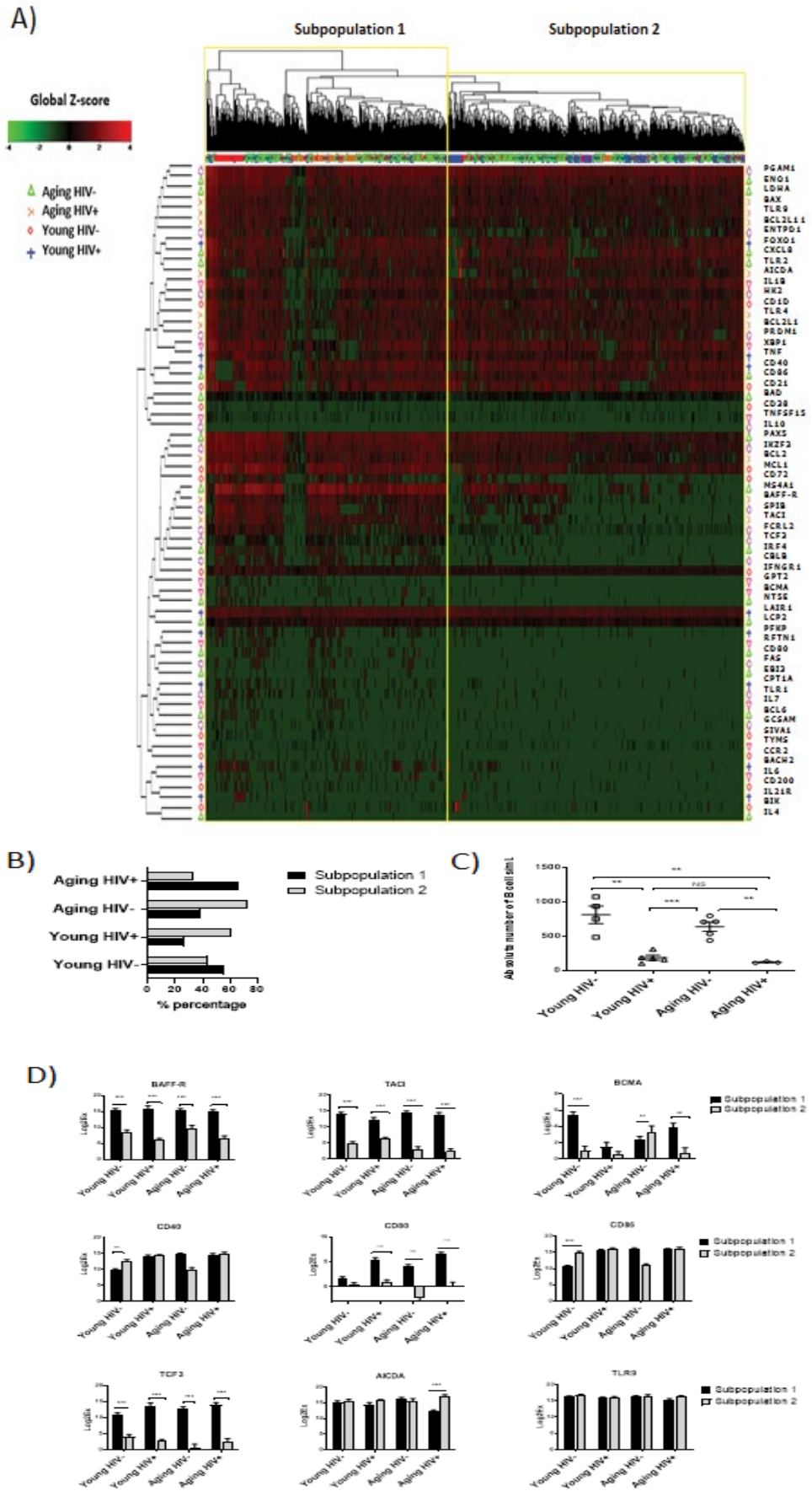

Figure 3. Single cell RT PCR heatmap depicting 2 distinct subpopulations present within sorted IgM memory B cells in young HIV-, young HIV+, aging HIV-, and aging HIV+ groups. A)1621 Cells used for pre-immunization single cell RT PCR analysis were isolated and sorted from 17 donors: 4 young HIV- individuals, 5 aging HIV- individuals, 5 young HIV+ individuals, 3 aging HIV+ individuals. Single cell RT PCR was performed on cells isolated from each donor individually. B) Subpopulation 1 and 2 distribution in all study groups. C) Absolute number of IgM memory B cells in study groups. D) Expression levels of genes that play a role in immune response to PPS antigens in subpopulation 1 as compared to subpopulation 2. P-values <0.05 were considered significant. *p <0.05, ** <0.01, ***p <0.001.

the extensive heterogeneity that exists within a seemingly homogeneous population. Hierarchical clustering analysis demonstrated that IgM memory B cells clustered into separate branches of the dendrogram and identified two distinct subpopulations (subpopulation 1 and subpopulation 2) with unique phenotypic characteristics that were present in all study groups. Interestingly, these populations did not display no significant correlation with the expression of IgD which was detected in similar proportions of single cells in both subpopulations (Supplementary Figure 2). To understand the significance of these findings, we analyzed expression levels of the genes directly or indirectly involved in responses to polysaccharide antigens in these subpopulations. We found that these genes at the single cell level demonstrated a highly multifaceted expression patterns that could not be detected by bulk cell analysis.

The pre-immunization IgM memory B cell subpopulation 1 was characterized by significantly higher levels of expression of BAFF-R and TACI in all study groups as compared to subpopulation 2. BAFF-R and TACI play a critical role in B cell function, homeostasis, regulation of Activation-Induced cytidine Deaminase (AID) and production of polysaccharide specific antibodies [26-30]. Studies have shown that TACI up regulation augments antibody production in response to TI-2 antigens whereas its absence or downregulation leads to unresponsiveness to TI-2 antigens [26,31-33]. Furthermore, adjuvants that specifically upregulate TACI expression, such as CpG-ODN have been shown to effectively increase the immune responsiveness to pneumococcal vaccine [34]. Together, this suggests that subpopulation 1 with high levels of TACI and BAFF-R is more likely to dominate in T cell independent immune response and therefore from here on will be the focus of the discussion.

Next, we examined the expression of CD80 (B7-1) and CD86 (B7-2), co-stimulatory molecules expressed on the surface of antigen presenting cells including B cells [35]. Increased levels of expression of these molecules on B cells have been previously reported in inflammatory diseases such as lupus erythematosus and also in HIV [36,37]. However, here we demonstrate that CD80 and CD86 molecules are overexpressed only in one subpopulation of IgM memory B cells specifically in young and aging HIV+ and aging HIV- groups. This is an important finding as predominance of IgM memory B subpopulation with higher levels of expression of CD80/CD86 could potentially result in modified B cell function, reflect B cell hyperactivity and translate into impaired vaccine specific responses in the elderly and HIV+.

We also examined expression levels of TCF3, a gene that encodes transcription factors E12 and E47 that play an important role in B cell differentiation and regulation of mature B cell function [38]. We found that subpopulation 1 had significantly higher levels of expression of TCF3 in all groups. Furthermore, we found that subpopulation 1 was metabolically more active and had higher levels of expression of signalling genes.

Based on these findings, we hypothesized that aging HIV+ individuals have low frequencies of subpopulation 1 pre-immunization which in turn can have an adverse effect on their ability to respond to TI-2 antigens. We compared the distribution of IgM memory B cells and found that in young HIV- group 56% of IgM memory B cells belonged to subpopulation 1 whereas in aging HIV+ group 68% of cells belonged to subpopulation 1. However, when we determined the absolute number of IgM memory B cells present in all groups, we found that aging HIV+ individuals have 3-fold lower number of IgM memory B cells. Collectively, these findings suggest that aging HIV+ individuals despite having higher prevalence of subpopulation 1, have low absolute numbers of IgM memory B cells likely associated with diminished ability to produce functional pneumococcal antibodies.

We also performed an assessment of post-immunization PPS-specific IgM memory B cells. We identified two distinct subpopulations with characteristics similar to the ones observed in pre-immunization profiling of IgM memory B cells including high levels of expression of BAFF-R, TACI and co-stimulatory molecules CD80/86. In contrast to pre-immunization IgM memory B cells, levels of AICDA in both subpopulation 1 and 2 were significantly lower post-immunization in the aging HIV- and HIV+ cell populations suggesting an impairment in the induction of AID which contributes to dysregulated B cell function previously documented in the elderly [39,40]. Further investigation of PPS-specific IgM memory B cells is ongoing.

Conclusion and Future Prospects

Collectively, our study demonstrated that the immune response to pneumococcal vaccination in HIV-positive population and the elderly is complex and shaped by multiple factors including HIV infection, age, and number of B cells and phenotypic characteristics of IgM memory B cells. The presence of two distinct subpopulations with both pre-immunization unselected and post-immunization PPS-selected IgM memory B cells is intriguing and demonstrates the advantages associated with single cell RT PCR. We recognize that our study has limitations. First, our sample size was small, which limits the power of the study; however we were able to observe statistically significant differences between the study groups. Second, vaccination regimen in young HIV- group was different from other study groups. These individuals did not receive Prevnar-13 prior to receiving Pneumovax-23. Generally, healthy young adults are not required to receive pneumococcal vaccination because they are not considered to be at high risk of IPD. In this study, this group was included as a positive control and was used as reference of a robust immune response and the results for this group must be interpreted with care. Furthermore, single cell analysis was performed pre-vaccination at the baseline for all groups therefore the results were not impacted by the vaccination regimen. Third, the studies of PPS-specific IgM memory B cells in HIV+ and aging HIV- groups are still ongoing and therefore specific trends cannot be determined yet. Finally, functional studies of the identified subpopulation in both pre- and post-immunization IgM memory B cells are ongoing.

Our future studies will continue evaluating polysaccharide-specific IgM memory B cells and potential benefits of rescuing levels of AID in order to increase CSR and SHM which would result in more robust immune response to pneumococcal vaccination in aging HIV+ individuals.

Conflict of Interest

All authors have no potential conflict of interest.

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Clinical Trial Registration

NCT03039491

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