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Race-related differences in functional antibody response to pneumococcal vaccination in HIV-infected individuals

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ABSTRACT

Background: Both HIV positivity and African American (AA) ethnicity are associated with increased incidence of invasive pneumococcal disease (IPD). Poor immune response to pneumococcal polysaccharide-based vaccines may contribute to the race related increased frequency of IPD in African American HIV positive individuals.

Methods: Caucasian and AA HIV-infected (HIV+) individuals 40–65 years old with $CD4^+$ T cells/μl (CD4) >200 on antiretroviral therapy (ART) received either the 13-valent pneumococcal conjugate vaccine (PCV) followed by the 23-valent pneumococcal polysaccharide vaccine (PPV) or PPV only. Serum IgG, IgM and opsonophagocytic antibody responses to serotypes 14 and 23F as well as serum IgG and opsonophagocytic antibody responses to serotype 19A were measured pre- and post-vaccination. We measured serum markers of inflammation in all participants and performed single cell gene expression profiling at the baseline by HD Biomark in Caucasians and African Americans.

Results: There were no significant differences in pre-immunization inflammatory markers or post-vaccination IgG and IgM concentrations between Caucasian and African American participants. However, we found significantly lower opsonophagocytic activity in response to serotypes 14 and 19A in the AA group compared to the Caucasian group. There was no association between inflammatory markers and immune response to vaccination, however we found extensive biomodal variation in gene expression levels in single IgM+ memory B cells. Differentially expressed genes may be related to differences in the immune response between ethnic groups.

Conclusions: Distinct racial differences were found in the functional immune response following either PPV and/or PCV/PPV immunization in HIV-positive adults, although these differences were serotype dependent. Decreased ability to respond to vaccination may in part explain racial disparities in pneumococcal disease epidemiology.

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1. Introduction

Streptococcus pneumoniae is a major cause of morbidity and mortality worldwide. Prior to the introduction of pneumococcal conjugate vaccines in 2000, *S. pneumoniae* caused 500,000 cases of pneumonia, 50,000 cases of bacteremia, and 3000 cases of meningitis per year in the United States alone, resulting in 40,000 deaths[1]. Since the institution of routine childhood vaccination with the pneumococcal conjugate vaccine, there has been a dramatic reduction in incidence of pneumococcal disease. Nevertheless, pneumococcal disease remains a major cause of community acquired pneumonia [2] and invasive pneumococcal disease (IPD) is still responsible for more than 3100 deaths yearly in adults in the US alone [3].

Although age is an important risk factor for IPD, a number of other factors are known to increase the risk for IPD in adults. These include ethnicity [4], low socioeconomic status, chronic underlying diseases such as chronic obstructive pulmonary disease (COPD), heart disease, diabetes and renal disease, and high risk behaviors such as smoking and alcohol abuse [4-8].

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Furthermore, a number of immune deficiencies [4,6-9] including HIV, increase the risk of IPD significantly. Moreover, despite the widespread availability of antiretroviral therapy, HIV-infected individuals remain at a 35-fold increased risk of IPD compared to age-matched HIV-negative [10].

A disproportionately high number of African American (AA) individuals are affected by HIV in the USA. Although only 12% of the US population is African American, 43% of the total number of persons living with HIV in the US are African American [11]. Several studies have shown that African American race is also associated with increased incidence of IPD in the HIV positive population similar to the observation made in HIV negative individuals [12-14]. We, and others, have previously shown that the immune response to both pneumococcal polysaccharide (Pneumovax®) (PPV) and conjugated polysaccharide (Prevnar®) (PCV) vaccines are suboptimal in the HIV positive adult populations [15-19]. It has been suggested that poor immune response to pneumococcal polysaccharides may contribute to the race related increased incidence of IPD in African American HIV positive individuals[14,20]. A recent prospective study that compared pre- to post-immunization capsule-specific IgG levels for 4 serotypes in AA versus Caucasian HIV-positive individuals failed to demonstrate race-related differences in immune response [21].

However, poor correlations between IgG antibody concentration and functional or opsonophagocytic antibody activity in adults have inferred that OPA is a more accurate surrogate of protection [22].

We report the immune response to 3 pneumococcal serotypes before and after revaccination with either PPV alone or PCV followed by PPV in Caucasian and AA HIV-positive adults by measuring both serum antibody levels and OPA as well as inflammatory markers before vaccination. Furthermore, we utilized systems biology approach to examine whether differential gene expression on the single cell level, has an impact on production of functional antibodies.

2. Materials and methods

2.1. Design and study population

HIV-positive subjects were recruited at the University of Toledo Medical Center or at the Medical University of South Carolina (MUSC). The study was monitored and approved by the Institutional Review Board at the University of Toledo and the Institutional Review Board at MUSC. Written, informed consent was obtained from all subjects. HIV positive individuals who agreed to participate in the study were randomized using a random assignment generator to receive either PCV/PPV (days 0 and 56) or PPV (day 0) alone; individuals who agreed to participate in the study, but were receiving vaccination as a part of routine clinical care were assigned and analyzed in their respective groups based on vaccination regimen in a non-randomized manner. Consequently, there was a smaller number of participants in PCV/PPV Caucasian group as compared to African American group. The study participants were enrolled over the course of 4 years from 2013 to 2017. Exclusion criteria included: active infection (except HIV), PPV < 5 years prior, pregnancy, immunosuppressive medications, and history of cancer, autoimmune disease, bleeding disorders, immunoglobulin therapy, organ transplantation, splenectomy, and end stage renal or liver disease. Volunteers were questioned about any prior hospitalizations consistent with pneumococcal infection. Eligibility criteria for HIV+ participants were further defined as CD4 > 200 cells/μl at the time of vaccination (Day 0), regardless of nadir CD4 count history (Table 1), HIV viral load ≤400 copies/ml, and ART for ≥1 year. All subjects had previously been immunized with PPV (≥5 years earlier). HIV+ individuals received either PCV/PPV (days 0 and 56) or a single dose of PPV (day 0).

2.2. Antibody ELISA

Blood samples were collected at days 0 (pre-immunization) and 30 for PPV only recipients and days 0, 63 and 90 for the PCV/PPV recipients. Serum samples were used to measure pneumococcal capsular polysaccharide-specific IgG responses pre- and 1 month post-vaccination with PPV (post-PPV) to serotypes 14, 19A and 23F. Serotype-specific IgG and IgM serum levels were detected by enzyme-linked immunosorbent assay (ELISA) as previously described [16,23].

2.3. Opsonophagocytic assay

Opsonophagocytic killing assay (OPA) was performed as previously described [16,24] to determine functional antibody responses. Data were analyzed using the Opsotiter1 software program (University of Alabama at Birmingham). OPA titers were defined as the reciprocal of the serum dilution that killed 50% of target bacteria (compared to serum-free control) during 45 min of incubation at 37 °C.

2.4. Inflammatory marker analysis

Luminex analysis of 10 inflammatory markers, BAFF, APRIL, IL-10, IL-1Ra, IL-8, IL-6, sCD40L, IP-10, IL-1B, and TNF-α, was performed on pre-immunization serum samples obtained from all participants by EveTechnologies (Calgary, AB, Canada).

2.5. Single cell qPCR

Single cell gene expression experiments were performed using Fluidigm 96.96 qPCR Dynamic Array microfluidic chips and HD Biomark. Peripheral blood mononuclear cells (PBMC) were collected from African American and Caucasian volunteers pre-vaccination on day 0 and stained with fluorochrome-conjugate mAbs using the following anti-human antigens: CD19, CD27, and IgM. IgM memory B cells (CD19 + CD27 + IgM+) were sorted using Beckman Coulter MoFLo Astrios sorter. Single IgM memory B cell capture and specific target amplification were carried out using Fluidigm C1 Single Cell Auto Prep System and Single Cell Auto prep array Integrated Fluidic Circuits (IFCs). Chip priming, cell loading, lysis, reverse transcription and pre-amplification was performed according to Fluidigm's recommended protocol. Pre-amplified cDNA samples from single cells were analyzed by qPCR using 96.96 Dynamic array IFC and the Biomark HD system and DELTA gene assay (Fluidigm) designed for 64 human transcripts.

2.6. Statistical analysis

Participant characteristics were represented as mean (range) for numerical values and number (percentage) for categorical values. Serotype-specific serum IgG, IgM (μg/ml) and OPA titers were reported as geometric mean concentrations or titers (95% confidence interval), respectively. IgG, IgM levels and OPA titers were log-transformed to approximate normal distribution prior to statistical analysis. Pre- to 1-month post-vaccination comparison within the groups were calculated using paired *t*-test; differences within the groups were calculated using Wilcoxon rank-sum test. Correlations were determined by Pearson's correlation coefficient. All statistical analyses were performed using Prism software (GraphPad). Single cell qPCR data was analyzed using Fluidigm Real Time PCR analysis software with Auto Ct Threshold method and also Singular package for R. ANOVA was performed and violin

Table 1
Baseline characteristics.

Race	African American (AA)			Caucasians (Cau)		
Characteristic	PPV (n = 15)	PCV/PPV (n = 13)	PPV&PCV/PPV (n = 28)	PPV(n = 20)	PCV/PPV (n = 8)	PPV&PCV/PPV (n = 28)
Demographic						
Mean Age (range)	51.2 (44–62)	53.2(40–63)	52(40–63)	52.1(42–64)	54.9(50–60)	52.8(42–64)
Male %	12(80)	11(85)	23(82)	19(95)	8(1 0 0)	27(96)
Clinical History						
Prior PPV ≥ 5 years (%)	15(1 0 0)	13(1 0 0)	28(1 0 0)	20(1 0 0)	8(1 0 0)	28(1 0 0)
Receiving ART ≥ 1 year (%)	15(1 0 0)	13(1 0 0)	28(1 0 0)	20(1 0 0)	8(1 0 0)	28(1 0 0)
Laboratory Data						
HIV viral load (copies/ml) < 400 (%)	14(93)	13(1 0 0)	27(96)	20(1 0 0)	8(10)	28(1 0 0)
CD4 T cell count (cells/μl) Mean current	442	336	393	324	218	294
(Range nadir)	(102–852)	(76–938)	(76–938)	(26–896)	(4–455)	(4–896)

plots of differentially expressed genes that were ranked by p-value were created. *P* values < 0.05 were considered significant.

3. Results

3.1. Subjects

Baseline characteristics of the 56 participants (28 Caucasian and 28 AA) included in this study are shown in Table 1. We analyzed each racial group according to vaccination protocol (PPV alone, PCV/PPV or two groups combined: both PPV and PCV/PPV shown in graphs as post-vaccination). Age, CD4 counts at enrollment and HIV viral load were similar between the two ethnic groups and vaccination protocols. All individuals were treated with ART ≥ 1 year. All participants had been immunized with PPV ≥ 5 years prior.

3.2. Serum antibody levels to serotypes 14, 19A and 23F post both PPV and PCV/PPV

Post-vaccination IgG levels were significantly higher in all groups and for all serotypes as compared to pre-vaccination levels, however IgM levels to PPS14 were significantly higher only in Caucasian group but not in AA group pre-to post vaccination (Fig. 1). Serum IgM concentrations to serotype 19A were not measured because a reference serum standard is not established. There were no significant differences in either pre- or post-vaccination IgG and IgM antibody concentrations between AA and Caucasians regardless of vaccination protocol.

3.3. Serum OPA titers to serotypes 14, 19A and 23F

Significant increases in serotype-specific OPA titers were observed in all vaccination regimens for both AA and Caucasian groups (Fig. 2). There were no differences in baseline OPA titers between the groups/vaccination regimens. However, post-immunization OPA titers were significantly higher in the response to PPS14 in all Caucasian groups, regardless of immunization protocol compared to the AA groups. OPA titers to PPS19A were also significantly higher in the Caucasian versus AA group immunized with PPV only, however in response to PCV/PPV there was no significant difference between ethnic groups. In response to PPS23F there were no detectable differences in OPA titer in any of the groups, irrespective of race or immunization strategy.

3.4. Correlations between post-PPV antibody levels and OPA titers

In the post-immunization response to PPS14 the AA and Caucasian HIV+ groups showed a good correlation between anti-

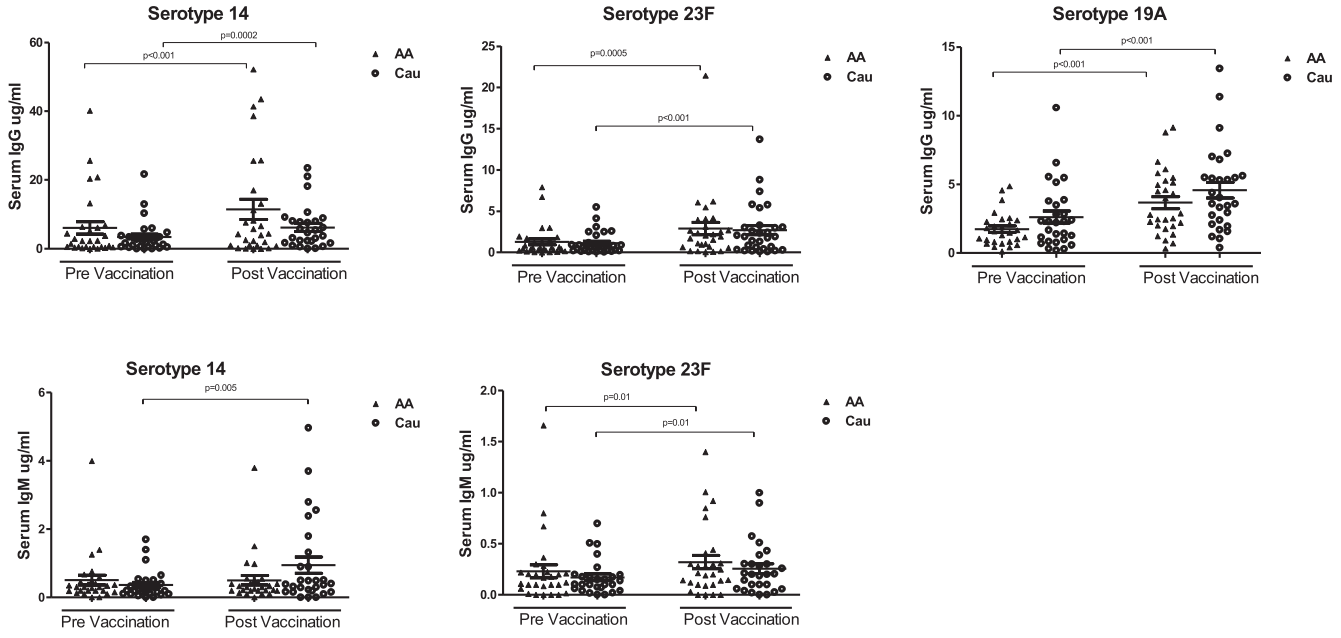

Fig. 1. Polysaccharide-specific serum antibody levels to serotypes 14, 23F and 19A. Serum IgG and IgM antibody responses tested for PPS14, PPS23F, and 19A in Caucasian and African American HIV-positive individuals pre- and post-immunization with Pneumovax-23 (PPV) or Prevnar-13 /Pneumovax-23 (PCV/PPV). 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.

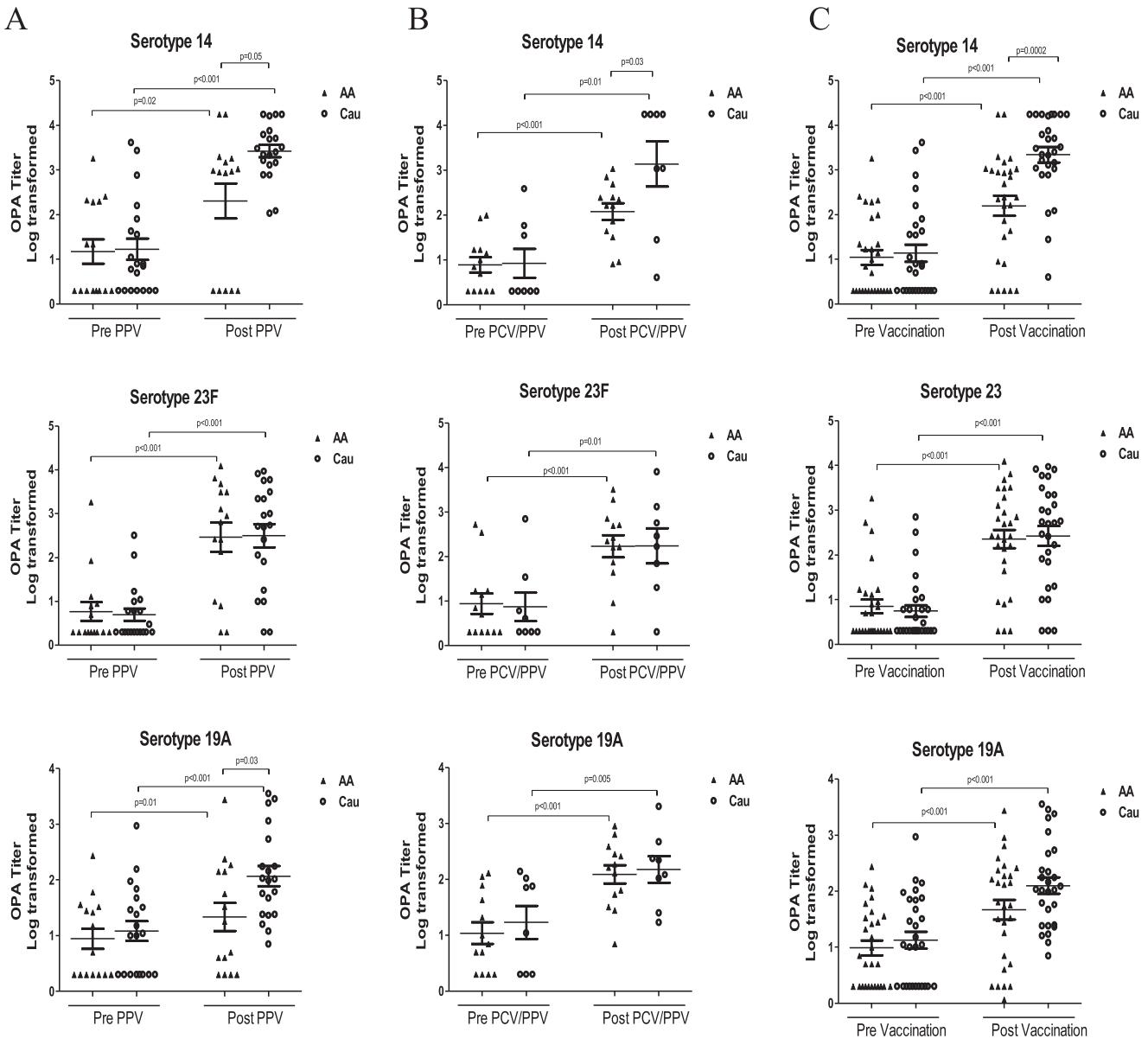

Fig. 2. Serum OPA titers to serotypes 14, 23F, and 19A. Serotype-specific serum OPA titers were determined in HIV-positive Caucasian and African-American individuals pre- and post-vaccination with either **A**-Pneumovax-23 (PPV) or **B**-Prevnar-13 (PCV)/Pneumovax-23 (PCV/PPV). All Caucasian versus African American participants in groups **A** and **B** together depicted in **C**. Changes in OPA titers in a single group were compared using paired *t*-test and changes between the groups were compared using Wilcoxon sign rank *t*-test.

PPS14 IgG and OPA with $r^2 = 0.66$ with $p = 0.0002$ for AA group and $r^2 = 0.45$ with $p = 0.016$ for Caucasian group (Fig. 3).

The correlation between post-vaccination IgG antibody concentration and OPA in response to PPS19A was significant in the AA ($r^2 = 0.51$, $p < 0.006$) but not in Caucasian group ($r^2 = 0.2$, $p > 0.3$) There was no significant correlation between post-vaccination IgG antibody response and OPA in response to PPS23F for both Caucasian group and AA group, regardless of vaccination strategy.

3.5. Inflammatory profiles

We measured a total of 10 inflammatory markers: BAFF, APRIL, IL-10, IL-1RA, IL-8, IL-6, sCD40L, IP-10, IL-1B, and TNF-alpha in pre-immunization serum of HIV-positive individuals in both cohorts to define inflammatory activity and its potential consequences or correlation with functional antibody activity. We compared each inflammatory marker in the group of Caucasian participants against the group of AA participants. We found no significant differences in any of the measured inflammatory markers measured between groups (Fig. 4).

3.6. Single cell qPCR

We performed single cell gene expression profiling on a specific subset of IgM memory B cells that play an important role in T-cell independent immune response at the baseline before vaccination. Results from our study have shown that there are significant differences between IgM memory subsets isolated from Caucasian and AA groups in terms of gene expression levels (Fig. 5). We have found that there are 33 genes that are differentially expressed between AA and Caucasian groups, including genes involved in metabolic activity (HK2, PFKP, LDHA, ENTPD1, ENO1, TYMS), lymphocyte signaling (MS4A1, FCRL2, CD1D, CXCL8, CD80, CBLB, RFTN1, CD40, CCR2), T-cell independent immune response (BAFF-R, TLR1, IL21R, TLR4), transcription factors (SPIB, IRF4, PAX5, IKZF3, BCL6, FOXO1), apoptotic activity

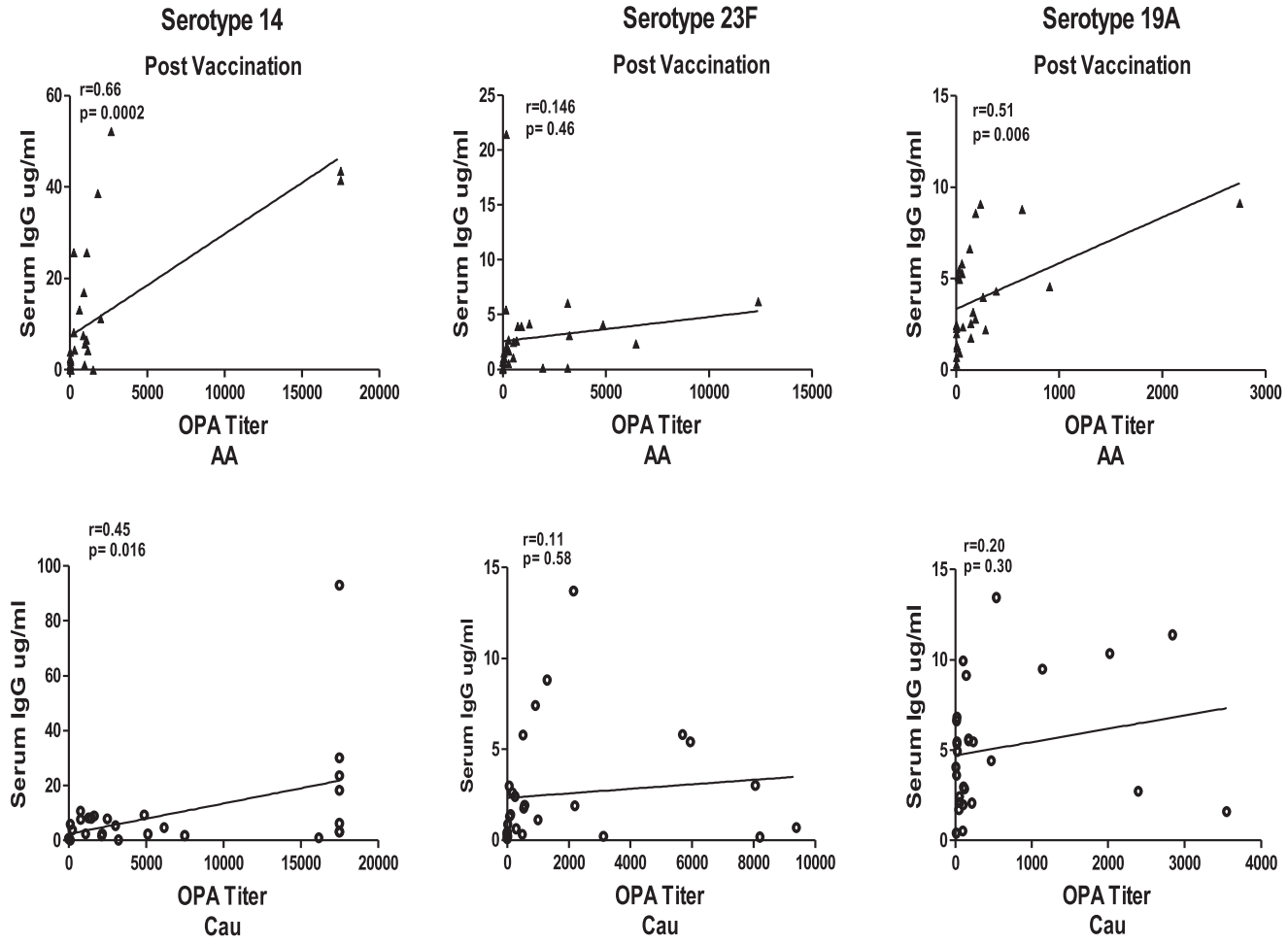

Fig. 3. Correlation between post-vaccination IgG antibody levels and OPA titers in all HIV-positive African-American and Caucasian individuals regardless of vaccination protocol. Correlations were determined by Pearson's correlation coefficient.

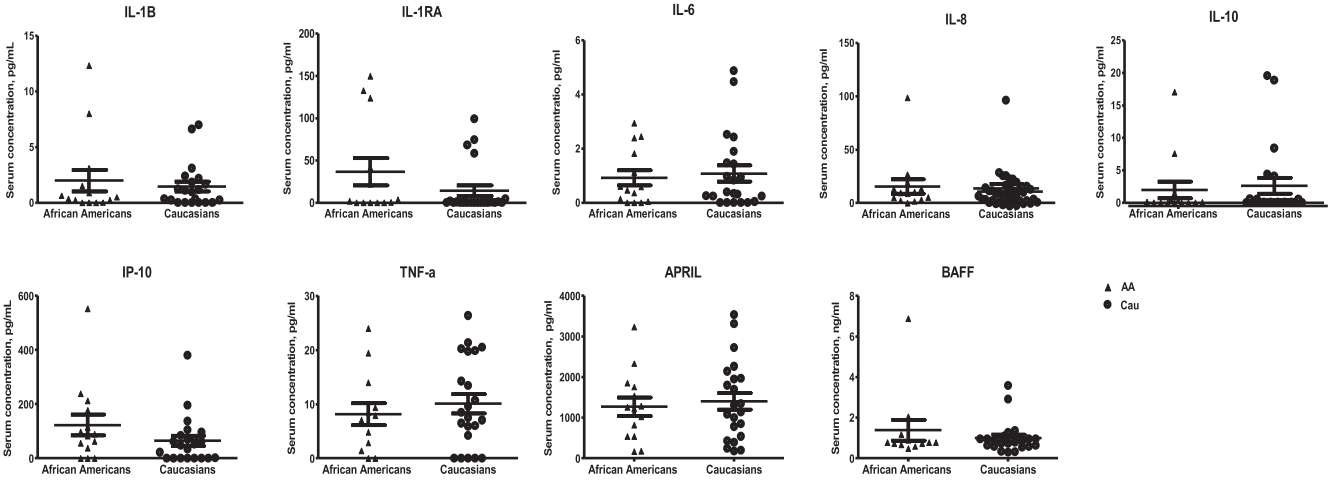

Fig. 4. Serum cytokine and chemokine levels. Serum inflammatory markers were measured using Luminex assay in HIV-positive African-American and Caucasian individuals prior to immunization. Difference between the groups were compared using Wilcoxon sign rank test; no significant differences were found.

(BCL2, BIK, MCL1, BAX), as well as cytokine and receptors (IL6, IL-7, INFGR). Gene expression levels were significantly higher in AA as compared to Caucasians. Interestingly, despite overall higher levels of expression of these genes, the bimodal distribution was more frequently observed in AA group as compared to Caucasian group.

4. Discussion

It has been well established that race is a significant risk factor in IPD in both HIV negative and HIV positive populations [4,12,14,29]. A retrospective case controlled study of HIV positive adults with pneumococcal disease found that pneumococcal dis-

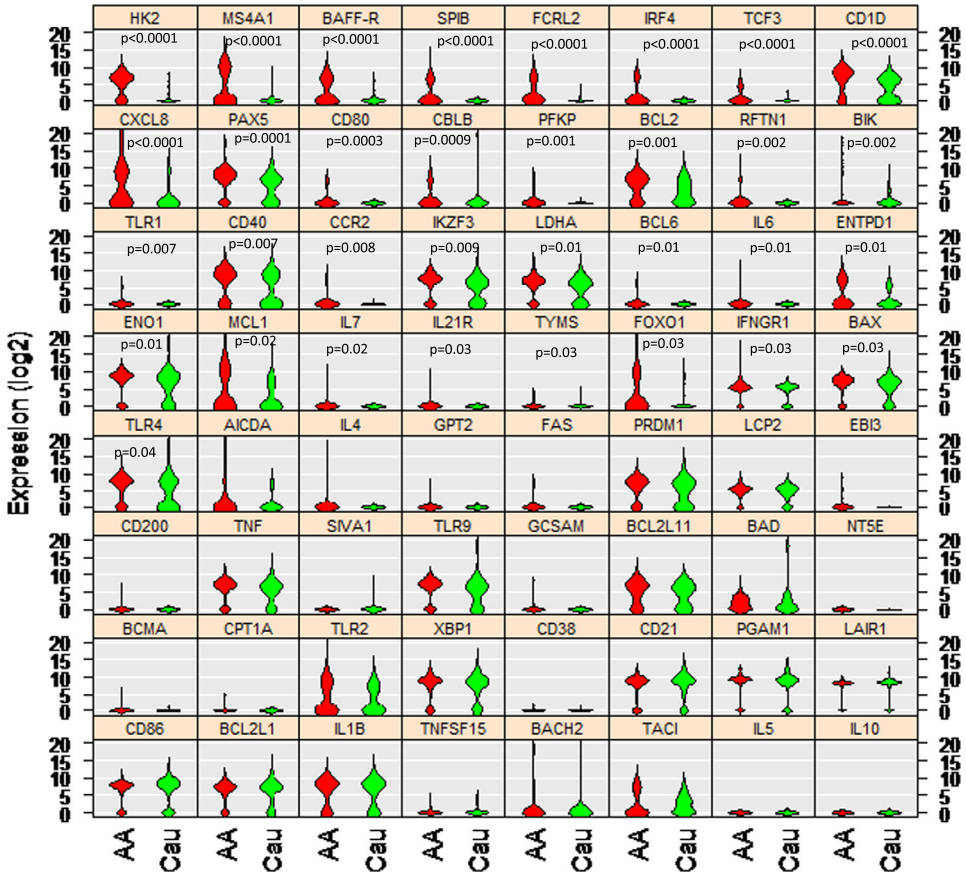

Fig. 5. Multimodal variation in gene expression levels. Violin plots of differentially expressed genes that are ranked by p-values. 64 genes were examined in pre-immunization IgM memory single B cells isolated from HIV-positive African-American and Caucasian individuals.

ease was more common in African American HIV positive persons compared to HIV positive persons of other ethnicities [14]. Moreover, the 23-valent pneumococcal polysaccharide vaccine (PPV23) efficacy was markedly better, 76% in Caucasians, compared to black patients, 24%, despite similar CD4 T cell counts [14]. Similarly, French et al. found that PPV23 was ineffective in a HIV-infected African population [20]. Based on these studies, it has been speculated that poor immunological response to pneumococcal polysaccharides may be in part responsible for this racial bias. A recent study however, reported no differences in PPS-specific IgG response between Caucasian and AA participants[21], but functional antibody studies were not performed.

Although serological criteria for evaluation of pneumococcal vaccines in infants have been established, the correlates of protective pneumococcal immunity in adult populations have yet to be determined [30]. The opsonophagocytic assay (OPA) is generally regarded as a better measure of protection compared to antibody concentrations as it mimics the host phagocytic, or functional, response [22,31]. Correlations between serum IgG antibody and OPA tend to vary greatly and are generally low in the elderly [32]. Moreover, PPS-specific IgM antibody concentrations also contribute significantly to OPA activity [33]. Based on the observations that both PPS-specific IgM and IgG opsonize pneumococci, and OPA appears to be the best correlate of protection. Here, we report an evaluation of the immune response to pneumococcal vaccination to 3 serotypes by measuring the PPS-specific IgG and IgM concentrations and OPA in HIV-positive African American and Caucasian individuals. The participants in our study were selected for participation in this study based on age (40–65 years), CD4 T cell count, low/undetectable HIV viral load and duration of ART, as each of these factors can influence vaccine responses [16,17,33-35]. We studied the immune response to the common clinical pathogens serotypes 14, 19A, and 23F as these serotypes are: 1. included in both PCV13 and PPV23, 2. represent both negatively charged and neutral polysaccharides, 3. Vary in immunogenicity, generating high (PPS14) to moderate (PPS23F) to moderately low (19A) immune responses and 4. have been widely studied in various populations in our laboratory with ample well standardized samples available for controls (14 and 23F) or 5 are important emerging serotypes (19A). We also examined immune responses to poorly immunogenic serotypes such as serotype 3, a serotype with low invasive potential, but high reported mortality rates and 7F, a serotype with very high invasive potential. However, as expected, the response rates in both groups were very low and there were no significant differences in OPA titers between ethnic groups (unpublished data). Serotypes that classically induce poor immune responses are less suitable for studies in what is expected to be a low responding population(s).

Although all participants were previously immunized with the 23-valent PPV and could potentially have various amounts of residual (functional) antibody levels, we found no differences in pre-immunization PPS-specific IgM, IgG or OPA between groups.

Similar to the findings described by Crum-Cianflone et al. [21], we also did not find significant differences between AA and Caucasian HIV-positive groups in post-immunization PPS-specific IgG levels against all serotypes measured (Fig. 1).

In contrast, we found significant differences in the functional antibody response between the AA and Caucasian populations post-immunization with the polysaccharide vaccine in the response to 2 of the 3 serotypes tested, namely 14 and 19A. Prior

immunization with the conjugate vaccine was able to overcome the difference between ethnicities in the case of serotype 19A but not in the response to serotype 14. This may be attributed to the small group size of the Caucasian group immunized with PCV/PPV (n = 8), and relative low OPA titers in response to PPS 19A as compared to serotype 14 for PCV/PPV vaccination regimen.

Several investigators have noted a poor correlation between quantitative antibody response as measured by ELISA and opsonophagocytic activity [32,36,37]. Therefore, individuals with antibody levels thought to be commensurate with protection, may not necessarily be protected against disease. Reduced or absent antibody activity, as determined by opsonophagocytic assay could be related to race-related distinctions in pre-vaccination gene expression levels in IgM memory B cells. Studies have shown, that IgM memory B cells is a predominant population of PPS-responding B cells, regardless of vaccination regimen in both healthy adults and HIV-positive individuals [25] and that absence of IgM memory B cells leads to high susceptibility to bacterial infection and inability to respond to polysaccharide antigens as seen in newborns, asplenic or splenectomized individuals, and patients with immune deficiencies [26-28]. In agreement with other studies, we demonstrated significantly different profiles of gene expression between African American and Caucasian ethnic groups[38]. We observed higher levels of expression in 33 genes on the single cell level in AA as compared to Caucasian which could in part influence immune response to pneumococcal vaccination. Despite the fact, that some genes are expressed more in AA group, only the Caucasian group demonstrated superior immune response as determined by OPA to PPS 14 and PPS19A. This could be attributed to more frequently observed bimodality within the single B cell population in AA which suggests that there is a greater variation in levels of gene expression between individual cells in AA as compared to Caucasian group and therefore in part explains lower functional antibody activity and more notable discrepancies between serum antibody and opsonophagocytic titers produced.

It has been postulated that increased levels of inflammation may lead to poorly functional antibody responses. Poor immune response to influenza vaccination was found to correlate with increased inflammation in the elderly population [39,40]. We measured 10 inflammatory markers in the pre-immune serum of all study participants and found no difference in any of the markers between study groups. These results suggest that factors other than inflammation are responsible for the interracial differences in functional antibody activity. It is important to mention however, that we specifically studied a cohort of HIV+ individuals with well controlled disease (at least 1 year on antiretroviral therapy and suppressed viral loads) without concurrent HIV-related co-infections. The fact that the disease is well-controlled could have impacted the detection of any possible differences in the expression of inflammatory markers and racial differences. A cohort of individuals with high viral loads and very low CD4 counts might display different pre-immunization inflammatory states and racial differences.

In conclusion, in this small but uniform cohort of HIV positive Caucasian and AA individuals between 40 and 65 years of age, we made some unique observations. First, as in previous studies, we found no significant differences in IgG or IgM antibody responses between Caucasian and AA participants in the immune response to immunization with either PPV or PCV/PPV only. However, when we measured opsonophagocytic activity, likely more reflective of protective immunity, we found significant differences between races in response to 2 of 3 serotypes measured. Furthermore, we demonstrated that gene expression levels between single cell IgM memory B cell populations are significantly different between ethnicities that might be a contributing factor to differences in immune response to pneumococcal antigens.

Our study has several limitations. Although, we are reporting the immune response to 3 serotypes, the results are significant. The serotypes evaluated in this study are diverse and elicit different levels of immune response and therefore provide a template of possible behavior in response to other serotypes. Furthermore, our results demonstrated that there was a significant difference in geometric mean antibody titers (GMT) between African-Americans and Caucasians for serotypes 14 and 19A, however we have not tested if (a) our results will hold up in a larger future study, and show that African Americans truly have a lower average GMT or (b) African Americans have a subgroup that has a lower GMT and a subgroup that may be similar to Caucasian population. At this point, our study supports the existence of a subgroup of African Americans that has lower GMT and in this subgroup lower OPA titers corresponded to lower anti-PPS IgG antibody concentrations. The subgroup of African Americans with higher OPA titers consistently had higher anti-PPS IgG antibody concentrations.

Our future studies will focus on mechanistic aspects of the immune response which will include characterization of polysaccharide-specific B cells and identification of variations in intercellular gene expression that shapes polysaccharide-specific B cell responses in different ethnic groups. These studies will significantly increase our knowledge of the genetic identities of B cells in HIV+ individuals of African American and Caucasian ethnicities and help to uncover B cell complexity and diversity. We will gain new insights into mechanisms underlying B cell dysfunction phenotype in HIV+ individuals that leads to poor responses to pneumococcal vaccination.

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Conflict of interest

All authors have no potential conflicts of interest.

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