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The controversial impact of B cells subsets on immune response to pneumococcal vaccine in HIV-1 patients

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SUMMARY

Background: Chronic HIV infection leads to severe perturbations of the B cell populations and hyporesponsiveness to vaccines. The associations between circulating B cell subpopulations and the antibody response to pneumococcal polysaccharide vaccine in antiretroviral-naïve and treated patients were studied.

Methods: Sixty-six HIV-infected adults were grouped according to antiretroviral therapy (ART) and CD4+ cell count; 31 were ART-naïve and 35 were ART-treated, and they were matched for age, CD4 cell count, and duration of HIV infection. All subjects were immunized with the 23-valent polysaccharide vaccine against *Streptococcus pneumoniae*. Pre- and post-vaccination B cell subpopulations were assessed by flow cytometry. Serum IgG concentrations for vaccine serotypes were quantified by ELISA at baseline and at 4 and 48 weeks post-vaccination.

Results: Patients under highly active antiretroviral therapy (HAART) had significantly higher antibody levels against pneumococcal vaccine antigens, while an adequate number of patients responded to vaccination. Memory B cells were diminished over time, although treated patients maintained higher levels of all subsets studied, with the exception of activated memory and isotype-switched memory B cells.

Conclusions: Low concentrations of total B cells and exhausted memory B cells was the strongest independent predictor of poor pneumococcal vaccine responsiveness, emphasizing that B cell subset disturbances are associated with a poor vaccine response among HIV-infected patients.

1. Introduction

Streptococcus pneumoniae infections are the most common cause of bacterial pneumonia among HIV-infected patients and account for high morbidity and mortality.[1] The introduction of highly active antiretroviral therapy (HAART) has led to a decline in the incidence of invasive pneumococcal disease, although it still affects HIV-infected more often than healthy individuals, even those with preserved CD4 cell counts.[2–7]

The guidelines still recommend vaccination of HIV-infected people with the 23-valent polysaccharide vaccine (PPV-23)[8] despite its challenge from the conjugate polysaccharide vaccine. Despite scheduled immunization and the introduction of HAART,[9–12] the risk of pneumococcal infection among these immunocompromised hosts remains high.[13–15]

B cells comprise one the most dysfunctional lymphocyte populations in patients with HIV infection.[16] During chronic viral replication, functional perturbations of B cells occur, including polyclonal activation,[17] hypergammaglobulinemia,[16] dysregulation of isotype switching, variation in the proportions and absolute numbers of circulating B cells, and impaired immune responses to immunization.[18–20] Significant heterogeneity is also obvious among

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memory B cells, and despite their ambiguous functional heterogeneity, many phenotypic subpopulations have been recognized.[21]

The loss of memory B cells is one aspect of dysfunction in HIV infection.[22] Moreover, there is evidence of alterations in B cell populations against T cell-independent antigens such as pneumococcal polysaccharides.[18] A reduced frequency of IgM+ memory B cells, which mediate memory responses against pneumococcal infection, is also observed.[23] Most activated B cells undergo apoptosis after infections lapse, except for a few cells comprising the resting memory B cell compartment, necessary for a rapid secondary immune response.

During the natural course of HIV-1 infection, the B cell subsets are altered, including resting memory B cells, which are severely depleted.[24] Additionally, defective B cell populations, including activated memory and exhausted memory B cells, rise in HIV-1-infected individuals, while they circulate at very low levels in healthy individuals.[25] These aberrant cells express a low level of CD21 on their surface and present features of immune activation[26] and cellular exhaustion.[25]

HAART initiation reduces polyclonal B cell activation[20,25] and normalizes limited numbers of naïve and memory B cell subsets.[18–20,27] However, neither the amount of circulating isotype-switched memory B cells nor their functional activity are restored by HAART,[28,29] which might lead to impaired immunity, even in treated patients.[11,12] The loss of memory is reflected in the decline in antigen-specific memory B cells after vaccination, which are not restored by HAART.[30,31] HAART has only a limited effect on the normalization of the B cell compartment. Resting memory B cells are maintained if HAART is initiated early post primary HIV infection.[32] It remains, though, to be elucidated whether HAART restores certain B cell defects. Furthermore, viremia has been associated with certain B cell defects.[30] However, the impacts of HIV viremia and the level of nadir CD4 cell counts have not been fully clarified, except in a few studies.[32]

Although antibody levels are useful as a surrogate marker of protection and have been used during recent decades as the gold standard assay, they have limitations and cannot fully describe the immune response to vaccines; consequently other markers have been established to assess immunogenicity and protection of immunization more precisely.[33–40]

The aim of this study was to fully assess the implication of memory B cells in the antibody response to the 23-PPV in HIV patients. Specifically, it was sought to record and evaluate associations between circulating B cell subpopulations in the peripheral blood and the antibody response during a 48-week period, pre and post immunological stimulation with the recommended vaccine against *S. pneumoniae* in HIV-1 patients. Two patient groups of distinct infection status were studied, those treated successfully with HAART and those who were HAART-naïve.

2. Materials and methods

2.1. Study participants and ethics approval

This was a longitudinal study involving 66 HIV-1 patients, of whom 31 were antiretroviral-naïve and had preserved CD4 cells (CD4 cell count above 500 copies/mm^3) and 35 were on successful HAART, with satisfactory viral suppression (HIV-1 viral load below 50 copies/ml); these patients were matched for age, CD4 cell count, and duration of HIV infection. All clinical, epidemiological, and laboratory data, including age, gender, HIV-1 transmission route, co-morbidities, HIV-1 viral load, current CD4 T cell count, and nadir CD4 cell count, were recorded or measured.

All patients were immunized for the first time against *S. pneumoniae*. Individuals who had been on HAART for less than 6 months or who had an HIV RNA above 50 copies/ml while being under HAART, those who had a CD4 T cell count below 200 cells/ml, and those who missed the first visit (4th week) post-immunization were excluded.

All patients were followed-up in the Infectious Diseases Unit of AHEPA University Hospital in Thessaloniki Greece. The ethics committee of the study institution approved the study protocol and all participants submitted a written informed consent.

2.2. Immunization and blood sample collection

All patients underwent blood sampling and then received 0.5 ml of the 23-valent polysaccharide vaccine Pneumovax 23 (Merck and Co., Inc.) via intramuscular administration. Patients returned 4 and 48 weeks post vaccination for further blood sampling. Fifteen millilitres of fresh whole blood was collected in ethylenediaminetetraacetic acid (EDTA) tubes for B cell staining; 10 ml more of whole blood was collected into heparin-coated, pyrogen-free tubes for phagocytosis assessment and 15 ml in tubes without anti-clot agent for antibody measurement via ELISA. Blood samples were analysed within 2 h of collection.

2.3. B cell immunophenotyping

The following mouse anti-human fluorochrome-conjugated monoclonal antibodies of Immunostep Company were used: CD19-PerCP, CD27-PE, IgM-FITC, IgD-FITC, and CD21-FITC. Results were expressed as the subset percentage of the total B cell fraction. Sample processing and analysis of results were performed in the XL Epics cytometer (Beckman Coulter Company, Miami, FL, USA). Upon addition of 10 μl of the above combined monoclonal antibodies, 100 μl of each blood sample was incubated in the dark for 10 min. Subsequently, red blood cells were thawed upon ingestion of 2 ml of Lysis Buffer (Becton Dickinson Biosciences, San Jose, CA, USA) and incubated for another 20 min at room temperature. The cell staining, input capture, and flow analysis were performed promptly in a blinded pattern. B cells were assessed prior to vaccination. The different B cell subsets were characterized at baseline as follows: total B cells (CD19+), memory B cells (CD19+CD27+, BMC), resting memory B cells (CD19+CD27+CD21high, RM), exhausted memory B cells (CD19+CD21lowCD27−, EM), IgM memory B cells (CD19+CD27+IgMhigh), isotype-switched memory B cells (CD19+CD27+IgM−, ITS), and activated memory B cells (CD19+CD21low+CD27+, AM). Results were expressed as B cell concentrations or as a percentage of the total B cell fraction. The cell staining, data capture, and flow analysis were performed in a blinded fashion.

2.4. ELISA

Prior to vaccination and at weeks 4 and 48, blood samples were collected, allowed to clot naturally, and the serum separated; ELISA was then performed immediately. Microwells were pre-coated with the *Pneumocystis jirovecii* Pneumonia (PCP) antigen (1–5, 6B, 7F, 8, 9N, 9 V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, 33F). Calibrators and controls were pre-absorbed against capsular polysaccharide (CPS) and samples were diluted in diluents containing CPS. The calibrators, controls, and diluted patient samples were then added to the wells and antibodies recognizing the PCP antigen bound, during the first incubation. Samples were run in duplicate.

The rest of the procedure was executed according to manufacturer's instructions.[41] In order to interpret the immune response to the vaccine, several criteria are used: the post-vaccination antibody titre, the fold-increase, and the frequencies of serotypes with a satisfactory response. Currently there is no global definition for the adequate response to pneumococcal polysaccharide vaccine. In this study a satisfactory antibody response was considered a two-fold antibody increase from the initial level.[42]

Table 1
Baseline characteristics of the study participants at inclusion

	HAART-naïve patients ($n = 31$)	Treated patients ($n = 35$)	p-Value
Age, years, mean ± SD	30.46 ± 7.12	33.15 ± 7.9	0.178
Gender, male/female, n (%)	31 (100.0%)/0 (0.0%)	31 (85.7%)/4 (14.3%)	0.065
Years of HIV infection, mean ± SD	3.84 ± 1.85	4.68 ± 4.44	0.345
CD4 cell count before vaccination, mean ± SD (copies/mm^3)	637.9 ± 270.4	708.7 ± 296.1	0.316
Nadir CD4 cell count, mean ± SD (copies/mm^3)	596.6 ± 239.1	315 ± 192.1	0.0005
VL before vaccination, median (IQR)	36 503 (165 080)	47 (0)	0.0005
HAART duration in months, mean ± SD	NA	35.6 ± 14.35	NA
Body weight, kg, mean ± SD	76.1 ± 10.22	73.0 ± 10.87	0.239
HCV infection, n (%)	1 (3.2%)	3 (8.6%)	0.616
HBV infection, n (%)	6 (19.4%)	3 (8.6%)	0.287
Current smoker, n (%)	21 (67.7%)	12 (34.3%)	0.013

HAART, highly active antiretroviral therapy; SD, standard deviation; VL, HIV RNA viral load; IQR, interquartile range; NA, not applicable; HCV, hepatitis C virus; HBV, hepatitis B virus.

2.5. Statistical analysis

Data were expressed as the mean ± standard deviation (SD) or median (interquartile range (IQR)) (in the case of violation of normality) for continuous variables, and as counts and percentages for categorical data. The Kolmogorov–Smirnov test was used for normality analysis of the parameters.

The one-factor repeated measures analysis of variance (ANOVA) model was used to compare the different time measurements of variables for each group. Pair-wise multiple comparisons were performed using the critical difference method of Tukey.

The vaccine antibody responses were calculated as the relative vaccine-specific IgG increase from pre-vaccination baseline to 4 and 48 weeks, thus providing a correlate for total vaccine response adjusted for the patient's IgG concentration of the 23 vaccine serotypes at the time of enrolment. The number of B cells and B memory cell subpopulations were compared between the two patient groups by Student's t-test, or the Mann–Whitney test in the case of violation of normality.

This was supplemented with multivariable regression analyses with adjustments for patient group (ART-naïve and treated patients) and current smoking status (yes/no). Logarithmic transformation was used when appropriate to obtain normality.

All tests are two-sided, and a p-value of <0.05 was used to denote statistical significance. All analyses were carried out using the statistical package SPSS version 16.00 (SPSS Inc., Chicago, IL, USA).

3. Results

The demographic, clinical, and laboratory data of the 66 HIV-1 patients are summarized in Table 1.

CD4 cell counts were higher in the treated group and their progress was different compared to naïve patients ($p = 0.024$), who had a downward trend in their CD4 cell counts. Concerning each post-vaccination time point separately, there was a significant variation at week 4 ($p = 0.029$) and week 48 ($p = 0.001$) in CD4 frequencies (data not shown). The two groups differed significantly concerning smoking habit ($p = 0.013$) and nadir CD4 cell count ($p < 0.0005$) (Table 1).

3.1. Antibody response to 23-PPV

Patients under HAART had significantly higher antibody levels at all three time points: at baseline ($p = 0.0005$) and at 4 weeks ($p = 0.006$) and 48 weeks ($p = 0.001$) post-vaccination. Fifty patients doubled their initial IgG level (responders), while 16 were non-responders (Table 2). Interestingly, the vast majority of non-responders were under HAART treatment. No correlation between an adequate response and current CD4 or nadir CD4 cell count was detected.

3.2. Most B cell subsets were lower among ART-naïve patients compared to treated patients at baseline

The frequency of total B cells, BMC, RM, and EM was higher in treated patients compared to naïve patients; in contrast, IgM memory B cells and ITS were higher in the naïve group. Furthermore, ART-naïve patients also preserved higher fractions of AM pre vaccination (Figure 1). These differences were not statistically significant. Memory B cells were diminished over time in both groups, although treated patients maintained higher levels of all subsets studied, with the exception of AM and ITS. There was no significant relationship between CD4 cell counts and the studied B cell subsets pre vaccination. In contrast, on multivariate analysis, low levels of nadir CD4 correlated with the diminished levels of resting ($p < 0.01$) and activated memory B cells ($p = 0.03$) (data not shown).

3.3. Memory B cells and IgM memory B cells demonstrated no correlation with vaccine IgG response

In unadjusted linear regression analyses, the levels of memory B cell and IgM memory B cell counts did not predict the vaccine response at all time points. Similarly, regression analyses adjusted for patient group showed no effect of memory B cell and IgM memory B cell concentrations on IgG vaccine response.

Table 2
Antibody response to 23-PPV in naïve and treated HIV patients[a]

	HAART-naïve patients ($n = 31$)	Treated patients ($n = 35$)	p-Value
Antibodies (log) pre-immunization, mean ± SD	3.16 ± 0.98	4.4 ± 1.43	0.0005
Antibodies (log) 4th week, mean ± SD	4.62 ± 1.38	5.46 ± 1.04	0.006
Antibodies (log) 48th week, mean ± SD	0.29 ± 0.5	0.65 ± 0.33	0.001
Responders	26 (83.8%)	24 (68.5%)	0.047
Non-responders	5 (16.1%)	11 (31.4%)	0.047
Fold increase, mean ± SD	6.72 ± 6.8	8.18 ± 13.41	NS

23-PPV, 23-valent pneumococcal polysaccharide vaccine; HAART, highly active antiretroviral therapy; SD, standard deviation; NS, not significant.

[a] Treated patients maintained higher levels of antibodies against *Streptococcus pneumoniae* pre- and post-immunization. Most patients responded successfully to the vaccine, irrelevant of HAART intake. However, there were twice as many treated non-responders as naïve non-responders.

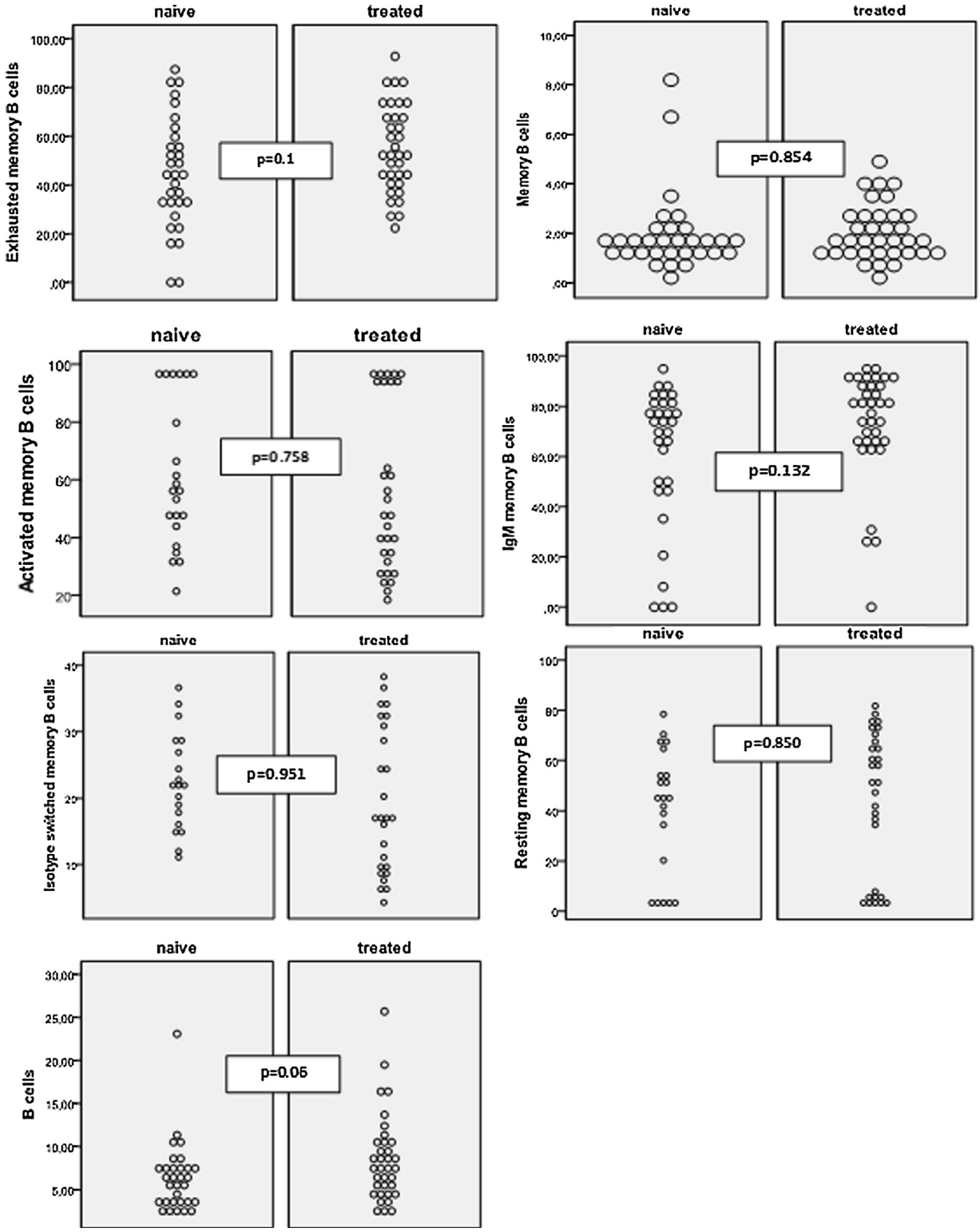

Figure 1. Distinct frequencies of total B cells and other B memory subpopulations in the peripheral blood of HIV viremic and aviremic (treated) individuals before immunization with the PPV-23 vaccine against *Streptococcus pneumoniae*. No significant differences in the levels of total B cells and certain memory B cell subsets were detected among antiretroviral-naïve and treated patients at baseline. HAART had no effect on the fluctuation of the initial B lymphocyte levels in HIV-1 infection.

3.4. Total B cell count and exhausted memory B cells predicted the antibody response to the vaccine

The vaccine-specific IgG concentration measured 4 weeks post vaccination correlated positively with total baseline B cell ($p = 0.02$) and exhausted memory B cell subsets ($p = 0.07$) in the unadjusted (Figure 2) and adjusted linear regression analyses. No other baseline B cell compartment was associated with the antibody response and preservation of the antibody concentrations post vaccination over the 48-week study period (data not shown).

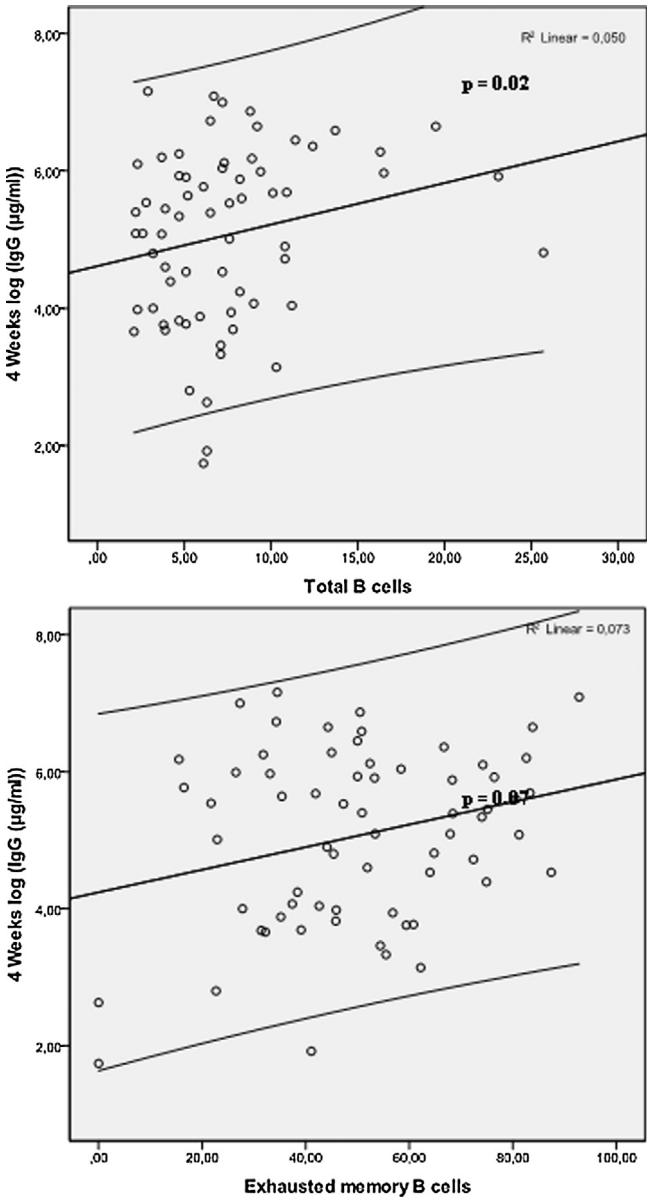

Figure 2. Linear regression plots: pre-vaccination baseline B cell subpopulations as predictors of the IgG antibody response. Scatter plots with the best fitted line. Association between the two B cell subsets (log cells/μl) plotted against the IgG vaccine-specific antibody concentration (log IgG μg/ml) at 4 weeks post-immunization, which were found to be significant, implying a positive predictive correlation of the initial levels of total B cells and the exhausted memory B cell subpopulation and the antibody response to PPV-23.

3.5. Contribution of viremia and nadir CD4 cell count

In order to investigate the role of different factors associated with B cell alterations, demographic, virological, and immunological factors at baseline were evaluated by multivariate analysis. Age correlated with activated and resting memory B cells, while gender did not seem to affect these populations. HIV RNA at baseline showed no correlation with AM, RM, or IgM memory B cell counts at baseline. The nadir CD4 cell count correlated with low levels of resting memory and activated memory B cells (data not shown).

To evaluate more precisely the accumulated results from the previous analysis, the possibility of a linear association of B cell subpopulation frequencies with HIV RNA viral load and CD4 T cell counts at all three time points were further investigated separately (before vaccination and at week 4 and week 48 post-vaccination). Activated B cells at baseline were associated with the baseline viral load ($r = 0.54$, $p = 0.01$), and resting memory B cells were moderately negatively correlated with viral load at baseline ($r = -0.45$, $p = 0.026$) (data not shown). No association was observed between CD4 cell counts and AM or RM cells at any of the investigated time points (data not shown).

The effect of HAART on distinct B cell subpopulations, along with the vaccine effect during the study period, is summarized in Table 3. Moreover, certain differences in B cell frequencies are illustrated compared to healthy individuals. There were significant variations in distinct memory B cell subsets in HIV-1 patients. Vaccination with the 23-PPV had an immunological effect on certain B cell subsets like AM, EM, RM, and ITS memory B cells, triggering alterations in their counts in the peripheral blood of HIV-infected patients. Moreover, HAART intake appears to have controversial implications in a few of these memory B cell populations.

4. Discussion

HIV-1 infection in its natural course leads to significant B cell defects, including hyperactivation, cell switch, and finally the onset of cell subsets that are normally lacking or appear at low levels in healthy individuals, and HIV patients present poor antibody responses to polysaccharide vaccines. The introduction of HAART triggered further study of these populations, concluding that these alterations are reversible with successful antiviral treatment, suggesting a causal relationship with viremia. The effect of viremia in B cell divergences has not been assessed thoroughly except in a few studies,[32] and consequently several differences have been recorded among ART-naïve and treated patients. This study, in line with those of other authors, showed that viremia is correlated with specific B cell populations. Patients with a viral load >50 copies/ml had elevated activated B cells and a decline in resting memory B cells.[32]

It was found that ART-treated patients had higher levels of several B cell subsets before vaccine administration. Moreover, it was determined that naïve patients with continuous viral replication preserved increased AM and ITS memory B cell counts, while nadir CD4 predicted low RM and ITS over time. It has been confirmed that HAART has a restoring effect on their population, triggering their increase, which is in line with the present results, in which treated patients had higher levels of RM B cells compared to naïve patients.[20] Similarly, activated memory B cells are expanded in chronic viral replication, which is in line with the present data; these showed that naïve patients maintained higher levels of this subset compared to the treated group, confirming the normalizing effect of HAART. With regard to isotype-switched memory B cells, it is known that this subset is dependent on T cell help, which has been thought to be defective in ART-treated adults compared to naïve; hence it is believed that the present results may reflect the better immunological status of naïve patients, reflected in higher nadir CD4 cell levels compared to treated patients. However, it should be noted that these differences are minor.[43]

Furthermore, it was observed that total B cells, memory B cells, and ITS memory B cells correlated with IgG responses at baseline. Finally, ART was associated with increased total B cells and the EM B cell compartment, which seemed to predict IgG responses.

HIV-1 infection impairs memory B cells, and seropositive patients display a low antibody response in T cell-independent (TI) antigens, such as those included in the 23-valent polysaccharide vaccine, irrespective of HAART, which further declines over time. These data were also confirmed in the present study, along with the fact that the majority of non-vaccine responders were ART-treated; the data further enhance the aspect that the antibody

Table 3
Effect of HAART and polysaccharide vaccine administration on different B cell subpopulations at weeks 4 and 48, compared to the frequencies of B cells in healthy adults[a]

	Viral load before vaccination in the treated group: <47 copies/mm^3 (undetectable) Viral load before vaccination in the naïve group: 36 503 (165 080) copies/mm^3					
	Baseline B cell subpopulations	Treatment effect	B cell subpopulations 4 weeks	Vaccination effect from baseline	B cell subpopulations 48 weeks	Vaccination effect from baseline
Total B cells (NR 4.9–18.4%)						
Treated	8.25	Increase	8.49	No change	7.64	No change
Naïve	6.62	NA	6.36	No change	5.92	No change
Memory B cells (NR 7.2–18.9%)						
Treated	2.16	Increase	2.28	Increase	1.91	Decrease
Naïve	1.98	NA	2.07	Increase	1.76	Decrease
Resting memory B cells (NR 44–78.3%)						
Treated	40.89	No change	60.63	Increase	59.61	Decrease
Naïve	40.66	NA	44.62	Increase	46.55	Decrease
Exhausted memory B cells (NR 7.2–11.2%)						
Treated	55.19	Increase	46.36	Decrease	45.28	Decrease
Naïve	47.49	NA	37.99	Decrease	39.55	Decrease
IgM memory B cells (NR 7.3–32.5%)						
Treated	20.02	Increase	24.64	No change	28.73	No change
Naïve	23.99	NA	24.44	Increase	28.24	Increase
Isotype- switched memory B cells (NR 6.5–29.1%)						
Treated	22.29	No change	26.11	Increase	38.73	Increase
Naïve	22.42	NA	25.03	Increase	37.28	Increase
Activated memory B cells (NR 1.0–3.6%)						
Treated	58.87	Decrease	39.48	Decrease	40.68	Decrease
Naïve	61.62	NA	54.79	Decrease	52.78	Decrease

HAART, highly active antiretroviral therapy; NR, normal range; NA, not applicable.

[a] Activated memory B cells increase in HIV-infected individuals, with conflicting HAART impact on normalization of their frequencies and activity. It is regarded to restore their expansion, especially if the initiation of HAART is prompt. The fact that activated memory B cells are expanded in chronic viral replication is in line with the data of the present study, which show that naïve patients maintain higher levels of this subset compared to treated patients, confirming the normalizing effect of HAART. These cells are prone to extrinsic apoptosis during the natural course of HIV infection. Due to the decline in their frequencies post vaccination in this study, irrespective of HAART, it is speculated that this is attributed to PPV-23 administration. Resting memory B cells are depleted during HIV infection, whereas their levels are high in healthy adults. It has been confirmed that HAART has a restoring effect on their population, triggering their increase, which is in line with the results of the present study, in which treated patients had higher levels of RM B cells compared to naïve patients. Regarding the subset of exhausted memory B cells, it is known that they accumulate in HIV-infected patients irrespective of viral control and HAART intake, and this has been attributed to high plasma viral loads, chronic immune activation, or disease progression. Additionally, their levels may change independent of immune activation or viral replication. Moreover, similar to healthy individuals, HIV controllers have been detected to have a high frequency of exhausted, tissue-like B cells, despite having low to undetectable viral loads. This suggests that the accumulation of dysfunctional exhausted memory B cells may be linked to intrinsic infection-induced alterations in the B cell compartment, which in the case of the present study could be attributed to the initial impairment of the CD4 T cell counts of the treated patients group (low nadir CD4 cell counts), despite current HAART intake. Isotype-switched memory B cells are known to depend on T cell help, which has been thought to be defective in ART-treated adults compared to naïve, so it is believed that the results of this study may reflect the better immunological status of naïve patients, imprinted on higher nadir CD4 cell levels compared to treated patients. However, these differences are minor.

response alone does not constitute an efficient protection index for pneumococcal infection in immunocompromised individuals.[42,44]

In this study, vaccination with the 23-PPV elicited satisfactory antibody responses in both groups, in accordance with previous studies.[40,42] Studies investigating long-term serological responses to 23-valent PPV in HIV patients have produced inconsistent results.[45–48]

Treated patients had higher levels of antibodies throughout the study period, although their progress over time was similar, irrespective of HAART intake. It was found that HIV viral suppression was associated with higher rates of antibody response.[42] This is consistent with other reports, which have also implied a negative correlation between plasma HIV RNA load and serological responses to PPV-23 that could be restored by HAART.[47,48] A moderate response to vaccine in treated patients probably reflects the exhaustion of the immune system along with the natural course of HIV infection.[42]

A recent study assessing the effectiveness of PPV-23 also suggested that, irrespective of CD4 cell count at immunization, the vaccine gave no benefit when it was administered to patients with high viremia.[49] This may be related to continuous HIV replication, which probably perturbs B cell function, or may be correlated to the premature exhaustion of B cells, which results in ineffective humoral responses to antigen stimulation.[25,50] The present study also suggests that CD4 cell restoration upon HAART improved the antibody responses of HIV-infected patients receiving 23-valent PPV during the study period.

No significant correlation between baseline CD4 cell counts and the studied B cell subsets was identified, which is in line with previous data.[30] In contrast, on multivariate analysis, low levels of nadir CD4 correlated with the diminished levels of resting and activated memory B cells. This implies an intense effect of initial CD4 cell count levels, despite the subsequent immune restoration by following HAART, in the preservation and functionality of vital B cell populations.

The phenotype of the B cells responsible for immune responses to the polysaccharide pneumococcal vaccine has been controversial, and this was the reason for the present investigation into most of the correlated B cell subsets. More precisely, IgM and isotype-switched memory B cells have a significant role in the immune response to 23-PPV. The present study is in line with those reported by other authors,[43] who tried to investigate the effect of B cell subsets on the final response to 23-PPV. Total B cells and BMCs were elevated in treated patients compared to naïve patients both pre and post vaccination, as shown in other studies as well.[19–21]

It has been confirmed that patients with diminished or a lack of IgM memory B cells, respond modestly to polysaccharide vaccine and are susceptible to invasive pneumococcal disease.[51,52] Several studies have demonstrated the loss of IgM and/or switched memory B cells in HIV patients, which were also confirmed in the present data. However, IgM memory B cells are not solely responsible for anti-polysaccharide antibody formation. Isotype-switched memory B cells have also been shown to produce effective antibodies post vaccination in vitro.[53,54] In agreement

with other authors, higher levels of isotype-switched memory B cells were seen in treated patients compared to naïve patients,[33] which correlated with the baseline levels of IgG against *S. pneumoniae*.

Moreover, it is speculated that the association of the IgG response with total B cells and exhausted memory B cells reflects that these two B cell compartments participate in mounting an adequate response to PPV-23. Exhausted memory B cells comprise a rare population expanded in chronic HIV infection irrespective of HAART and viral replication.[55] It is known that EM accumulate in HIV-infected patients irrespective of viral control and HAART intake, and this has been attributed to high plasma viral loads, chronic immune activation, or disease progression. Additionally, their levels may change independent of immune activation or viral replication. Moreover, similar to healthy individuals, HIV controllers have been detected to have a high frequency of exhausted B cells despite having low to undetectable viral loads. This suggests that the accumulation of dysfunctional exhausted memory B cells may be linked to intrinsic infection-induced alterations in the B cell compartment, which in the case of the present study could be attributed to the initial impairment of the CD4 T cell counts of the treated patients group (low nadir CD4 cell counts), despite current HAART intake.[55]

This study has some limitations. The peripheral blood B cell population was studied since this is by far the easiest accessible compartment and the most widely investigated, and the peripheral blood B cell subsets are only representative of cells participating in the response. The data indicate that total B cells and the exhausted memory B cell compartment are the only peripheral populations that can be used to predict the IgG response to PPV-23, although other significant associations are reported, which may lead to further investigations on the role of B cells in effective vaccine responses.

This study emphasizes the fact that the disturbance of certain B cell subsets is associated with a poor vaccine response among HIV-infected patients and supports the idea that B cell defects play an important and independent role in the antibody response over time. Moreover, antibody assessment cannot comprise an index of effective protection against *S. pneumoniae* in immunocompromised hosts. In conclusion, an integrated study of all implicated aspects of the immune response may be a better approach to the evaluation of immunogenicity of the 23-PPV in HIV-infected patients.

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