SARS-CoV-2 Humoral and Cellular Immune Responses in COVID-19 Convalescent Individuals With HIV

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Abstract

Background

SARS-CoV-2-specific immune response features in people with HIV infection (PWH) remain to be fully elucidated. We aimed to evaluate the impact of HIV over humoral and cellular responses in COVID-19 convalescent PWH.

Methods

Blood samples from 29 PWH with preserved CD4+T-cell counts on ART and 29 HIV-negative (HIVneg) donors were included. SARS-CoV-2-specific IgG levels and IgG titers were determined by ELISA. Antibody neutralization capacity was evaluated against the reference B1 strain SARS-CoV-2. IFN-γ-secreting cells were detected by ELISpot using SARS-CoV-2 Spike, RBD, or Nucleocapsid protein or overlapping peptide pools. Frequency and phenotype of T, B and NK cells and levels of soluble cytokines and chemokines were assessed by flow cytometry.

Results

SARS-CoV-2-specific antibodies were detected on 65.5% of PWH and 79.3% of HIVneg individuals, with no differences in serum IgG levels and anti-SARS-CoV-2 neutralizing antibodies. All donors exhibited SARS-CoV-2-specific cellular immunity, including those with undetectable antibody responses. PWH showed diminished percentages of antibody-secreting cells compared to HIVneg cohort, with similar B cell proportions between groups. PWH presented an increment in T follicular helper (Tfh, CD4+CXCR5+) percentage, which negatively correlated with IgG titers. Additionally, CD4+PD1+ and CD8+HLA-DR+ cell frequencies were augmented in PWH. Moreover, PWH presented a high proportion of CD95+, CD25+, NKp46+, HLA-DR+, and CD38+/HLA-DR+ NK cells. Both groups displayed similar Tregs frequency, effector/memory, and T-helper profile for CD4TL, exhaustion and memory phenotypes for CD8TL and subtle differences in classical monocytes. Profile of circulating cytokines and chemokines was significantly different between both groups. Magnitude of IFN-γ responses to S or N proteins, and RBD was lower in PWH compared to HIVneg donors. Correlation analysis of immune and clinical parameters showed a distinct immune landscape in the PWH group.

Conclusions

PWH showed a distinctive immune profile although severity of COVID-19 was not exacerbated. PWH with conserved CD4+T-cell counts exerted both humoral and cellular responses against SARS-CoV-2. Even though cellular response was lower compared to HIVneg individuals, PWH achieved similar antibody

responses with a high neutralization capacity. These data reinforce the impact of ART, not only in controlling HIV but also other infections.

Introduction

The outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), etiologic agent of coronavirus disease 2019 (COVID-19), has raised concerns around the world (1). In Argentina, the first cases were recorded at the beginning of March 2020 (2). The predisposing factors for severe COVID-19 are aging, diabetes, cardiac disease, and obesity among others, which condition the host's immune response to the virus (3). Although human immunodeficiency virus (HIV) infection induces immune suppression mainly because of CD4+ T cell depletion, this chronic viral infection does not appear to be a risk factor for severe COVID-19 (4–6). Nevertheless, data regarding the impact of HIV on the susceptibility and severity of infection with SARS-CoV-2 are insufficient in the literature. It is known that, in immune-competent persons, effective response against SARS-CoV-2 involves the development of a functional antibody response and cellular immunity, which drives antibody production and cytotoxic effectors to viral eradication (7).

Studies on humoral immune responses against SARS-CoV-2 in HIV-immunosuppressed individuals are scarce and controversial. On one hand, it has been described that antibodies against SARS-CoV-2 are detected in people with HIV (PWH) with previous history of COVID-19, although with lower conversion rates when compared to HIV-naïve persons (8, 9). On the other hand, it was shown that similar antibody responses can be achieved by PWH on ART with complete HIV suppression compared to HIV-uninfected individuals (10). In addition, memory cellular immunity could be assessed after SARS-CoV-2 infection in some cohorts of PWH (9). A deeper understanding about this issue is fundamental to applying an optimal vaccination strategy to this population. Yet, an integrative analysis of the level and quality of antibody or T-cell response capacity against SARS-CoV-2 has not been tackled previously.

We aimed to study the immune landscape that occurs after COVID-19 in PWH. Therefore, antibody testing, phenotypic analysis of peripheral blood leukocytes, plasma cytokines assessment, and determination of memory responses against SARS-CoV-2 were performed in a cohort of PWH. Afterwards, we compared these observations with those seen in a cohort of HIV-negative (HIVneg) individuals convalescent from COVID-19. Our findings provide insights for understanding long-term SARS-CoV-2-specific T cell and humoral immunity in the context of HIV infection.

Methods

Individuals enrolled. This is a cross-sectional study. Between April and December 2020 peripheral blood samples from donors to the Argentinean Biobank of Infectious Diseases (BBEI) with confirmed COVID-19 diagnosis were collected at the convalescent stage: 29 PWH with preserved CD4+T-cell counts on ART and 29 HIV-negative individuals (HIVneg) were included. Written informed consents were obtained from

all participants. Research was conducted according to protocols approved by the institutional review board of Fundación Huésped, Argentina.

Sample processing: Blood samples were processed within 4 hours from withdrawal. Serum and plasma were aliquoted and stored at -80°C. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque density gradient centrifugation (GE Healthcare, Sweden). Cells were preserved in a solution of fetal bovine serum (FBS, Sigma-Aldrich, USA) supplemented with 10% DMSO (Sigma-Aldrich) and stored in liquid nitrogen until thawed the day of analysis.

Antibody assessment: SARS-CoV-2-specific IgG antibodies were evaluated in plasma samples from all donors by ELISA (COVIDAR kit, Laboratorio Lemos S.R.L., Argentina). Normalized optical density (NOD) values were calculated by subtracting the cut-off value to each donor sample OD value, and the resulting value was divided by the mean positive control OD value. Additionally, SARS-CoV-2-specific IgG was titrated by making 2-fold serial dilutions of plasma.

Virus neutralization: Vero-E6 cells were maintained in DMEM medium (Sigma-Aldrich) plus 2 mM L-glutamine (Sigma-Aldrich), 100 U/ml penicillin (Sigma-Aldrich), 100 µg/ml streptomycin (Sigma-Aldrich) and 10% FBS (Gibco BRL, USA). SARS-CoV-2 strain (lineage B.1, hCoV-19/Argentina/PAIS-G0001/2020, GISAID Accession ID: EPI_ISL_499083) was kindly provided by Dr. Sandra Gallego (InViV working group). Serial 2-fold dilutions of decomplemented plasma were incubated with 200 plaque-forming units (PFU) of SARS-CoV-2 for 1 h at room temperature, in triplicates. Then, mixtures were added to 80% confluent Vero-E6 cell monolayers in 96-well plates and incubated at 37°C for 1 h. Afterward, cells were washed and culture medium with 2% FBS was added. After 72 h, plates were fixed with 4% paraformaldehyde for 20 min at room temperature and stained using a 0.5% crystal violet dye solution in acetone and methanol. Neutralization titer was calculated as the inverse of the highest plasma dilution that showed an 80% cytopathic inhibitory effect.

Flow cytometry and CBA analysis. Recently thawed PBMCs were stained with the following fluorochrome-conjugated antibodies (Abs) for *ex vivo* study: CD3, CD4, CD8, CD25, CD127, CD196 (CCR6), CD185 (CXCR5), CD183 (CXCR3), CD57, PD1, CD27, CD45RA, CD62L, CD19, ICOS, HLA-DR, CD38, CD14, CD16, CD56, CD69, CD95, NKP46, NKP30 and NKG2D (all from BD Biosciences). Dead cells were excluded using the BD Horizon™ Fixable Viability Stain 510 from BD Biosciences. Negative control samples were incubated with irrelevant isotype-matched mAbs in parallel with experimental samples. Sample acquisition and analysis were carried out on a BD FACSAria™ Fusion Flow Cytometer using the BD FACSDiva software (BD Biosciences). Data were analyzed with FlowJo Software (Version 10, BD Biosciences). Anomalies in flow cytometry data were eliminated using FlowAI (FlowJo Software, BD Biosciences). Gating strategy is shown in Supplementary Figure 1A, B and C. Uniform Manifold Approximation and Projection (UMAP) (nonlinear, dimension reduction) analysis was performed (n = 26, 13 individuals in PWH and 13 individuals in HIVneg group) using FlowJo plugins. Additionally, several cytokine and chemokine concentrations from plasma samples were determined using the Th1/Th2/Th17

and Chemokine CBA kits (BD Biosciences) following the manufacturer's instructions and analyzed using the FCAP Array™ software Version 3.0 (BD Biosciences).

SARS-CoV-2 specific cellular responses. IFN-γ-secreting cells were detected using enzyme-linked immunospot (ELISpot) assays conducted as described previously (11). Briefly, PBMC were plated on sterile 96-well plates (MultiScreen IP plates; Millipore), coated with mouse anti-human IFN-γ monoclonal antibody (BD Biosciences) at $2x10^5$ cells/well. SARS-CoV-2 Spike, RBD, Nucleocapside proteins (kindly provided by Dr. A. Gamarnik, Leloir Institute, Buenos Aires, Argentina), Spike or Nucelocapside peptide pools (BEI Resources, NIAID, NIH. NR-52402, and NR-52404) were titrated beforehand and added in duplicate wells (final concentration 10μg/ml for proteins and 2μg/ml for peptide pools). Negative (medium plus 0.05% DMSO) and positive (PHA 10μg/ml Sigma-Aldrich) controls were included for each donor. Plates were developed using biotinylated anti-human IFN-γ monoclonal antibody, streptavidin-peroxidase complex, and AEC (3-amino-9-ethylcarbazole) substrate reagent set (BD Biosciences). Plates were scanned on an ImmunoSpot reader and specific spots were counted using the ImmunoSpot software (Cellular Technology Ltd.). Results were expressed as the ratio of Ag-specific spot-forming units (SFU)/10^6 PBMC to the negative-control values. Cut-off value was set at 25 SFU/10^6. In samples where negative control had a value of zero, a value of 12.5 SFU/10^6 was assigned for calculations. Additionally, when the negative control value was above 25 SFU/10^6, the cut-off was calculated as the media + 1 SD of negative replicate results.

Data analysis: Clinical, demographic and laboratory data associated with each donor was kept at the Noraybanks software database (Noraybio, Spain). Statistical analyses were performed using GraphPad Prism 8.4.3 (GraphPad Software). All tests were considered statistically significant when the *p* values were <0.05.

Results

PWH with conserved $CD4^+$ T cell counts mount robust antibody responses against SARS-CoV-2 without clinical worsening of COVID-19

We aimed to study the impact of HIV infection over the immune response against SARS-CoV-2 in COVID-19 convalescent individuals. The median age of PWH was 44 years (IQR: 34.7-53.5) and 22 out of 29 individuals (75.8%) were male. Median CD4+ T cell count was 513 cells/μL (IQR: 351-873). Otherwise, the median age of HIV-negative donors was 41 years (IQR: 35-51.5) and 11/29 (37.9%) were male. All individuals met the confirmed case definition of COVID-19 (positive for SARS-CoV-2 by RT-PCR). In both groups 23 out of 29 individuals showed a mild to moderate COVID-19 presentation, whereas the remaining individuals presented severe forms of the disease (defined by the presence of related complications such as pneumonia, hypoxemia or need of oxygen (2)). The mean time from symptoms onset to sampling was 69 days (IQR: 31-93) for PWH and 41 days (IQR: 18.5-55) for HIVneg. Regarding the available clinical data, comorbidities could be documented in 3 PWH (1 case of arterial hypertension -AHT- + diabetes mellitus + hypothyroidism, 1 case of AHT and 1 case of diabetes mellitus) and in 5

HIVneg donors (1 case of AHT+ hypothyroidism, 1 case of AHT + asthma, 1 case of AHT + pituitary adenoma, 1 case of thrombocytopenic purpura and 1 case of obesity). In addition, both groups displayed similar symptom patterns with no statistical differences (Chi square test with Yates correction, significance level: 0.05; not significant for any symptom analyzed, Figure 1A). We could observe that 65.5% of PWH and 79.3% of HIVneg individuals showed detectable SARS-CoV-2-specific antibodies (Figure 1B), with similar amounts of total IgG, IgG titers and anti-SARS-CoV-2 neutralizing antibodies between groups (Figure 1C, D and E). Finally, a thorough analysis was performed among PWH, revealing that neutralization capacity correlated with IgG titers (r:0.70, p<0.001) and NOD values (r:0.65, p<0.001). Additionally, IgG titers were associated with NOD values (r:0.87, p:0.0001) and CD8+ TL counts (r:0.82, p<0.001) (Figure 1F), as was also observed by us in HIVneg donors ((2) and Figure 7).

PWH display a distinctive immune profile of peripheral leucocytes' populations

We next aimed to investigate the impact of HIV infection over the cell populations involved in SARS-CoV-2 immune response. Therefore, the phenotype of T, B, NK cells and monocytes was investigated by flow cytometry in peripheral blood samples from the donors involved in the study. Gating strategy and population definitions developed during this study is depicted in Supplementary Figure 1. Diminished percentages of circulating antibody secreting cells (ASC) were observed among PWH compared to HIVneg, with similar B cell proportions between groups (Figure 2A and B). Regarding the phenotype of $CD4^+$ T cell populations, Th1 lymphocytes were expanded in PWH compared to HIVneg, whereas both groups presented similar TH17 and Th1* (CD3+/CD4+/CCR6+/CXCR3+) populations (Figure 2C). Although PWH displayed augmented percentages of Tfh $CD4^+$ cells, the proportion of CXCR3-expressing Tfh subset, a population involved in antibody responses against viral infections and vaccination (12), were similar between groups (Figure 2D and E). Nevertheless, when examining the phenotype of Tfh cells by UMAP high dimensional analysis, we observed differences between PWH and HIVneg in the reduction 2D representations (Figure 2F), mainly due to the upregulation of CD27 and CXCR3 expression in several Tfh clusters (Supplementary Figure 2). Importantly, we observed a negative correlation between IgG titers and Tfh proportions in the PWH group, therefore highlighting the importance of this population on the immune response against SARS-CoV-2 (Figure 2G).

The frequencies of Tregs were similar for both groups (Supp. Figure 3A). As regards the exhaustion/activation profile of CD4+ T lymphocytes, we observed augmented PD-1+ frequency but not of CD57 nor of PD-1/CD57 co-expression proportions within the PWH group, therefore showing an increased activation state, without enhanced exhaustion/senescence among these individuals (Figure 3A) (13). In addition, a slight but significant increase of CD4+ T_{CM} cells was detected, as depicted in Supplementary Figure 3B. Concerning the phenotype of CD8+ T cells during the convalescent phase of COVID-19, a significant increment in the proportion of HLA-DR+ CD8+ T lymphocytes in PWH compared to HIV-negative individuals was detected (Figure 3B). No differences were observed neither in the CD38+ proportion nor the exhaustion/senescent profile or CXCR5+ distributions of CD8+ T cells between groups, although regarding the memory/effector profile, a significant lesser proportion of CD8+ T_{TM} cells was detected (Supp. Figure 3C and D, and data not shown).

Continuing our analysis, similar proportions of NK cells were observed in both groups as determined by the expression of CD56 (Figure 4A). Augmented proportions of CD25, CD95, NKP46, HLA-DR and HLA-DR/CD38 co-expressing cells among the PWH group were detected, therefore indicating an enhanced activation/exhaustion of peripheral NK cells in these individuals (Figure 4B to E, respectively). Finally, increased percentages of CD14++CD16- classical peripheral monocytes in the PWH group were observed compared to HIVneg individuals, whereas there weren't any differences regarding the proportion of total CD14+, CD14+CD16^{bright} non-classical or intermediate CD14++CD16^{dim} monocytes between groups (Supplementary Figure 4), therefore showing minor differences on monocyte's distribution or abundance (14).

Plasma cytokines / chemokines are differentially modulated in convalescent COVID-19 PWH

Continuing our task on characterizing the immune profile during the COVID-19 convalescence phase, plasma concentrations of several cytokines and chemokines were determined by flow cytometry using a multiplex assay. A marked decrease of IL-8/CCL8 and increased levels of IP-10/CXCL10 were observed in PWH individuals compared to HIV-negative COVID-19 convalescents (Figure 5), whereas any differences were found on MCP-1/CCL2, MIG/CXCL9 or RANTES/CCL5 plasma concentrations between groups (data not shown). Moreover, statistically diminished levels of IFN-γ, TNF-α, IL-17A, IL-6 and IL-10 plasma concentrations were noticed in PWH compared to HIVneg COVID-19 convalescent individuals, as shown in Figure 5. Notably, IL-8/CCL8 couldn't be detected in 65.2% of the PWH tested, whereas in the HIV negative group was undetectable in 22.9% of individuals (p = 0,0005, *Chi* square test with Yates correction).

Cellular immune responses against SARS-CoV-2 are diminished in PWH

Advancing on our studies, we aimed to determine T cell responses against SARS-CoV-2 antigens and peptide pools, as it was described elsewhere (9, 15). Hence, PBMCs from each donor were stimulated in the presence of proteins or peptide pools encompassing both Spike and Nucleocapside proteins and afterwards the IFN-γ-producing cells were determined by ELISpot. Our data demonstrated an overall diminished response against SARS-CoV-2 antigens, specifically against Spike, RBD and Nucleocapside whole proteins in the PWH group, with no differences in T cell responses against Spike or Nucleocapside peptide pools (Figure 6A and B). These data show that although PWH presented lower T cell responses against SARS-CoV-2 compared to HIV negative donors, these cells were able to collaborate to mount robust humoral immune responses against the pathogen.

Analysis of associations of immune cell subsets, serological determinations, cytokine/chemokines assessments and Ag-specific cellular immune responses in PWH and HIV negative participants

Finally, to examine differences in the immune landscape between PWH and HIV-negative participant groups, associations between clinical, serological, cytokine/chemokine plasma levels, numerous immune cell populations and SARS-CoV-2-specific cellular immune response parameters were performed by Spearman correlation analysis. Results can be evinced in Figure 7 for statistical significances and

Supplementary Figure 5 for Spearman R values. All in all, we could observe distinctive patterns of associations when comparing both cohorts. Several correlations were statistically significant in PWH but not in HIVneg: i) On comparing serological parameters and Tfh frequency, a positive correlation between ASC and Tfh proportions and negative correlations between nAb titers and ASC/Tfh percentages were found; ii) Among CD4+ T cell activation/exhaustion/memory-effector markers, positive correlations between PD1 and PD1/CD57 with Treg proportions or between exhaustion markers and CD4+ T_{CM}, CD4+ $T_{TM,}$ CD4+ T_{EM}, and CD4+ T_{EMRA}, Th1, Th1* and Th17 percentages were discovered, as well as negative correlations between Treg and CD4+ T_{Naive} or Th2 cells; iii) Numerous cytokines/chemokines levels correlated with Ag-specific cellular immune responses, finding negative associations between CCL5 concentration and Spike protein responses, IL-6 and Spike peptide pool–specific IFN-γ producing cells, or TNFα/ IL-10 levels and RBD or Nucleocapsid protein responses; iv) Ag-specific immune cell responses correlated with the number of symptoms, B lymphocytes, several CD8+ TL and NK activated/exhausted populations, and lastly, v) different cytokines/chemokines levels were associated to ASC/Tfh frequencies (Figure 7 and Supp. Figure 5). We couldn't observe any significant correlation between the magnitude of the cellular immune responses against viral antigens and anti-SARS-CoV-2 IgG titers neither in PWH nor HIVneg donors. These differences reveal the overall impact of HIV infection over the memory response against SARS-CoV-2 after COVID-19 infection.

Discussion

We aimed to investigate the impact of HIV infection over the immune profile of lymphocyte populations in PWH recovered from COVID-19, as well as the anti- SARS-CoV-2 humoral and cellular responses. Both PWH and HIV negative individuals displayed similar frequency of symptoms, with asthenia, myalgia, and anosmia being the most frequent, reflecting the prevalence of mild COVID-19 in the study groups. Preserved CD4+ T cell counts in PWH was accompanied by mild symptoms, as it was also described by others (16).

When studying the antibody profile assessed as NOD, IgG titer and IgG neutralization capacity, similar levels in both PWH and HIV-negative convalescent COVID-19 individuals were observed. It indicates that adaptive humoral immunity finds its way to an effective response against SARS-CoV-2, even in an immune-compromised environment, as it was also demonstrated by others (10). It has been established that Tfh cells contribute with B cells in germinal center of secondary lymphoid organs to generate efficient neutralizing and non-neutralizing antibody responses in SARS-CoV-2 infection. As Tfh are key determinants of humoral responses, this subset should be taken into consideration in the design of effective vaccination regimens (17). We observed a negative correlation between SARS-CoV-2 specific-IgG titers and the proportion of Tfh cells in the PWH group (Figure 2G). It has been deeply described that Tfh cells are expanded and exhibit a dysfunctional state during HIV infection, and that these defects persist even when ART treatment succeeds in controlling viral replication (18, 19). Moreover, it has been shown that CXCR3-expressing Tfh lymphocytes (also named Th1-like skewed Tfh cells) are expanded and that this expansion could be driven by persistent HIV antigen expression (20). We additionally observed a

central memory phenotype (as shown by an up-regulated CD27 expression) in Tfh cells from PWH, which paralleled with increased CD4+ T_{CM} lymphocytes within this group (shown in Supplementary Figure 3B). Therefore, we hypothesize that these differences in Tfh proportions and phenotype might be a preexisting trait, due to chronic HIV infection. Finally, although the proportion of total B lymphocytes were similar among groups, we detected reduced ASC proportions in the PWH group. It has been described that during COVID-19 infection, ASC were elevated in individuals with detectable SARS-CoV-2 compared to those that have cleared the virus, being this association stronger in the HIV-negative group (21). Moreover, prior to recovery, a case report from an HIV-negative individual with mild-to-moderate COVID-19 showed that ASC appeared in the blood at the time of viral clearance and peaked on day 8, then finally diminished (22). These data are in agreement with our results showing a diminished proportion of ASC in convalescent PWH. We postulate that a smaller amount of ASC could have been taken place during acute SARS-CoV-2 infection due to the immune disturbances related to chronic HIV infection. Our observations, in addition to previous data published by others, suggest that HIV infection would exert a negative effect on the subsequently antibody secretion to newly recognized antigens, as those from SARS-CoV-2. Contrary to expectations, we could not detect any differences in the antibody secretion's magnitude and the viral neutralizing capacity when we compared humoral immune responses from both PWH and HIV negative individuals. It indicates that Tfh are efficient in driving humoral responses against newly recognized pathogens, even in a chronic HIV-compromised environment.

Systemic CD4+ T cell activation is a hallmark for HIV chronic infection (23). As a whole, we observed slight differences in activation markers within PWH when comparing with HIV-negative persons. These similarities in CD4TL status between groups could be interpreted as a long and sustained cell activation in our HIVneg cohort that could lead to persistent clinical symptoms triggered during the acute phase COVID-19, as described previously (24).

Both CD8+ T cells and NK cells are key at controlling viral infections, including those carried on by SARS-CoV-2 (25, 26). Also, both cell types are affected during HIV infection, lowering their cytotoxic and cytokine-producing functions, therefore affecting anti-viral immunity (27, 28). We detected a marked NK and a less evident CD8+ TL activation state in PWH with respect to convalescent COVID-19 HIVneg individuals. These observations are in agreement with previous reports, showing an increment of activated but not exhausted CD8+ T cells in HIV+ viremic individuals compared to healthy donors in the COVID-19 convalescent state (29). Regarding NK cells, Antinori and coworkers have shown highly activated NK cells with augmented NKG2A and NKG2C proportions in convalescent COVID-19 HIV+ individuals (30), therefore reinforcing the idea of increased activation/exhaustion in HIV/SARS-CoV-2 coinfected patients. Our data suggest that enhanced NK activation might offset the failure in adaptive immunity derived from HIV infection.

It has been extensively shown that a cytokine storm takes place during acute COVID-19 infection, which is responsible for severe disease (7). These elevated pro-inflammatory cytokines are produced by monocytes, lymphocytes, and other cell types, as well as endothelial cells (7, 31). In most immune-competent individuals, after SARS-CoV-2 infection is cleared, cytokines and chemokines levels return to

normality (31). On the contrary, HIV infection imposes a different scenario, where chronic inflammation constitutes a distinctive trait that leads to excess risk of non-AIDS events (32). In our cohort, convalescent COVID-19 PWH presented significant diminished levels of IL-8, IFN-γ, TNF-α, IL-17A and IL-6, therefore suggesting a reduced pro-inflammatory capacity in both the monocyte/macrophage and the T lymphocyte compartments, which would correlate with a milder disease severity in COVID-19/HIV coinfection, also seen by others (31).

IL-6 and TNF-α are potent macrophage-induced cytokines with pro-inflammatory effects, partially responsible for the cytokine storm these patients experience (31). These cytokines were significantly diminished in PWH individuals compared to HIVneg donors, paralleling a mild to moderate COVID-19. As expected for chronic and virologically controlled HIV infection, IP-10 (the ligand of CXCR3) levels were higher in this group compared to HIV-negative donors (33), showing a distinctive kinetics pattern for this chemokine, as seen previously for both HIV and COVID-19 (31, 33). Finally, the T-cell derived pro-inflammatory cytokines IFN-γ and IL-17 and the regulatory IL-10 were diminished in plasma from COVID-19 convalescent PWH group, therefore displaying the disturbances provoked by HIV infection in the T cell compartment, as it was described (19, 34). IL-8, TNF-α and IL-6 constitute an inflammatory cytokine signature of COVID-19, but also a bridge to the subsequent adaptive immune response (35). Therefore, we hypothesize that the lower levels of these cytokines observed in the PWH cohort would result in a weaker cellular immune response against the virus. Moreover, the observed persistence of pro-inflammatory cytokines in the HIV negative cohort might be linked to the development of long COVID-19 symptoms, although until now there is no data available on this topic.

It is known that a Th1-skewed profile arises after SARS-CoV-2 infection, as was extensively demonstrated (7, 31, 36, 37). Our results showed a measurable IFN-γ response after stimulation of PBMC from all PWH tested in the presence of viral antigens. Notably, all the individuals tested responded to at least one antigen, independently of their serological status. However, we could not detect any correlation between the magnitude of cellular responses with any serological parameter tested. Otherwise, PWH presented lower levels of cellular response against viral proteins when compared to HIVneg, therefore showing some degree of anomalous response. These observations point out the imbalance elicited by HIV infection, also demonstrated by lower levels of plasma cytokines in convalescent PWH. Additional work will be required to assess potential T cell cross-reactivity against other human coronaviruses, as was reported in other studies (36–39).

One limitation of this study is that we did not perform an in-depth analysis of B cell-antigen specific responses. Further research, such as the analysis of fluorochrome-coupled Spike-specific B cells' frequency and phenotype, could add insights into the SARS-CoV-2 specific memory immune response. Another limitation we can evince is that the data presented along this work was derived from cryopreserved samples, which may underestimate the magnitude of the assessed T cell responses (40). Finally, the lack of a control group of PWH ART-naïve didn't allow us to fully elucidate the role of low CD4+ T cell counts over the immune response and clinical outcome of SARS CoV-2 infection.

Conclusions

HIV infection without treatment has been proposed as serious comorbidity of COVID-19, but with proper ART overall immunity against SARS-CoV-2 is not affected in these individuals (16). Since COVID-19 infection outcomes were similar in PWH compared with HIV negative participants, the differences in cellular immune responses and cell phenotypes between both groups may suggest an alternative immunity to SARS-CoV-2 in PWH instead of a dysregulated one. Our data support the landscape of increased activation/exhaustion, reduced cellular responses and plasma cytokines concurrent with effective antibodies responses against SARS CoV-2, reinforcing the idea of a significant impact of ART not only in HIV control but in the capacity of restrict other infections.

Declarations

Ethics approval and consent to participate

The study was carried out in line with the World Medical Association Declaration of Helsinki and approved by the Ethics Committee of Fundación Huésped, Buenos Aires, Argentina. Written informed consent was provided by all the participants.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

NL and MFQ conceived and designed experiments; DG, MBV, NL and MFQ analyzed and interpreted the data and wrote the manuscript. DG, MBV, AC, MLP and LC processed samples and performed experiments. SB, BWG, NL and YL recruited donors, collected samples and obtained clinical data. YL performed serological studies. VGP performed and analyzed flow cytometry data. GT, NL and YG

contributed reagents/materials and analyzed and interpreted the data. All authors contributed to the refinement of the report and approved the final manuscript.

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References

1. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497-506.
2. Longueira Y, Polo ML, InVi Vwg, Biobanco de Enfermedades Infecciosas Coleccion Cwg, Turk G, Laufer N. Dynamics of SARS-CoV-2-specific antibodies among COVID19 biobank donors in Argentina. Heliyon. 2021;7(10):e08140.
3. Apicella M, Campopiano MC, Mantuano M, Mazoni L, Coppelli A, Del Prato S. COVID-19 in people with diabetes: understanding the reasons for worse outcomes. Lancet Diabetes Endocrinol. 2020;8(9):782-92.
4. SeyedAlinaghi S, Karimi A, MohsseniPour M, Barzegary A, Mirghaderi SP, Fakhfouri A, et al. The clinical outcomes of COVID-19 in HIV-positive patients: A systematic review of current evidence. Immun Inflamm Dis. 2021.
5. Oral abstracts of the 11th IAS Conference on HIV Science, 18-21 July 2021. J Int AIDS Soc. 2021;24 Suppl 4:e25755.
6. Brown LB, Spinelli MA, Gandhi M. The interplay between HIV and COVID-19: summary of the data and responses to date. Curr Opin HIV AIDS. 2021;16(1):63-73.
7. Mazzoni A, Salvati L, Maggi L, Annunziato F, Cosmi L. Hallmarks of immune response in COVID-19: Exploring dysregulation and exhaustion. Semin Immunol. 2021:101508.
8. Liu Y, Xiao Y, Wu S, Marley G, Ming F, Wang X, et al. People living with HIV easily lose their immune response to SARS-CoV-2: result from a cohort of COVID-19 cases in Wuhan, China. BMC Infect Dis. 2021;21(1):1029.
9. Alrubayyi A, Gea-Mallorqui E, Touizer E, Hameiri-Bowen D, Kopycinski J, Charlton B, et al. Characterization of humoral and SARS-CoV-2 specific T cell responses in people living with HIV. bioRxiv. 2021.
10. Snyman J, Hwa SH, Krause R, Muema D, Reddy T, Ganga Y, et al. Similar antibody responses against SARS-CoV-2 in HIV uninfected and infected individuals on antiretroviral therapy during the first South African infection wave. Clin Infect Dis. 2021.
11. Quiroga MF, Angerami MT, Santucci N, Ameri D, Francos JL, Wallach J, et al. Dynamics of adrenal steroids are related to variations in Th1 and Treg populations during Mycobacterium tuberculosis

infection in HIV positive persons. PloS one. 2012;7(3):e33061.

12. Zhang J, Liu W, Wen B, Xie T, Tang P, Hu Y, et al. Circulating CXCR3(+) Tfh cells positively correlate with neutralizing antibody responses in HCV-infected patients. Sci Rep. 2019;9(1):10090.
13. De Biasi S, Meschiari M, Gibellini L, Bellinazzi C, Borella R, Fidanza L, et al. Marked T cell activation, senescence, exhaustion and skewing towards TH17 in patients with COVID-19 pneumonia. Nat Commun. 2020;11(1):3434.
14. van den Bosch TP, Caliskan K, Kraaij MD, Constantinescu AA, Manintveld OC, Leenen PJ, et al. CD16+ Monocytes and Skewed Macrophage Polarization toward M2 Type Hallmark Heart Transplant Acute Cellular Rejection. Front Immunol. 2017;8:346.
15. Ni L, Ye F, Cheng ML, Feng Y, Deng YQ, Zhao H, et al. Detection of SARS-CoV-2-Specific Humoral and Cellular Immunity in COVID-19 Convalescent Individuals. Immunity. 2020;52(6):971-7 e3.
16. Sharov KS. HIV/SARS-CoV-2 co-infection: T cell profile, cytokine dynamics and role of exhausted lymphocytes. Int J Infect Dis. 2021;102:163-9.
17. Boppana S, Qin K, Files JK, Russell RM, Stoltz R, Bibollet-Ruche F, et al. SARS-CoV-2-specific circulating T follicular helper cells correlate with neutralizing antibodies and increase during early convalescence. PLoS Pathog. 2021;17(7):e1009761.
18. Lindqvist M, van Lunzen J, Soghoian DZ, Kuhl BD, Ranasinghe S, Kranias G, et al. Expansion of HIV-specific T follicular helper cells in chronic HIV infection. J Clin Invest. 2012;122(9):3271-80.
19. Phetsouphanh C, Xu Y, Zaunders J. CD4 T Cells Mediate Both Positive and Negative Regulation of the Immune Response to HIV Infection: Complex Role of T Follicular Helper Cells and Regulatory T Cells in Pathogenesis. Front Immunol. 2014;5:681.
20. Niessl J, Baxter AE, Morou A, Brunet-Ratnasingham E, Sannier G, Gendron-Lepage G, et al. Persistent expansion and Th1-like skewing of HIV-specific circulating T follicular helper cells during antiretroviral therapy. EBioMedicine. 2020;54:102727.
21. Karim F, Gazy I, Cele S, Zungu Y, Krause R, Bernstein M, et al. HIV status alters disease severity and immune cell responses in variant SARS-CoV-2 infection wave. 2021.
22. Thevarajan I, Nguyen THO, Koutsakos M, Druce J, Caly L, van de Sandt CE, et al. Breadth of concomitant immune responses prior to patient recovery: a case report of non-severe COVID-19. Nat Med. 2020;26(4):453-5.
23. Sedaghat AR, German J, Teslovich TM, Cofrancesco J, Jr., Jie CC, Talbot CC, Jr., et al. Chronic CD4+ T-cell activation and depletion in human immunodeficiency virus type 1 infection: type I interferon-mediated disruption of T-cell dynamics. J Virol. 2008;82(4):1870-83.
24. Townsend L, Dyer AH, Naughton A, Kiersey R, Holden D, Gardiner M, et al. Longitudinal Analysis of COVID-19 Patients Shows Age-Associated T Cell Changes Independent of Ongoing Ill-Health. Front Immunol. 2021;12:676932.
25. Peng Y, Mentzer AJ, Liu G, Yao X, Yin Z, Dong D, et al. Broad and strong memory CD4(+) and CD8(+) T cells induced by SARS-CoV-2 in UK convalescent individuals following COVID-19. Nat Immunol. 2020;21(11):1336-45.

26. Antonioli L, Fornai M, Pellegrini C, Blandizzi C. NKG2A and COVID-19: another brick in the wall. Cell Mol Immunol. 2020;17(6):672-4.
27. Perdomo-Celis F, Taborda NA, Rugeles MT. CD8(+) T-Cell Response to HIV Infection in the Era of Antiretroviral Therapy. Front Immunol. 2019;10:1896.
28. Mikulak J, Oriolo F, Zaghi E, Di Vito C, Mavilio D. Natural killer cells in HIV-1 infection and therapy. AIDS. 2017;31(17):2317-30.
29. Karim F, Gazy I, Cele S, Zungu Y, Krause R, Bernstein M, et al. HIV status alters disease severity and immune cell responses in beta variant SARS-CoV-2 infection wave. Elife. 2021;10.
30. Mondi A, Cimini E, Colavita F, Cicalini S, Pinnetti C, Matusali G, et al. COVID-19 in people living with HIV: Clinical implications of dynamics of the immune response to SARS-CoV-2. J Med Virol. 2021;93(3):1796-804.
31. Azkur AK, Akdis M, Azkur D, Sokolowska M, van de Veen W, Bruggen MC, et al. Immune response to SARS-CoV-2 and mechanisms of immunopathological changes in COVID-19. Allergy. 2020;75(7):1564-81.
32. Deeks SG, Tracy R, Douek DC. Systemic effects of inflammation on health during chronic HIV infection. Immunity. 2013;39(4):633-45.
33. Lei J, Yin X, Shang H, Jiang Y. IP-10 is highly involved in HIV infection. Cytokine. 2019;115:97-103.
34. Van der Sluis RM, Kumar NA, Pascoe RD, Zerbato JM, Evans VA, Dantanarayana AI, et al. Combination Immune Checkpoint Blockade to Reverse HIV Latency. J Immunol. 2020;204(5):1242-54.
35. Buszko M, Nita-Lazar A, Park JH, Schwartzberg PL, Verthelyi D, Young HA, et al. Lessons learned: new insights on the role of cytokines in COVID-19. Nat Immunol. 2021;22(4):404-11.
36. Braun J, Loyal L, Frentsch M, Wendisch D, Georg P, Kurth F, et al. SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19. Nature. 2020;587(7833):270-4.
37. Le Bert N, Tan AT, Kunasegaran K, Tham CYL, Hafezi M, Chia A, et al. SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. Nature. 2020;584(7821):457-62.
38. Sekine T, Perez-Potti A, Rivera-Ballesteros O, Stralin K, Gorin JB, Olsson A, et al. Robust T Cell Immunity in Convalescent Individuals with Asymptomatic or Mild COVID-19. Cell. 2020;183(1):158-68 e14.
39. Grifoni A, Weiskopf D, Ramirez SI, Mateus J, Dan JM, Moderbacher CR, et al. Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. Cell. 2020;181(7):1489-501 e15.
40. Owen RE, Sinclair E, Emu B, Heitman JW, Hirschkorn DF, Epling CL, et al. Loss of T cell responses following long-term cryopreservation. J Immunol Methods. 2007;326(1-2):93-115.

Figures

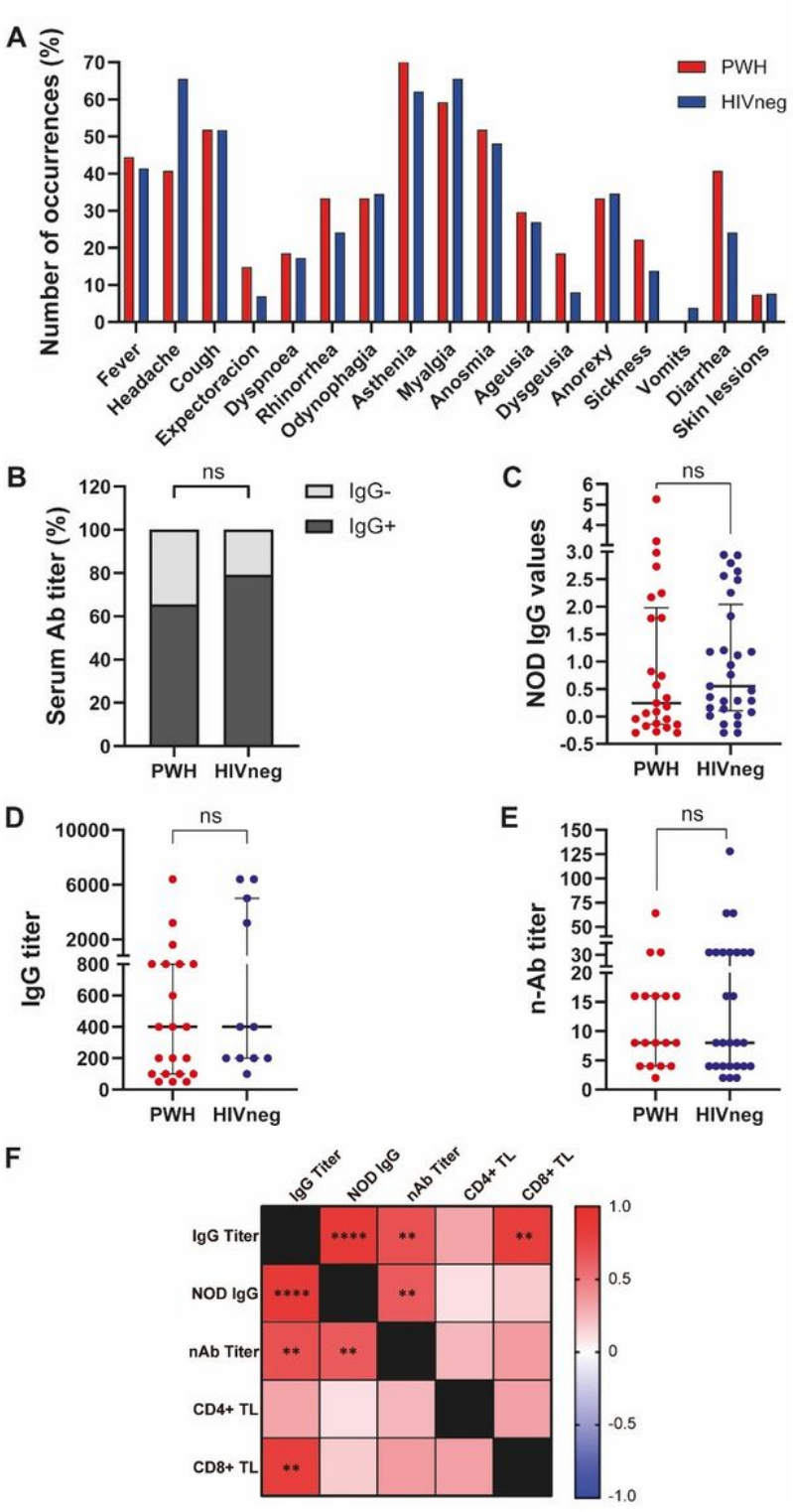

Figure 1

Clinical manifestations and anti-SARS-CoV-2 antibody responses in PWH and HIVneg individuals. (**A**) Frequency of occurrence of the depicted symptoms in PWH (red) and HIVneg (blue) cohorts. (**B**) Proportion of measurable IgG responses in PWH and HIVneg participants. Chi Squared test, ns. (**C**) IgG NOD values, (**D**) IgG titers, and (**E**) neutralizing Anti-SARS-CoV-2 antibodies were determined in plasma from PWH and HIVneg individuals as described in the *Materials and Methods* section. Mann-Whitney test

was used. $p < 0.05$ were considered significant. Data are expressed as median and interquartile range. (**F**) Heatmap depicting from red (+1) to blue (-1) Spearman Rank correlation values between each parameter. p values per correlation are shown in those boxes where statistics were significant. $^{**}p < 0.01$; $^{****}p < 0.0001$.

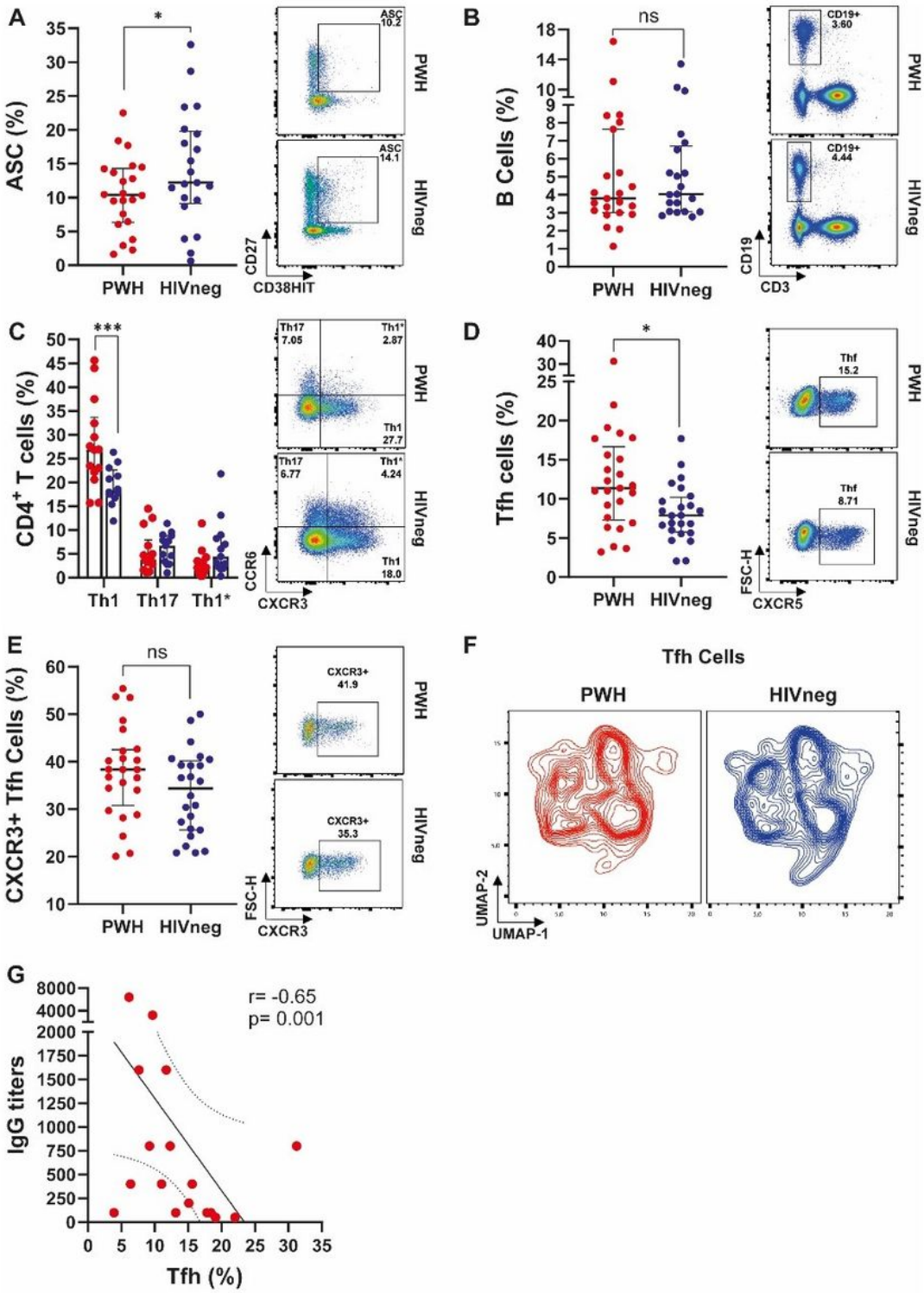

Figure 2

Analysis of antibody-secreting cells and CD4+ T-lymphocytes' phenotype in PWH and HIVneg donors. Frequency of (**A**) Analysis of antibody-secreting cells (ASC), (**B**) B lymphocytes, (**C**) CD4+ Th1, Th17 and Th1* (Th1/Th17) subpopulations, (**D**) Tfh, and (**E**) CXCR3+ Tfh cells in SARS-CoV-2 convalescent PWH (n =25) and HIV-negative (n =24) individuals via traditional gating, as showed in Supplementary Figure 1. Each dot represents an individual donor. Data are expressed as median and interquartile range. Representative flow cytometry graphs depicting each analyzed marker in PWH (upper panels) and HIVneg (lower panels) donors are shown. Significance was determined by two-tailed Mann–Whitney *U* test, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. (**F**) Profile of Tfh cell markers using Uniform manifold approximation and projection (UMAP) analysis between PWH (red) and HIVneg individuals (blue). (**G**) Spearman test (two-tailed) demonstrated a negative correlation between IgG titters and frequency of Tfh in PWH.

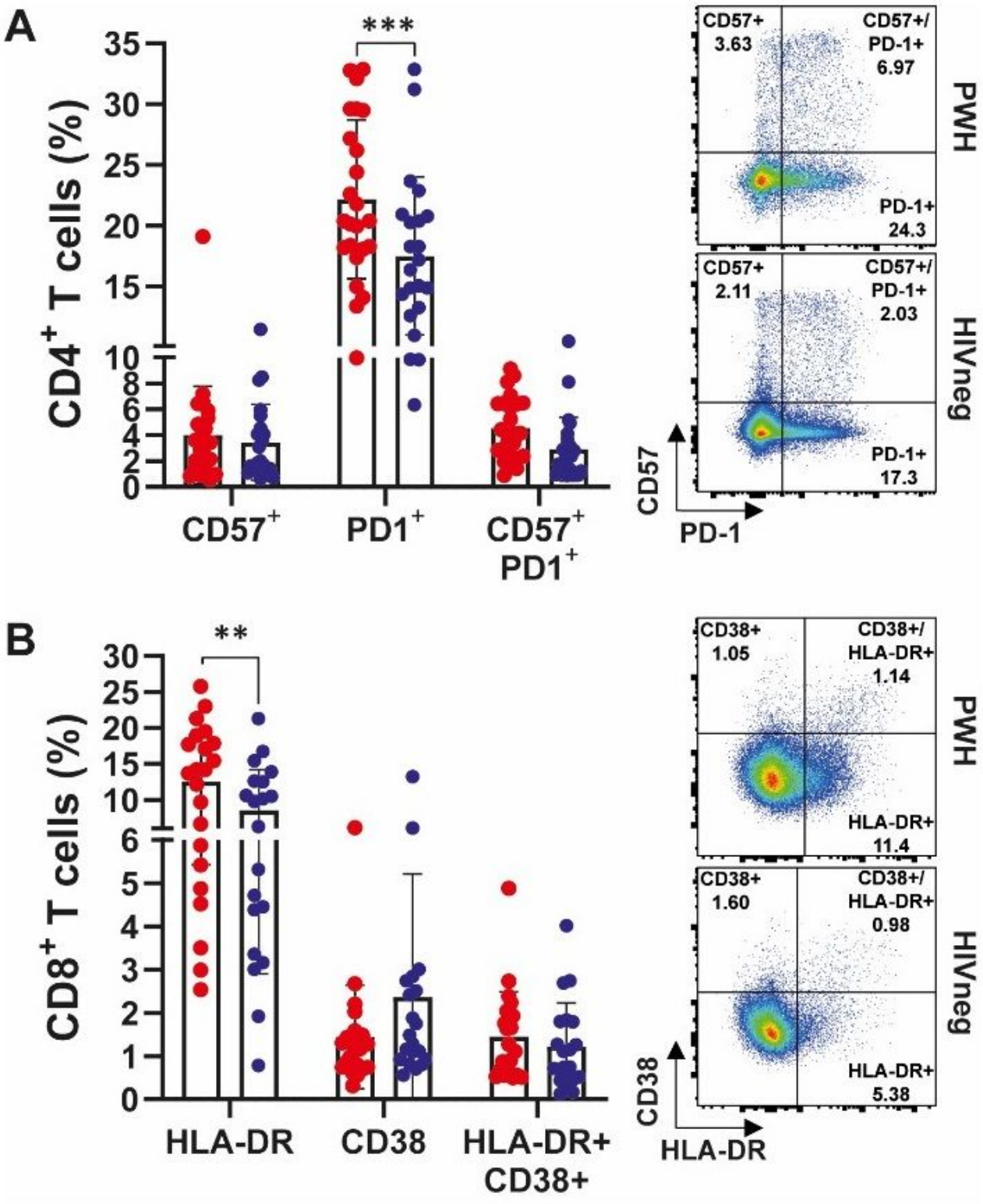

Figure 3

Study of activation/memory markers on CD4+ and CD8+ T cells from PWH and HIVneg donors.
(**A**) Frequency of CD4+ T lymphocytes expressing CD57, PD-1 or CD57/PD-1. (**B**) Proportion of CD8+ T lymphocytes expressing HLA-DR, CD38 or both. Markers were determined via traditional gating as depicted in Supplementary Figure 1 in SARS-CoV-2 convalescent PWH (n =25, red dots) and HIV-negative (n =24, blue dots) individuals. Each dot represents an individual donor. Data are expressed as median and

interquartile range. Representative flow cytometry graphs depicting each analyzed marker in PWH (upper panels) and HIVneg (lower panels) donors are shown. $^{**}p < 0.01$, $^{***}p < 0.001$. Significance was determined by two-tailed Mann–Whitney *U* test.

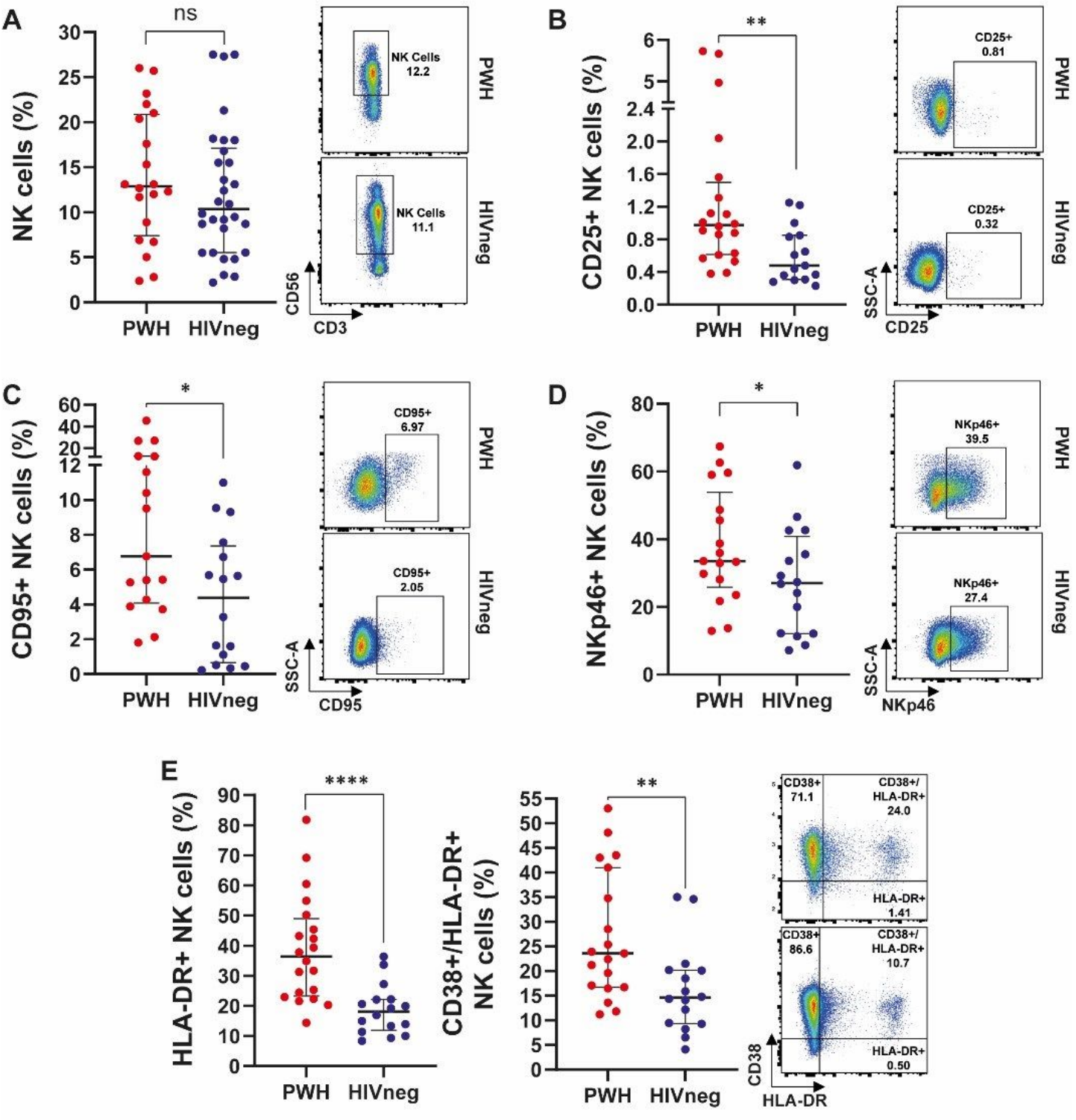

Figure 4

Analysis of peripheral NK cells' phenotype in PWH and HIVneg donors. Frequency of (**A**) total NK cells; **(B)** CD25+ NK cells; **(C)** CD95+ NK cells; **(D)** NKp46+ NK cells; **(E)** HLA-DR+ (left) and HLA-DR+/CD38+ co-expressing (right) NK cells in SARS-CoV-2 convalescent PWH (n =20) and HIV-negative (n =29) individuals via traditional gating, as showed in Supplementary Figure 1. Each dot represents an individual donor. Data are expressed as median and interquartile range. Representative flow cytometry graphs depicting each analyzed marker in PWH (upper panels) and HIVneg (lower panels) donors are shown. Significance was determined by two-tailed Mann–Whitney *U* test, *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$.

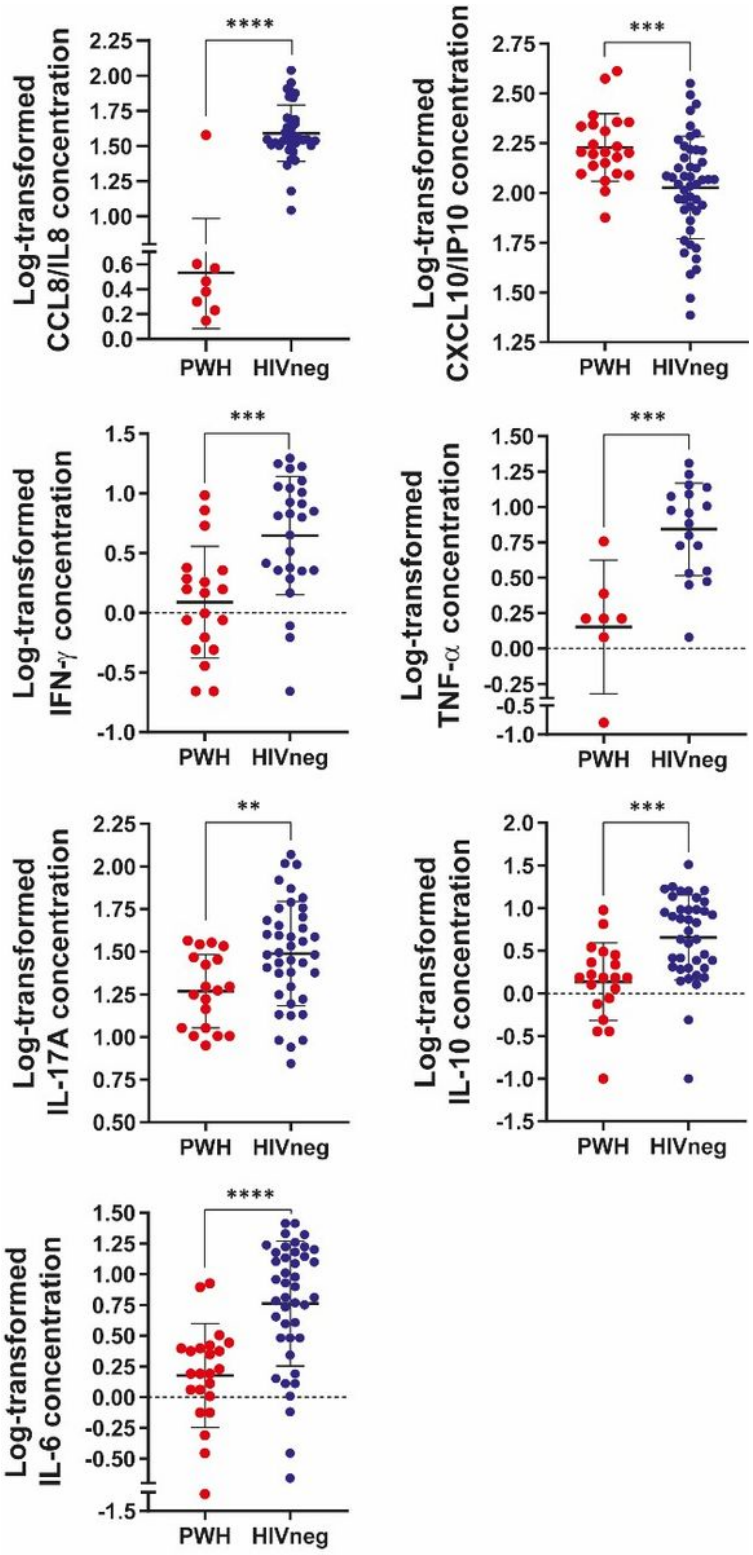

Figure 5

Assessment of serum cytokines and chemokines in PWH and HIVneg donors. The concentrations of CCL8/IL8; CXCL10/IP10; IFN-γ; TNF-α; IL-17A IL-10 and IL-6 were determined by a multiplex assay and flow cytometry. Data are depicted as the log-transformed concentration values (pg/mL). Each point represents an individual donor. Data are expressed as median and interquartile range. Significance was determined by two-tailed Mann–Whitney *U* test, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

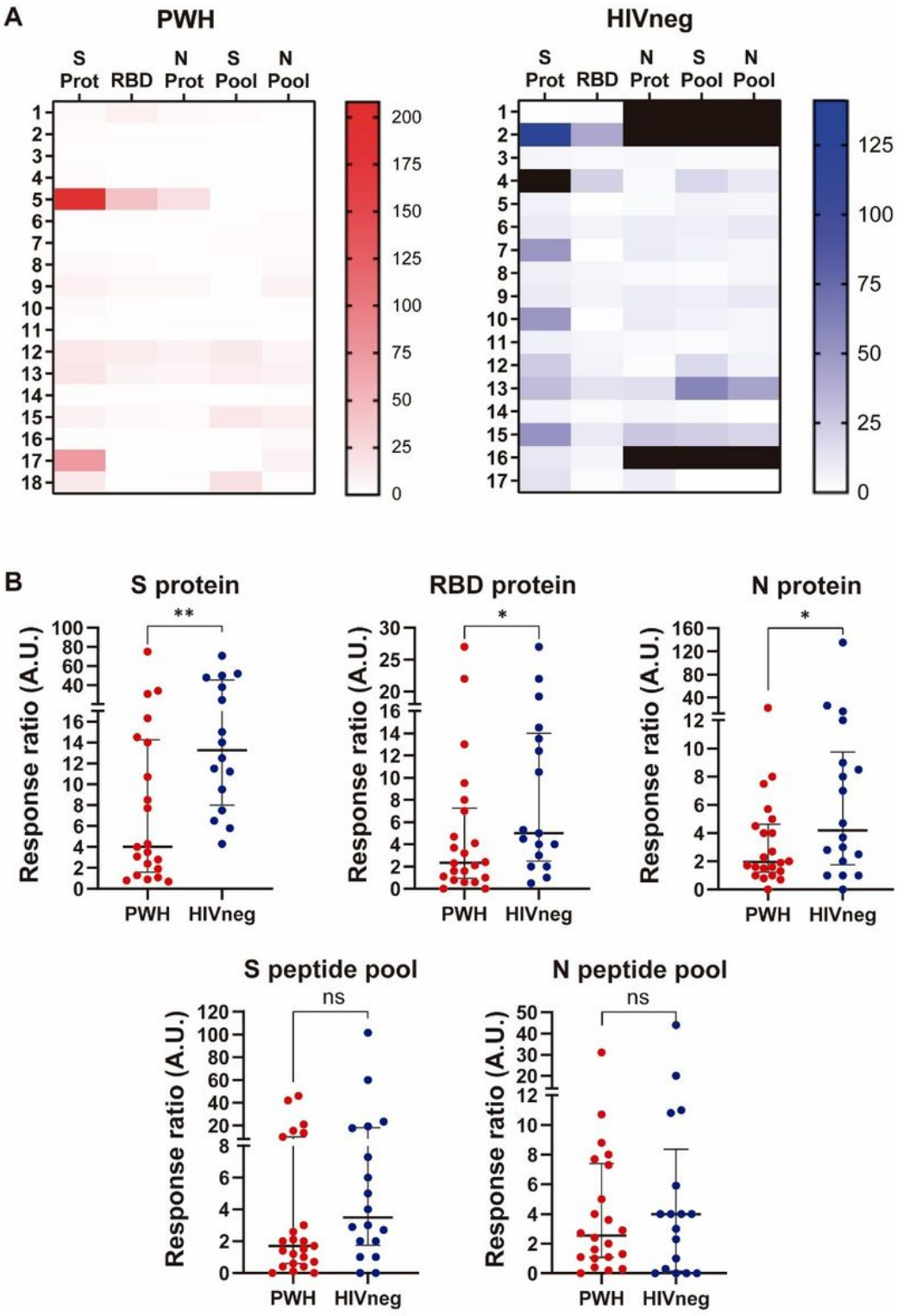

Figure 6

Cellular immune response after *in vitro* stimulation of PBMCs from PWH and HIVneg with SARS-CoV-2 protein antigens and peptide pools. IFN-γ ELISpot assays were performed to determine the frequency of Ag-experienced T cells in peripheral blood from the individuals enrolled. Stimulation of BPMCs with Spike (S) protein, RBD protein, Nucleocapside (N) protein or a peptide pool encompassing S protein or N protein was performed. Afterwerads, IFN-γ producing cells were determinined as illustrated in the *Materials and*

Methods section. (**A**) Heatmap depicting the relative-to-media magnitude of the IFN-γ ELISpot responses to the different SARS-CoV-2 proteins or peptide pools in PWH and HIVneg participant. Each row represent an individual. (**B**) In order to compare group differences, data were normalized to media levels. Each dot represents an individual donor. Data are expressed as median and interquartile range. Significance was determined by two-tailed Mann−Whitney U test, *$p < 0.05$; **$p < 0.01$. A.U.: arbitrary units.

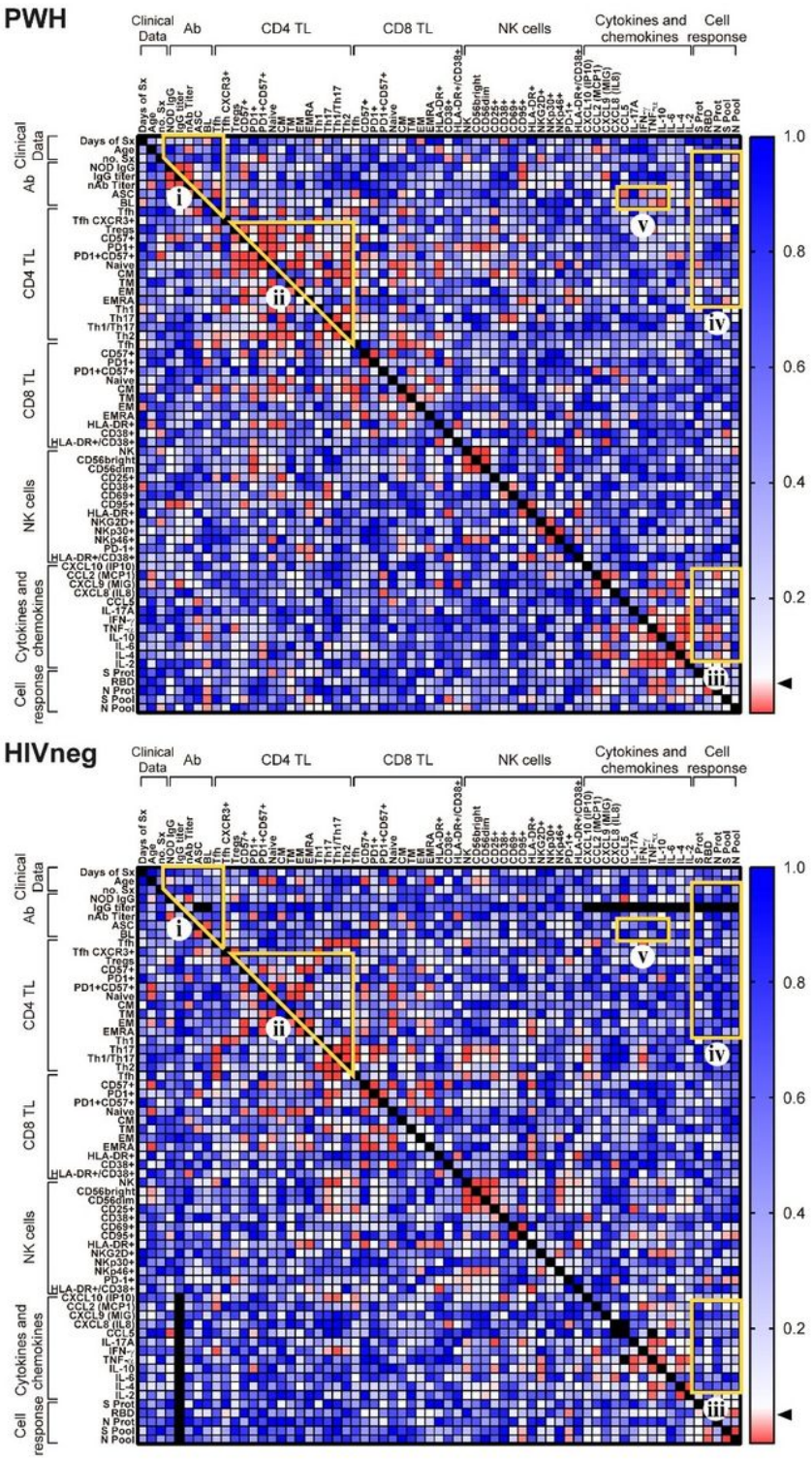

Figure 7

Immune parameters and clinical correlates in PWH and HIVneg donors. Statistically significant Spearman rank correlation values (*p*) are shown from red (0) to blue (+1.0). Arrow shows *p* = 0.05 value. *R*-values per correlation are shown in Supplementary Figure 4. Demarcated regions (yellow line) indicate a set of correlations that differ between PWH and HIVneg participant group. The regions with statistically significant correlations were denominated i, ii, iii, iv and v for a better analysis. Square brackets encompass clinical, serological, phenotypic, cytokine/chemokines' levels or cellular response parameters.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigures.docx