# ALTERATIONS IN B LYMPHOCYTE SUBSETS IN VIROLOGICALLY SUPPRESSED HIV-POSITIVE INDIVIDUALS DID NOT IMPACT 17DD YELLOW FEVER VACCINE IMMUNOGENICITY – ANRS 12403


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## BACKGROUND

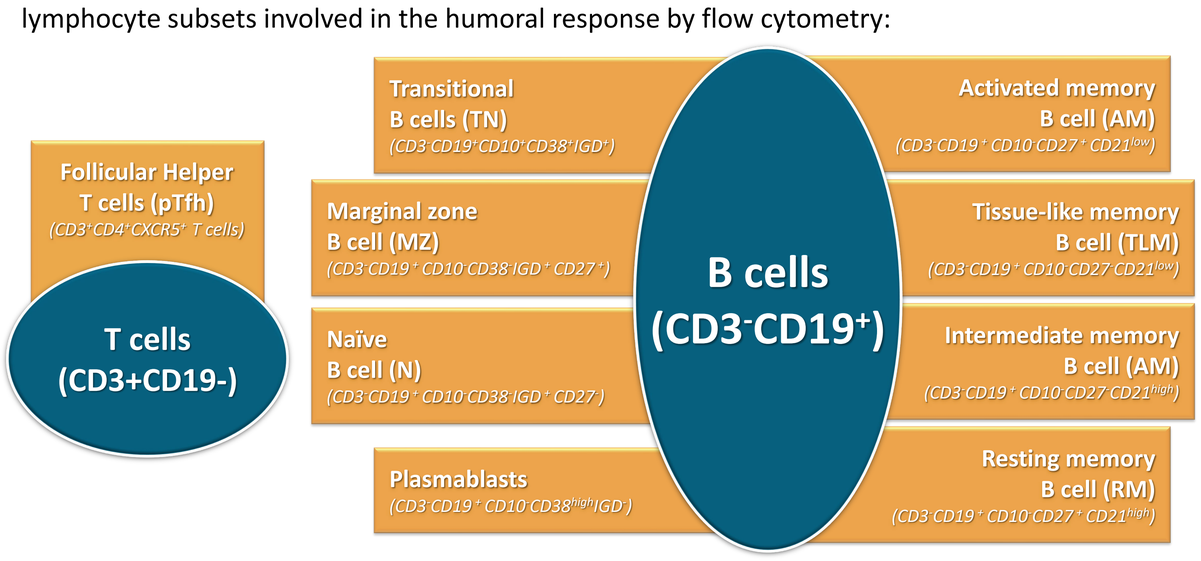
The HIV-1 infection is characterized by generalized deregulation in the immune system, resulting in altered dynamics of several components of cellular and humoral immune responses. Among the cellular subsets, B lymphocytes and T follicular helper cells stand out since their dynamics are crucial for the immune response against pathogens and vaccinal response. In addition, studies have demonstrated lower immunogenicity for some vaccines in people living with HIV (PLHIV).

The Yellow-fever vaccine 17DD (YFV-17DD) was recommended in Brazil only for PLHIV residents in areas with the circulation of yellow fever virus and with T CD4$^+$ cells counts >200 cells/mm$^3$. However, due to recent outbreaks, the Ministry of Healthy expanded the regions with YF vaccination recommendation, including Rio de Janeiro and São Paulo states, resulting in an increasing number of PLHIV with vaccine indication. Considering the paucity and limitation of previous studies with YFV PLHIV vaccinees, more research is necessary to evaluate the impact of HIV infection on YF vaccine immunogenicity.

## METHODS

The following results are part of a substudy developed in the context of a non-randomized phase IV clinical trial for evaluation of immunogenicity and safety of YFV in PLHIV conducted at the Evandro Chagas National Institute of Infectious Diseases (INI-FIOCRUZ), Rio de Janeiro, Brazil. We enrolled two groups of virologically suppressed PLHIV on antiretroviral treatment (ART)– one with T CD4 counts higher than 500 cells/mm$^3$ (CD4>500; n=20) and another with T CD4 values between 250-500 cells/mm$^3$ (CD4<=500; n=26) – and a group of HIV negative individuals (HIVneg; n=18).

For all participants, biological samples were collected at vaccination's day (D0) and on days 30 and 365 postvaccination (D30 and D365). Peripheral blood mononuclear cells (PBMCs) from D0 were stained with labeled monoclonal antibodies to evaluate the frequency of the following lymphocyte subsets involved in the humoral response by flow cytometry:




YFV-17DD immunogenicity was evaluated by neutralization levels measurement by micro plaque reduction neutralization test with horseradish peroxidase ($\mu PRN_{50}$-HRP) on D30 and D365.

## RESULTS

Clinical and demographic characteristics of the studied groups are shown in Table 1. As expected, changes in T cell populations were observed in individuals having CD4<=500, but no differences in age and sex were observed between groups.

Analyses of peripheral T follicular helper cells revealed similar frequencies of these cells in all evaluated groups (Figure 1). For B lymphocyte subsets analyses, the group CD4<500 presented lower frequencies of MZ B cells (p=0.006) and higher frequencies of N B cells (p<0.034) in comparison to HIVneg (Figure 1), while the frequencies of the TN B subset did not differ between groups. Regarding B memory subsets, both HIV groups presented lower frequencies of AM (p<0.014 for CD4>500; p<0.039 for CD4<500) and RM (p<0.005 for CD4>500; p<0.0001 for CD4<500) B cells, but no differences were observed for TLM and IM B cells subsets.

Neutralizing antibody levels at D30 and D365 postvaccination did not differ between the three evaluated groups (Figure 2A and B). Spearman analyses (Figure 2C) showed weak correlations between D0 and D30 T CD4 counts with MZ (Rho ≈ 0.38) and RM (Rho ≈ 0.5) B cells. YFV neutralization titers did not correlate to any of the subsets evaluated.

## CONCLUSIONS

Our data showed that B lymphocytes immune response is altered in HIV-infected individuals despite suppressed viral load and high T CD4 cells counts. Those individuals present disbalance in N, MZ, AM, and RM B cells frequencies when compared to HIV-uninfected individuals. Despite that, YFV-17DD neutralization titers were similar between HIV-infected and uninfected individuals and did not correlate with cell subsets, indicating that B cell responses against YFV-17DD one year after vaccination is not impaired in treated HIV-infected individuals.


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**Table 1. Clinical and demographic characteristics of the studied groups**

| | HIVneg | CD4>500 | CD4<500 | P-value |
|---|---|---|---|---|
| **Age [median (IQR)]** | 40,5 (31 - 49) | 41,5 (35 - 46) | 40,5 (28 - 50) | ns |
| **Sex** | | | | |
| - Male [n (%)] | 8 (44,4%) | 14 (70%) | 20 (76,9%) | ns |
| - Female [n (%)] | 10 (55,6%) | 6 (30%) | 6 (23,1%) | ns |
| **T CD4+ counts (cells/mm$^3$)** | | | | |
| - D0 [median (IQR)] | 1232 (1029 - 1531) | 776 (653 - 935) | 384 (315 - 436) | **<0.0001** |
| - D30 [median (IQR)] | 1007 (947 - 1569) | 750 (595 - 992) | 375 (320 - 450) | **<0.0001** |
| - D365 [median (IQR)] | 981 (0 - 1347) | 758 (599 - 1221) | 518 (331 - 637) | **<0.0001** |
| **T CD8+ counts (cells/mm3)** | | | | |
| - D0 [median (IQR)] | 572 (435 - 1038) | 909 (674 - 1433) | 877 (595 - 1256) | **<0.022** |
| - D30 [median (IQR)] | 511 (452 - 771) | 922 (654 - 1316) | 898 (652 - 1200) | **<0.016** |
| - D365 [median (IQR)] | 395 (0 - 688) | 870 (525 - 1139) | 703 (464 - 1170) | ns |
| **CD4/CD8 Ratio** | | | | |
| - D0 [median (IQR)] | 1,88 (1,4 - 2,75) | 0,79 (0,51 - 1,35) | 0,41 (0,32 - 0,58) | **<0.0001** |
| - D30 [median (IQR)] | 1,74 (1,39 - 2,46) | 0,77 (0,56 - 1,26) | 0,45 (0,3 - 0,67) | **<0.0001** |
| - D365 [median (IQR)] | 1,5 (0 - 2,37) | 0,87 (0,63 - 1,31) | 0,48 (0,3 - 0,98) | **<0.0003** |

*Statistical significance for categorical variables were evaluated using Chi-square test and for continuous variables were evaluated using Kruskal-wallis test; ns – non-significant*

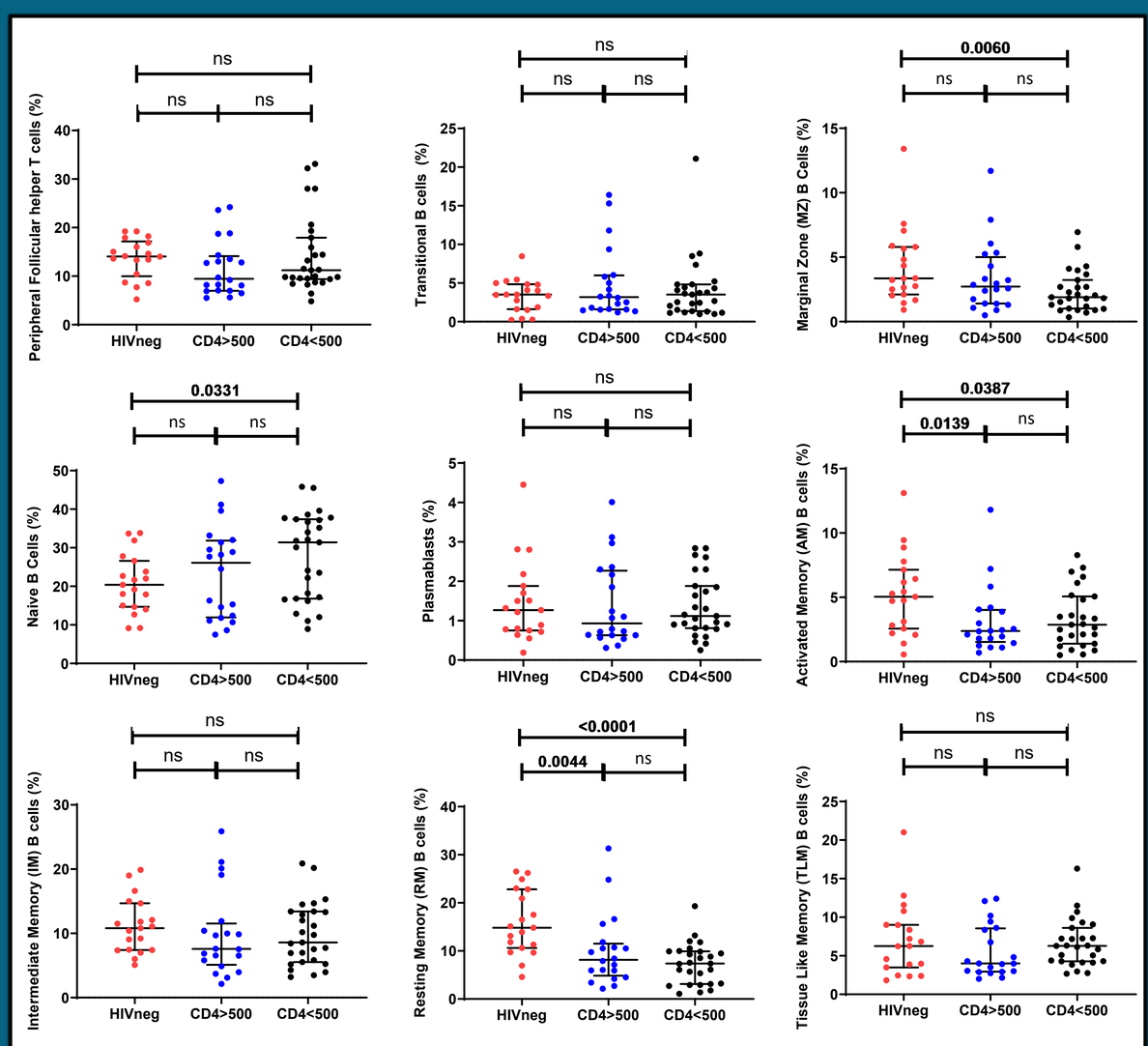


**Figure 1. Frequencies of prevaccination pTfh and B cell subsets among studied groups**
*The graphs represent the frequencies of peripheral T follicular helper cells (pTfh) and the evaluated B lymphocytes subsets, as described in the Y axis in PBMCs obtained from D0 samples; Horizontal bars represent median and 75$^{th}$/25$^{th}$ quartile ranges. Scatter dot plots were constructed with GraphPad Prism 9 and Mann Whitney test was used for comparison between groups. Non significant p-values are indicated as "ns".*

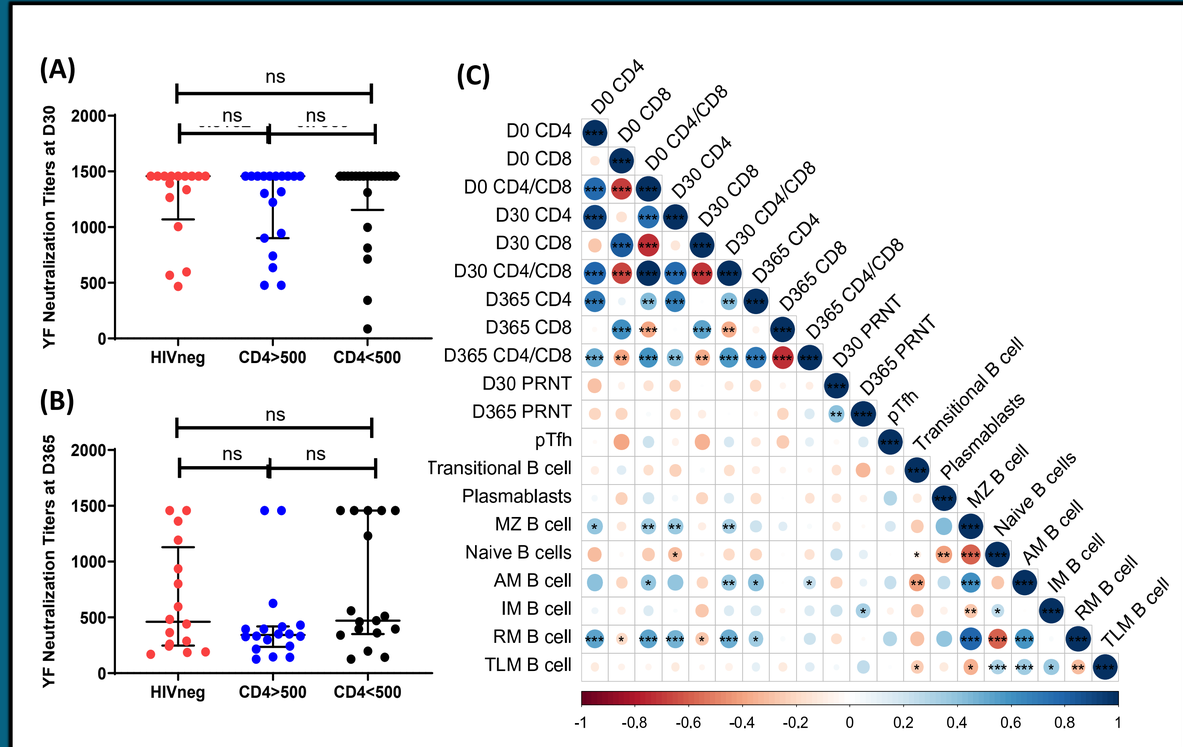


**Figure 2. Neutralization titers in response to YF-17DD vaccine and correlation with cell subsets evaluated in the study**
*Neutralization titers at D30 (A) and D365 (B) postvaccination among studied groups; (C) Correlation matrix between markers evaluated in the study; For graphs (A) and (B), Scatter dot plots were constructed with GraphPad Prism 9 and Mann Whitney test was used for comparison between groups; Horizontal bars represent median and 75$^{th}$/25$^{th}$ quartile ranges and Nonsignificant p-values are indicated as "ns". For graph (C), Sperman rank-order correlations analyses and construction of correlation matrix were performed with corrplot package in R v4.1.2; Circles' sizes and colors are equivalent to Spearman Rho values obtained for each comparison, as represent in the legend. Significant p-values were represented inside the circles as: * p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.*