# Decrease of Yellow Fever neutralizing antibody titers one year after vaccination is related to different NK cell repertoire in PLWH and non-HIV controls - ANRS12403.

Andressa da Silva Cazote[1], Laíse Nery da Silva Caldas[1], Daniel Granato da Costa Lima[1], Dalziza Victalina de Almeida[1], José Henrique Pilotto[1], Sheila Maria Barbosa de Lima[2], Adriana de Souza Azevedo Soares[2], Ygara da Silva Mendes[2], Ana Bispo de Filippis[3], Michelle Morata[4], Luciana Gomes Pedro Brandão[4], Marcellus Dias[4], Sandra Wagner Cardoso[4], Beatriz Grinsztejn[4], Luiz Antônio Camacho[5], Lara Esteves Coelho[4], Fernanda Heloise Cortes[1], Daniel Scott-Algara[6] & Carmem Beatriz Wagner Giacoia Gripp[1,*]

[1] Laboratory of AIDS and Molecular Immunology, Oswaldo Cruz Institute, Rio de Janeiro, Brazil.
[2] Laboratory of Virological Technology, Biomanguinhos, Rio de Janeiro, Brazil.
[3] Laboratory of Flavivirus, Oswaldo Cruz Institute, Rio de Janeiro, Brazil.
[4] National Institute of Infectious Diseases Evandro Chagas, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.
[5] National School of Public Health Sergio Arouca, ENSP/FIOCRUZ, Rio de Janeiro, Brazil.
[6] Pasteur Institute, Paris, France.

* Correspondence: cbwggripp@gmail.com

AS-IAS-2023-04855

## BACKGROUND

Yellow fever (YF) vaccine has been successfully used to control YF disease and prevent new outbreaks. The 17DD YF vaccine is considered safe and highly effective vaccine in people living with HIV (PLWH), but the immunogenicity mechanisms are not fully understood. We hypothesized that, despite HIV viral load (VL) suppression, PLWH may have reduced production and maintenance during the time of YF-neutralizing antibodies (NAbs) and that different NK cells repertoire could participate in this process.

## METHODS

This project was nested in a longitudinal study that investigated YF vaccine safety and immunogenicity in PLWH and non-HIV controls (CTRL). NK cell repertoire was analyzed by the detection of NCR, KIR and NKG2 families (Figure 1). Analysis were conducted in PLWH with baseline CD4+ T cell counts ≥ 200 cells/mm³ and suppressed VL (n=25), and in CTRL (n=16), by flow cytometry, at pre-vaccination (Day 0), and at three moments post-vaccination (Days 5, 30 and 365). YF vaccine immunogenicity was evaluated by Nab levels measured through a micro plaque reduction neutralization test (μPRNT, cut-off ≥ 1:100) at Days 30 and 365. For the analysis, all participants were grouped according to HIV status and, for PLWH, CD4 counts as ≥200<500 or ≥500 cells/μL, and regardless HIV status and according to Nab titers at Day 365 as: low (>1:100 and < 1:500), moderate (≥500 and <1000) and high (≥1000).

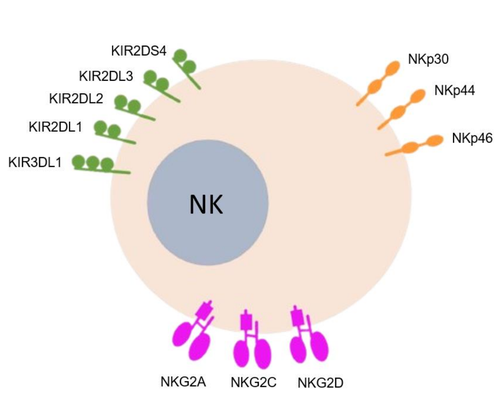


Figure 1 Mononuclear peripheral cells were phenotyped to five members of KIR family (KIR2DL1, KIR2DL2/DL3, KIR3DL1, KIR2DS4), to NKG2A/C/D, and to NCR members NKp30/44/46).

## RESULTS

First, NK profiles were investigated according to the participants' CD4 levels at Day 0 in PLWH. Significant differences were observed between CTLR and PLWH and within the groups along the vaccine follow-up visits.

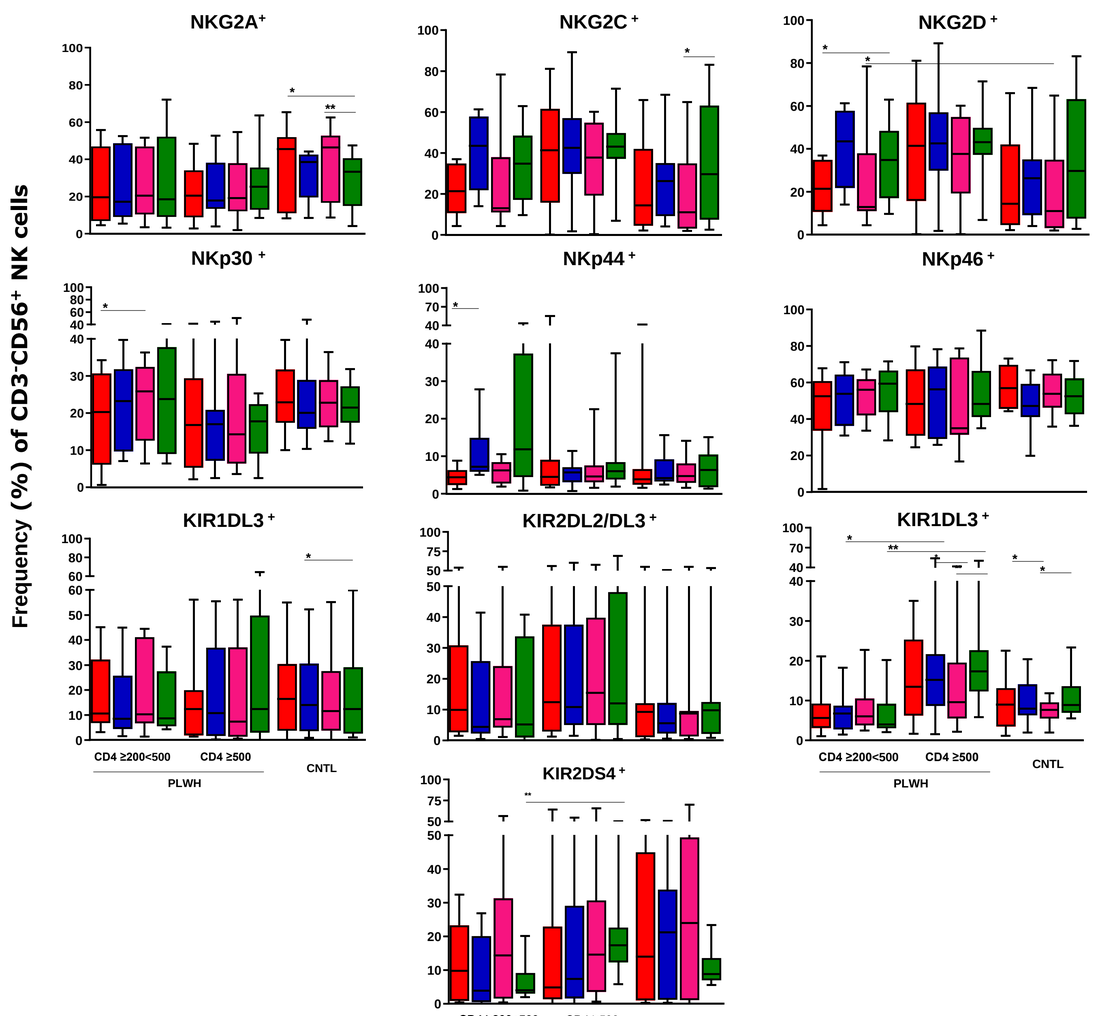


Figure 2. NK repertoire profiles among CTRL and PLWH according to CD4 status as ≥200<500 cell/μL and ≥500 cell/μL. Frequency of NK CD3-CD56+ expressing different receptors at Day 0 (red), Day 5 (blue), Day 30 (pink) and Day 365 (green). Results are shown as median and confidence intervals (25-75%). *p<0.05; **p<0.01.

In the next step, the Nab titers were evaluated. YF vaccine resulted in protective Nab titers at Day 30 in all participants. Nab titers decreased one year after vaccination, regardless of HIV status or CD4+ T cells counts (Figure 3).

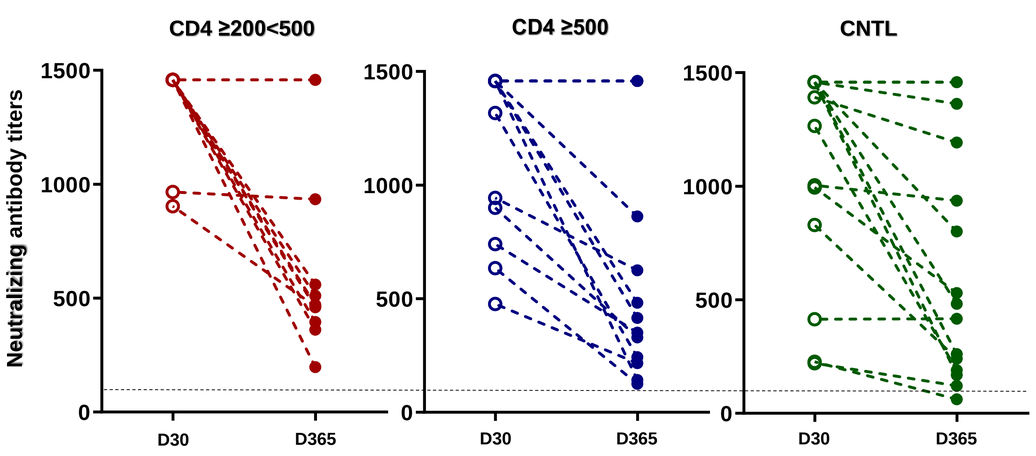


Figure 3. Neutralizing antibody titers after YF vaccine, at D30 and D365. Titers of Nabs from PLWH with T CD4+ counts ≥200<500/μL (red) and ≥500 /μL (blue), and from CNTL (green). Dashed black line defines μPRNT cut-off.

The NK repertoire was then analysed at different time points and correlated with Nab titers at Day 365, independent of HIV status. Interestingly, the frequencies of NKp30+ NK cells were significantly lower at Day 0 in individuals with low Nab titers compared to those with moderate NAb titers, while the frequencies of NKG2A+ NK cells were also lower in some visits, compared to those with high NAb titers (p<0.05) (Figure 4).

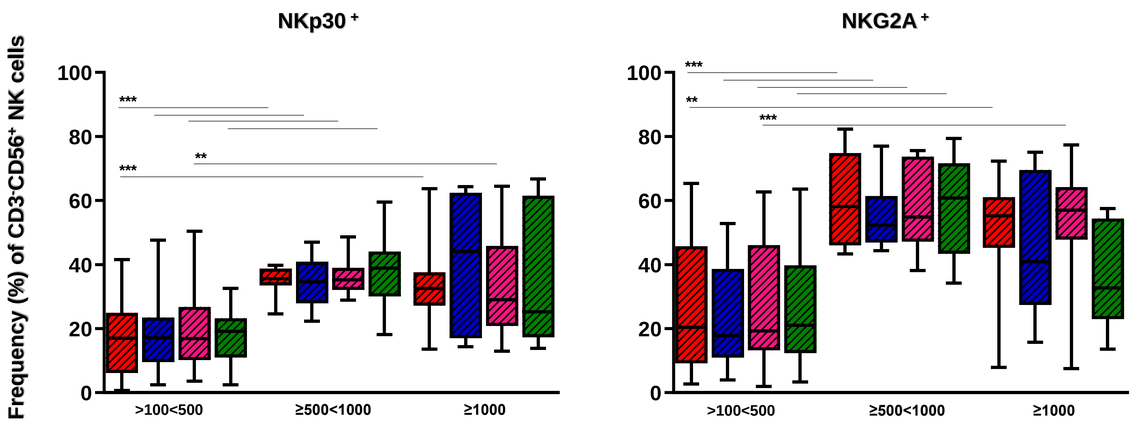


Figure 4. NK cell receptor expression according to Nab titers at Day 365, as >100<500, ≥500<1000, ≥1000. Frequency of NK CD3-CD56+ expressing NKp30 or NKG2A at Day 0 (red), Day 5 (blue), Day 30 (pink) and Day 365 (green). Results are shown as median and confidence intervals (25-75%). *p<0.05; **p<0.01; ***p<0.001.

## CONCLUSIONS

Our results suggest that the expression levels of NKp30 and/or NKG2A on NK cells at the time of YF vaccination may influence the levels of Nabs. As NKp30 is important for NK cell activation, while NKG2A is associated with high responsiveness of educated NK cells, early activation of these subsets may engage pathways involved in a better B cell response. Our results suggest that the immunogenicity of the YF vaccine, and thus the long-term duration of NAbs protection, may be influenced by the NK cell repertoire at the time of vaccination

ACKNOWLEDGMENT

Multiparametric Flow Cytometry Facility, Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro Brazil.

FINANCIAL SUPPORT: