Detection of anti-Interferon Alpha/Omega Neutralizing Autoantibodies in PLWH

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BACKGROUND

Anti-type I interferon (IFN-I) neutralizing autoantibodies (NAbs) have been associated with an increased risk of life-threatening viral infections, including SARS-CoV-2, influenza virus and more recently West Nile virus. Given the controversial role of IFN-I in HIV-1 infection, it is imperative to investigate anti-IFN NAbs and associated IFN pathway modulation in People living with HIV (PLWH). Therefore, we investigated the prevalence of anti-IFN-I NAbs in a cohort of PLWH, and examined their specificity, fluctuation over time, biological significance and clinical implications.

METHODS

Blood samples were collected from 385 virologically suppressed, antiretroviral therapy (ART)-treated PLWH at the Policlinico Umberto I, Sapienza University Hospital in Rome, Italy. Serum samples were tested for the detection of NAbs against IFN-α, IFN-β and IFN-ω using an antiviral bioassay (**Fig. 1**). Longitudinal measurements of NAbs over a ~3-year period were performed on serum samples from PLWH with anti-IFN-I NAbs. Expression levels of the IFN-induced antiretroviral restriction factors, APOBEC 3G/3F and ISG15, were measured by RT/real-time PCR in peripheral blood mononuclear cells (PBMCs) from 4 NAbs-positive and age- and sex-matched NAbs-negative (n=16) PLWH.

Table 1. Titers of and neutralizing antibodies (NAbs) to IFN-I (IFN-α and IFN-ω), demographic and clinical parameters of People living with HIV (PLWH)

	Patient N. 1	Patient N. 2	Patient N. 3	Patient N. 4	Patient N. 5	Patient N. 6	Patient N. 7	Patient N. 8	Patient N. 9	Patient N. 10	Patient N. 11
Gender	M	M	M	M	M	M	M	M	M	M	M
Age (Years)	77	50	51	58	52	30	62	65	66	70	73
Comorbidities	CPAP for COVID-19	Severe COVID-19, dead	Severe COVID-19	Hypertension, hypercholesterolemi, severe COVID-19	Kaposi's sarcoma	Kaposi's sarcoma	NA	NA	AIDS dementia complex with oropharyngeal candidiasis	NA	NA
CD4 T cell count nadir (cell/µl)	NA	NA	NA	NA	410	90	190	190	NA	94	229
CD4 T cell count (cell/µl)	304	86	500	500	1.163	90	1.275	677	698	522	384
IFN-α2 NAbs (TRU/ml)	530.000	5.680	<10	<10	22.757	11.387	249	7.964	8.960	53	<10
IFN-ω NAbs (TRU/ml)	2130	<10	18	10	<10	<10	<10	<10	31	<10	213

Data are expressed as single value for each subject. NAbs titers were calculated using the Kawade's method, and the titers were expresssed in Tenfold Reduction Units (TRU)/ml. All participants in the study are virologically suppressed and undergoing antiretroviral therapy (ART). Abbreviations: NA=Not available; CPAP=continuous positive airway pressure ventilation.

RESULTS

Anti-IFN-I NAbs were detected in ~3% of PLWH (11/385). The levels of NAbs against IFN-α varied widely, ranging from 53 to 530.000 tenfold reduction units [TRU/mL]). We found that 25% (5/11) of the NAbs-positive sera were able to neutralize IFN-ω but not IFN-β. All NAbs-positive PLWH were male, with a median age of 62 years (range 30-77) and median nadir CD4+ T cell counts (cells/µl) and CD4+ T cell counts of 190 and 500, respectively. Among them, four had been hospitalized for severe COVID-19 (two requiring mechanical ventilation and one with a fatal outcome); two had Kaposi's sarcoma, and one had AIDS dementia complex with oropharyngeal candidiasis (**Table 1**). Longitudinal evaluation of anti-IFN-I NAbs revealed persistence of anti-IFN-I NAbs, with titer fluctuation over time, in 27% of PLWH (3/11, PLWH N. 9, PLWH N. 10, PLWH N.11, **Fig. 2**, Panels A-C). PLWH N. 9 tested negative for NAbs in 2021 (shown as T0 in **Fig. 2**, Panel A), but the first anti-IFN-α/-ω NAbs-positive serum appeared 15 months later. Subsequent tests at 25, 33 and 36 months from the T0 were positive for the presence of NAbs, with anti-IFN-α NAbs titers increasing between T15 and T25, and anti-IFN-ω NAbs titers increasing between T15 and T33, before decreasing (**Fig. 2**, Panel A). PLWH N. 10 showed persistent anti-IFN-α NAbs at 6, 8 and 10 months after the first positive serum, with minimal variation in titers over time (**Fig.2**, Panel B). PLWH N. 11 showed anti-IFN-ω NAbs with increasing titer after two months (**Fig. 2**, Panel C). Reduced levels of APOBEC-3G/3F ($p<0.001$) and a trend toward lower expression of ISG15 mRNA were observed in PBMC from 4 anti-IFN NAB positive PLWH compared to negative PLWH (**Fig 3**, Panels A-C).

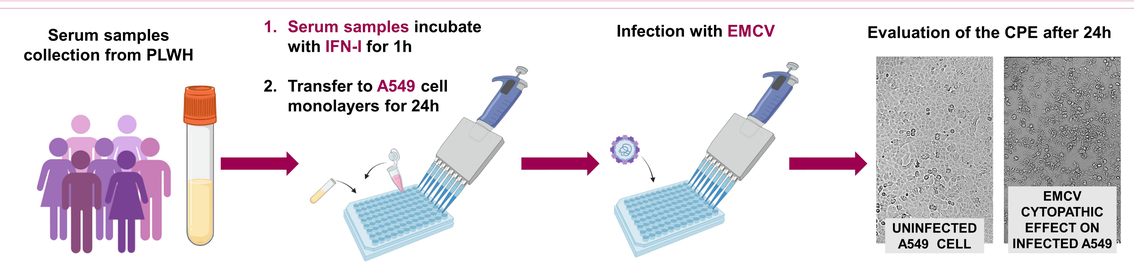


Fig. 1 Neutralizing antibodies (NAbs) to type I interferon (IFN-I) were assessed in a bioassay based on IFN-induced inhibition of encephalomyocarditis virus (EMCV) cytopathic effect (CPE) on human lung carcinoma epithelial cells (A549)

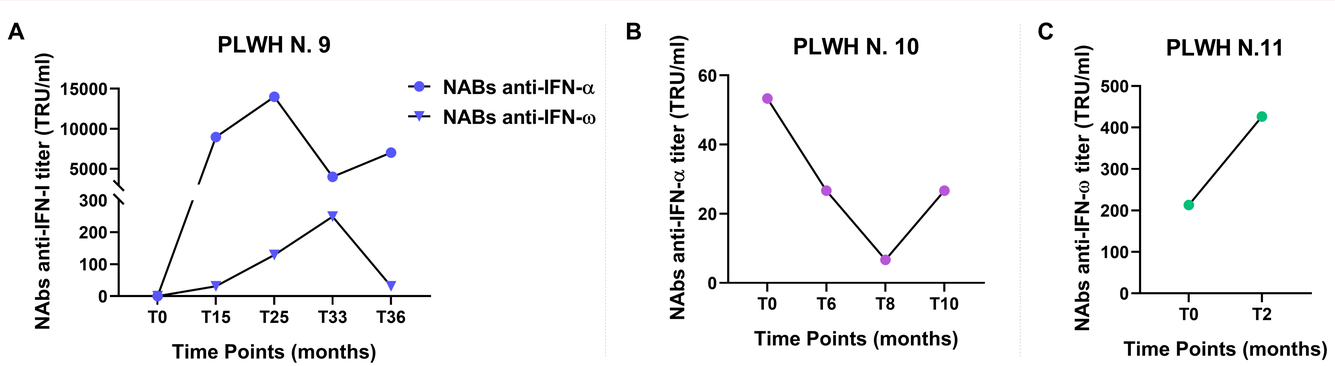


Fig. 2 **Persistence of anti-type I interferon (IFN-I) neutralizing antibodies (Nabs) in People living with HIV (PLWH).** Temporal changes in anti-IFN-α and anti-IFN-ω NAbs titers in NAbs-positive PLWH at different time points. Serum samples were available at different time points only for PLWH N.9 (**Panel A**), PLWH N.10 (**Panel B**) and PLWH N.11 (**Panel C**). The first serum tested for NAbs detection is indicated as "T0", and all subsequent time points denoted as "T" followed by the number of months elapsed from T0.

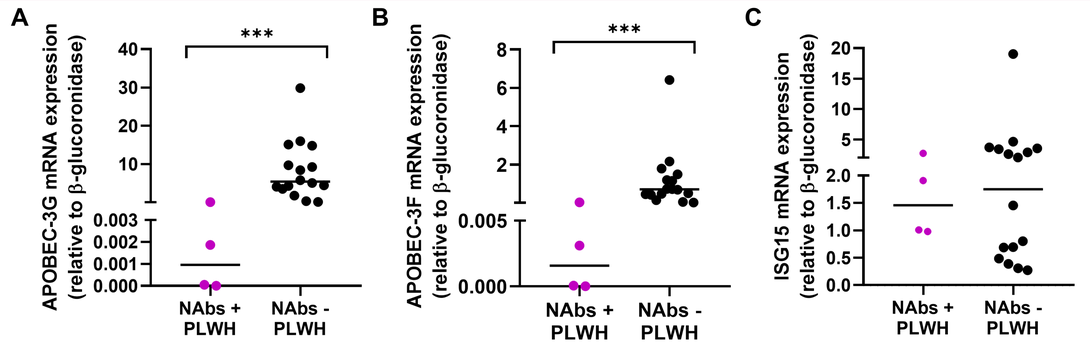


Fig. 3 Expression levels of APOBEC-3G/3F and ISG15 measured by Real Time PCR in peripheral blood mononuclear cells (PBMCs) collected from anti-type I interferon (IFN-I) neutralizing antibodies (NAbs)-positive People living with HIV (PLWH) (n=4) compared to NAbs-negative PLWH (n=16). Anti-IFN-I NAbs-positive PLWH and anti-IFN-I NAbs-negative PLWH are indicated as "NAbs + PLWH" and "NAbs – PLWH", respectively. Statistical analysis of transcript levels of APOBEC-3G/3F and ISG15, relative to β-glucoronidase ($2^{-\Delta Ct}$ method), was performed using Mann-Whitney test. Median value of gene expression levels are reported for each study group, with a black horizontal line. ***$p<0.001$

CONCLUSIONS

These results highlight the presence of anti-IFN-α/ω NAbs in the serum of PLWH, capable of reducing the expression of IFN-induced retroviral restriction factors. Notably, these NAbs persist over time in some PLWH and are associated with worsening disease. Our findings suggest that assessment of anti-IFN-I NAbs could emerge as a promising novel candidate biomarker, potentially improving clinical management of PLWH.