UNIVERSITY OF MIAMI

IL-21 ALTERS TFH DYNAMICS, IMPROVES FLU VACCINE RESPONSE IN OLD SIV+ NHPS UNDER ART

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ABSTRACT

HIV/aging contribute to inflammaging (chronic low-grade systemic inflammation) and immune senescence (accelerated aging of the immune system). Immune dysfunction, in the form of impaired antibody (Ab) responses to vaccines such as influenza (flu) vaccination, is observed in aging and HIV infection. Given the central role of IL-21 in Ab responses we hypothesized that administration of IL-21 as a flu vaccine adjuvant in aged, ART treated, SIV+ Rhesus Macaques (RM) would result in significant improvement in the quality of pTfh and B cell function alongside improved germinal center reactions, resulting in improved Ab responses to vaccination.

In this study flu vaccination was administered with (N=4) and without (N=4) subcutaneous IL-21 in a prime, boost, boost series at 3-month intervals to old ART treated, SIV+ (IV SIVmac239) RM. IL-21 was given (50µg/kg) on d-2, d0, d5 post each vaccine dose. Blood was collected on d0, d5, d14 and d42; and lymph node tissue was collected on d14 after each vaccine dose. Serum was analyzed for flu Ab titers, and PBMC with multicolor flow cytometry using panels for detailed phenotypic characterization of peripheral blood T follicular helper (pTfh) cells and CD4 memory populations.

In results analyzed to date, pre-prime H3N2 HAI titers of controls (mean=1:55) did not differ from IL-21 treated animals (mean=1:100). Titers increased significantly (p=0.0018) in IL-21 treated animals from 1:100 at baseline to 1:283 post boost 1 (PB1) and were significantly higher (P=<0.0001) than the PB1 control mean titer of 1:60 (Fig. 1A). We did not observe baseline differences in pTfh frequency between groups (Fig. 1A). IL-21 treated animals had significant (p=0.0118) expansion of pTfh, as measured by the fold change of pTfh frequency from day of Boost 1 (B1) to 14 days PB1, correlating with H3N2 HAI titers 14 days PB1 (R2=0.6978, P=0.0193, Fig. 1B). We also observed that the frequency of PD1+ pTfh cells was significantly higher (p=0.0188) in IL-21 treated animals (mean=27%) compared to controls (mean=16.7%) on the day of B1 and correlated with H3N2 HAI titers 14 days PB1 (R2=0.728, P=0.0146).

These findings suggest IL-21 has a significant adjuvant effect, improving flu vaccine titers in old, ART treated, SIV+ RM. As no baseline pTfh differences were observed, these results highlight that IL-21 may be directly or indirectly inducing a shift in pTfh cell kinetics and phenotype, warranting further investigation as a potential vaccine adjuvant.

BACKGROUND

- HIV/aging contribute to inflammaging (chronic low-grade systemic inflammation) and immune senescence (accelerated aging of the immune system)
- Immune dysfunction, in the form of impaired antibody (Ab) responses to vaccines such as influenza (flu) vaccination, is observed in aging and HIV infection
- IL-21 is critical in the generation and function of CD4+ T-follicular helper (Tfh) cells, which provide cognate help to B-cells for high affinity antibody production 12,13
- IL-21 is paramount for LN germinal center formation and B cell Ab responses to T dependent and independent antigens

HYPOTHESIS

Administration of IL-21 as a flu vaccine adjuvant in aged, ART treated, SIV+ Rhesus Macaques (RM) will result in significant improvement in the quality of pTfh and B cell function alongside improved germinal center reactions, resulting in improved Ab responses to vaccination.

AIMS

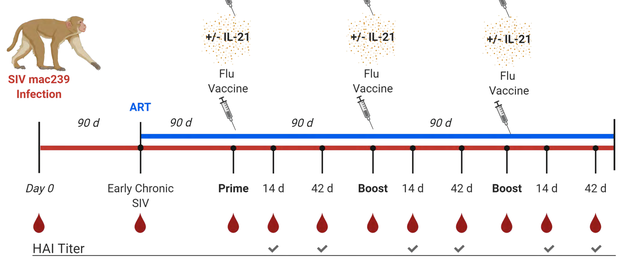
- Investigate serum antibody levels to systemic influenza vaccination in IL-21 treated animals in comparison with non-IL-21 treated animals
- Examine changes in immune cells in PBMC and lymph nodes by multicolor flow cytometry and tissue staining.

METHODS

Flu vaccination was administered with (N=4) and without (N=4) subcutaneous IL-21 in a prime, boost series at 3-month intervals to old ART treated, SIV+ (IV SIVmac239) RM. IL-21 was given (50µg/kg) on d-2, d0, d5 post each vaccine dose. Blood was collected on d0, d5, d14 and d42; and lymph node (LN) tissue was collected on d14 after each vaccine dose. LN tissue was stained and imaged via quantitative, multiplexed confocal imaging for the comparative analysis of lymph node immune cell populations Tfh. Serum was analyzed for flu Ab titers, and PBMC with multicolor flow cytometry using panels for detailed phenotypic characterization of pTfh) cells and CD4 memory populations. Comparison of two groups was performed using a Mann-witney T test. Multiple cmparisons were performed using 2-way ANOVA. Correlation was performed using simple linear regression. Statistical analysis was performed using GraphPad Prism 8.3.1 .

Rhesus Macaque Groups			
Age	SIV	IL-21	n
Old	+	+	4
Old	+	-	4
Old	-	-	4
Young	-	-	4
Total			16

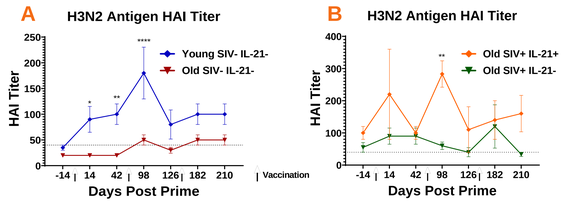
Systemic Immunization Scheme



IL-21 has a significant adjuvant effect, improving flu vaccine titers in old, ART treated, SIV+ Rhesus Macaques

RESULTS

HAI titers highlight age-associated immune impairment and IL-21 Induced improvement of Ab response



- H3N2 HAI titers in SIV- young RM are higher than old SIV- RM demonstrating our NHP model's recapitulation of an age-associated impairment of influenza vaccine response (**Fig. 1A**)
- IL-21+ old SIV+ animals had improved titers over IL-21- old SIV+ post boost 1 (**Fig. 1B**)

Figure 1. Serum hemagglutination titers for H3N2 Influenza antigen was performed at pre-vaccine, 14 days post-each vaccine dose and 42 days post each vaccine dose

pTfh gating strategy

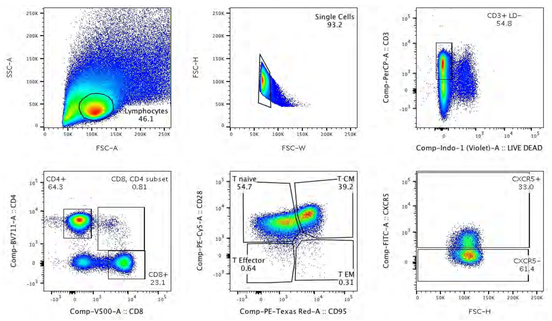
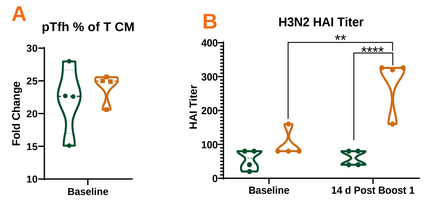


Figure 2. pTfh Gating Strategy CXCR5+ expression on CD4+ T central memory (CD95+CD28+CD4+CD3+, live single lymphocytes)

Analysis of pTfh reveals association between pTfh expansion and Ab response to vaccination



Old SIV+ IL21- Old SIV+ IL21+

- No baseline differences in pTfh frequency between groups (**Fig. 3A**)
- Pre-prime H3N2 HAI titers of controls (mean=1:55) did not differ from IL-21 treated animals (mean=1:100) (**Fig. 3B**)
- Titers increased in IL-21 treated animals from 1:100 at baseline to 1:283 post boost 1 (PB1) and were significantly higher than the PB1 control mean titer of 1:60 (**Fig. 3B**)
- IL-21 treated animals had significant expansion of pTfh, as measured by the fold change of pTfh frequency from day of Boost 1 (B1) to 14 days PB1 (**Fig. 3C**), correlating with H3N2 HAI titers 14 days PB1 (**Fig. 3D**)

Figure 3. (A,C,D) Multicolor flow cytometry was employed for Immunophenotyping of Rhesus Macaque PBMCs to investigate Tfh cell subsets. **(B)** Serum hemagglutination titers for H3N2 Influenza antigen

Multiplexed confocal imaging for the comparative analysis of Tfh and IL-21+ immune cell populations

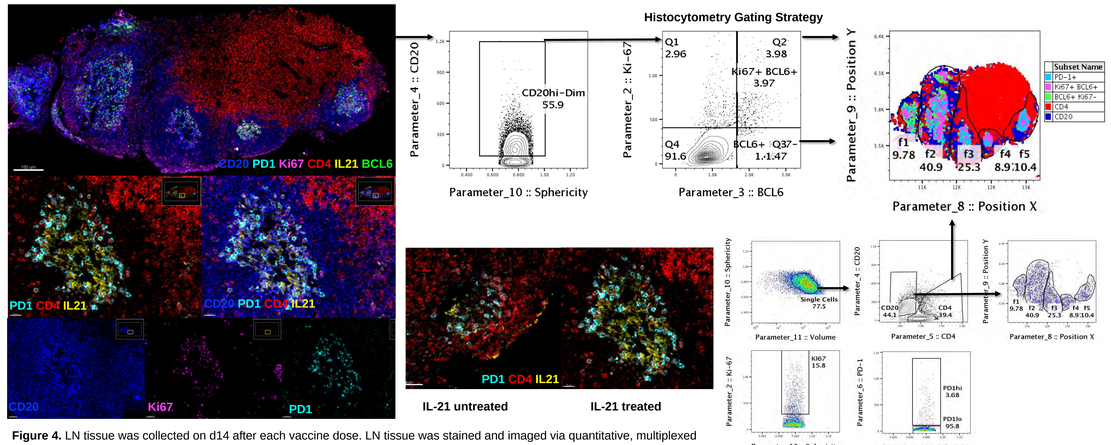
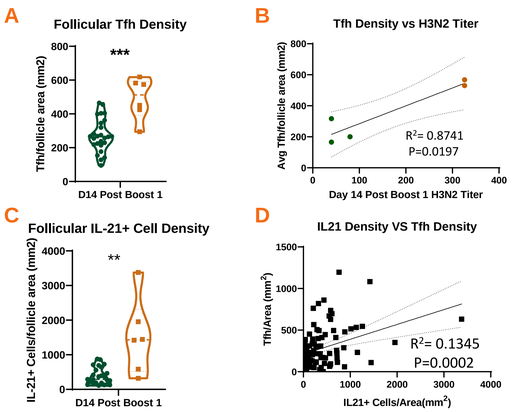


Figure 4. LN tissue was collected on d14 after each vaccine dose. LN tissue was stained and imaged via quantitative, multiplexed confocal imaging for the comparative analysis of lymph node immune cell populations including Tfh, B cells and IL-21+ cells.

RESULTS (cont.)

Higher Tfh and IL-21 density in IL-21 treated RM



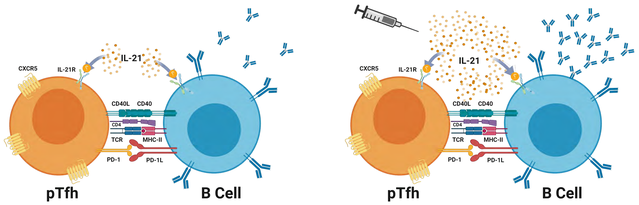
- Tfh (CD4+PD-1hi) density per LN B cell follicle was significantly higher in IL-21 treated animals compared to untreated (**Fig. 5A**)
- Correlation analysis revealed a significant association between average follicular Tfh cell density per animal and H3N2 HAI titers (**Fig. 5B**)
- The density of IL-21+ cells per follicle was significantly higher in IL-21 treated animals compared to untreated (**Fig. 5C**)
- Further, the density of IL-21+ cells per follicle associated with the density of Tfh per follicle (**Fig. 5D**)

Figure 5. LN tissue was collected on d14 after each vaccine dose. LN tissue was stained and imaged via quantitative, multiplexed confocal imaging for the comparative analysis of lymph node immune cell populations including Tfh, B cells and IL-21+ cells.

SUMMARY

- No baseline differences were observed in pTfh frequency nor H3N2 HAI titers
- IL-21 treated old, ART treated, SIV+ RM have improved H3N2 flu vaccine titers
- A significant expansion of pTfh in blood was observed from the day of boost 1 to 14 days post boost 1 in IL-21 treated animals
- pTfh expansion during the 14 days following boost 1 correlated with H3N2 titers
- We observed higher density of Tfh and IL-21+ cells in the IL-21 treated animals 14 days post boost 1
- IL-21 density per LN follicle correlates with Tfh density per follicle
- IL-21 may be directly or indirectly inducing a shift in Tfh/pTfh cell kinetics, warranting further investigation as a potential vaccine adjuvant

MODEL



REFERENCES

1. Pallikkuth S, de Armas L, Rinaldi S, Pahwa S. T Follicular Helper Cells and B Cell Dysfunction in Aging and HIV-1 Infection. Front Immunol. 2017;8:1380. doi:10.3389/fimmu.2017.01380
2. Pallikkuth S. et al. Interleukin-21 administration to rhesus macaques chronically infected with simian immunodeficiency virus increases cytotoxic effector molecules in T cells and NK cells and enhances B cell function without increasing immune activation or viral replication. *Vaccine*. 2011;29:9229-9238.doi:10.1016/j.vaccine.2011.09.118
3. Vogelzang A, et al. A Fundamental Role for Interleukin-21 in the Generation of T Follicular Helper Cells. *Immunity*. 2008;29(1):127-137. doi:10.1016/j.immuni.2008.06.001

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