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Abstract # 303

Immune activation and defective pTfh are associated with impaired immunity in aging HIV+ persons

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ABSTRACT

Background: With improved antiretroviral treatment (ART) and increasing survival, the aging HIV infected population is rising. HIV infection and aging are independently associated with waning immunity including poor vaccine induced antibody responses, but the combination of HIV and aging needs further investigation.

Methodology: This study represents initial observations from a larger ongoing study in young (Y) and old (O) people in the presence (+) or absence (-) of HIV infection. Four groups n=12 each, O+ median age (Δ) 61yr; O- Δ 62yr; Y+ Δ 38yr, and Y- Δ 34yr were investigated. HIV+ were well controlled on ART with<48 HIV RNA copies/ml and mean±SD CD4 counts (cells/mm^3) of 602.7±273.6 (O+) and 654.3±313.8 (Y+). Investigations included Hemagglutination inhibition (HAI) titers at pre- (T0) and 4 weeks post (T2) seasonal 2011-2012 influenza vaccination, immune activation (IA), and Immunologic analysis of peripheral T follicular helper cells (pTfh) and B cells. Differences between groups were analyzed by Student *t*-test and correlation between variables was determined by Pearson correlation and linear regression analysis.

Results: At T0, seroprotection [HAI titers ≥1:40] was less frequent in Old (O+ 25%, O- 33%), compared to Young (Y+ 83% and Y- 91.6%). At T2, a 4-fold increase in HAI titers was noted in 75% of Y- [peak mean±SD titer, 240±217] but only in 50% of each of the other groups, with O+ exhibiting lowest peak titer (55±27). At T0, the O groups compared to Y had higher CD4 IA (CD38+HLADR+) and lower frequencies of pTfh (CD4+CD45RO+CXCR5+), with highest and lowest values respectively noted in O+. Following vaccination, HAI titers at T2 correlated with pTfh changes (expansion, upregulation of ICOS expression and intracellular IL21) and memory B cell changes (expansion, upregulation of IL21R and increases in H1N1 specific Ab responses in Elispot assay). The magnitude of these responses was in the order Y->Y+>O->O+. CD4 T cell IA at T0 was negatively correlated with HAI titers at T2. In 5 day PBMC cultures, addition of exogenous IL-21 augmented ICOS expression on pTfh in all four groups, and H1N1 specific Ab responses in ELIspot assay were augmented in all except the O+ group.

Conclusion: Aging and HIV infection were independently associated with pTfh defects that contribute to B cell impairment, but the presence of HIV infection and aging together exhibited the worst immune profile, with highest underlying immune activation and poorest serologic response following influenza vaccination. Supplementation of IL-21 ex-vivo was partially restorative of the observed immune defects with better effects noted in HIV+ young than in HIV+ old, indicating the need for additional investigations e.g. targeting immune activation in the HIV+ aging population.

BACKGROUND

- Physiological aging is associated with impaired Ab responses to influenza (flu) vaccine and is attributed to immune senescence, which is also a feature of HIV infection [1].
- We recently reported that Ab response to influenza vaccine in post menopausal women with HIV infection is impaired compared to HIV negative controls, and is inversely correlated with cellular immune activation and inflammatory cytokines [2,3].
- We have previously reported that impaired antibody response to the monovalent Influenza A (H1N1) 2009 vaccine in HIV-infection is associated with impaired function of peripheral CD4+ T follicular helper (pTfh) cell subset [4].

OBJECTIVES

1. To determine the independent and combined effects of aging and of HIV infection on antibody responses to 2011-2012 seasonal influenza vaccine
2. To study the cellular components (B cells, pTfh) influencing antibody response and impact of underlying immune activation in the context of aging and HIV infection.

STUDY SUBJECTS

Study population:

70 participants, all women, old (O, age 57-72yr, post-menopausal) and young (Y, age 25-43yr); HIV+ (+) and HIV negative (-).

HIV+: all were on combination antiretroviral therapy (cART) >6 months with HIV RNA <100 copies/ml

Influenza Vaccination:

Single i.m. dose: 15 mg; 0.5 ml of inactivated influenza vaccine [FLUARIX® GSK, containing: A/California/7/2009 (H1N1), A/Perth/16/2009 (H3N2) and B/Brisbane/60/2008].

METHODS

Blood Samples: pre-vaccination (T0) and wk4 (T2)

Antibody Titers: Hemagglutination inhibition assay (HAI) using vaccine as antigen

Seroprotection: HAI titer of ≥1:40

Immunology Assays:

CD4 T cells

- *Immune activation markers:* CD38+HLADR+
- *pTfh phenotype:* CD4+CD45RO+CXCR5+
- *pTfh function:* Induction of ICOS, and IL-21 at 12 hr post-stimulation of PBMC with H1N1 Ag.

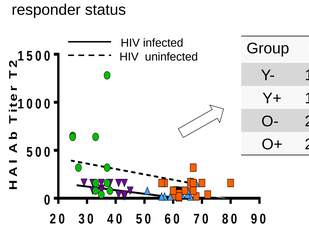
B cells

- *Memory phenotype:* CD20+CD21hiCD10-CD27+
- *Expression of IL21 Receptor*
- *Memory B cell function:* Antibody secreting cells (ASC) by ELIspot 5 d post-stimulation of PBMC with H1N1 antigen

RESULTS -1

Vaccine responder (VR): Titer ≥1:40 plus 4-fold increase at 4 weeks post-vaccination (T2)

Vaccine non-responders (VNR): Not meeting responder status



Group	n	VR/VNR
Y-	15	10/5
Y+	15	6/9
O-	20	6/14
O+	20	6/14

Following serology, we selected 48 participants for further analysis (Table 1)

Table 1: Characteristics of study population, n=48

Group	Study group	n	VR/ VNR	Mean Age in yrs (SD)	Mean HAI titer (SD) at T0	Mean HAI titer (SD) at T2
1	Y-	12	7/5	34.5 (5)	**55 (37.29)** #	**240 (217.6)**#☆Δ
2	Y+	12	5/7	37.8 (4.1)	**42.5 (20.06)** #	**125 (80)** #
3	O-	12	5/7	62 (5.9)	**42.5 (55.54)**	**72.5 (58.33)**
4	O+	12	6/6	61 (2.6)	**23.3 (10.7)**	**55 (27.14)**

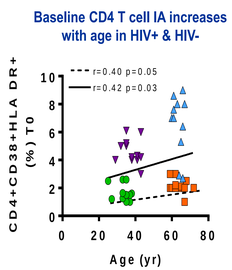
# significant compared to group 4, ☆Significant compared to Group 2, Δ significant compared to group 3, VR=Vaccine responder; VNR= Vaccine non-responder
T0 = At entry, T2 = 4 weeks post influenza vaccine

RESULTS-2

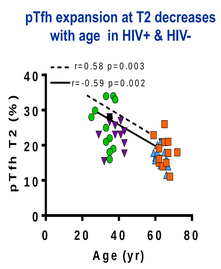
Correlations of Age



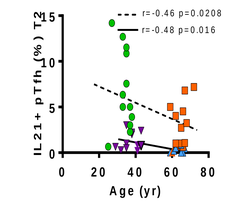
Baseline CD4 T cell IA increases with age in HIV+ & HIV-



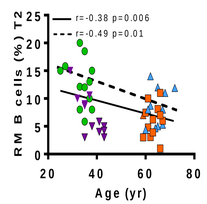
pTfh expansion at T2 decreases with age in HIV+ & HIV-



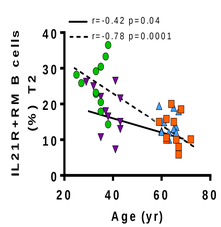
pTfh function at T2 decreases with age in HIV+ & HIV-



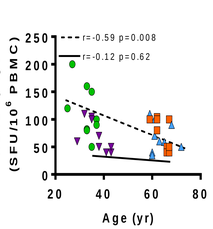
RM B cell expansion at T2 decreases with age in HIV+ & HIV-



IL21R+ RM B cells at T2 decrease with age in HIV+ and HIV-



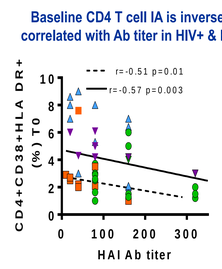
Memory B cell responses at T2 decrease with age in HIV-



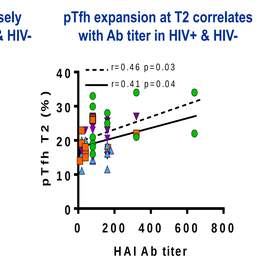
RESULTS-3

Correlations of HAI Ab Titer

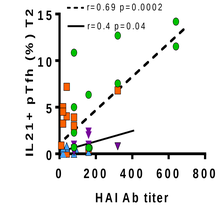
Baseline CD4 T cell IA is inversely correlated with Ab titer in HIV+ & HIV-



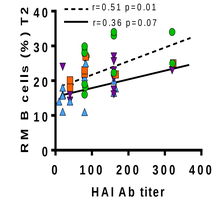
pTfh expansion at T2 correlates with Ab titer in HIV+ & HIV-



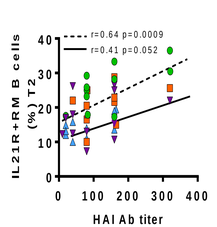
pTfh function at T2 correlates with Ab titer in HIV+ & HIV-



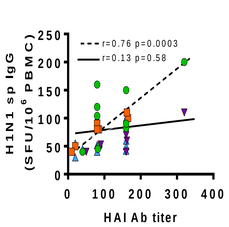
RM B cell expansion at T2 correlates with Ab titer in HIV-



IL21R+ RM B cells at T2 correlate with Ab titer in HIV-



Memory B cell responses at T2 correlates with Ab titer in HIV-

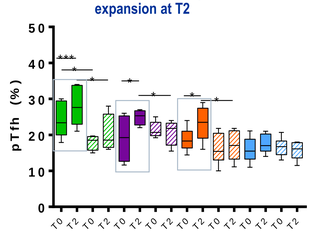


RESULTS-4

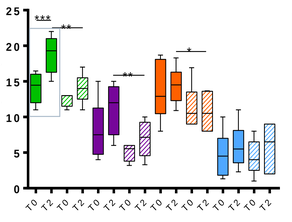
Results at T0 and T2 in VR and VNR in HIV+ and HIV- young and old



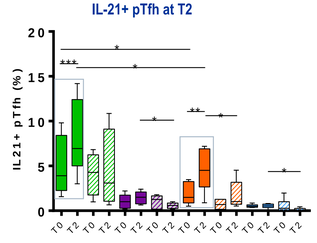
Y-, Y+ and O- VR show pTfh expansion at T2



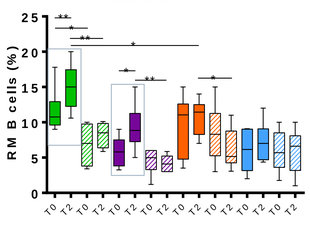
Y- VR show expansion of ICOS+pTfh at T2



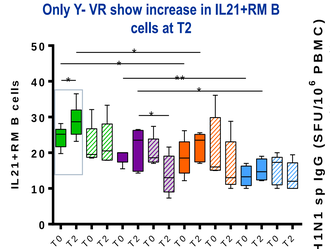
Y- and O- VR show expansion of IL-21+ pTfh at T2



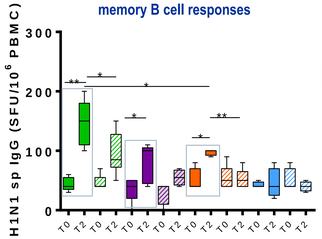
Y-, Y+ VR show expansion of RM B cells at T2



Only Y- VR show increase in IL21+RM B cells at T2



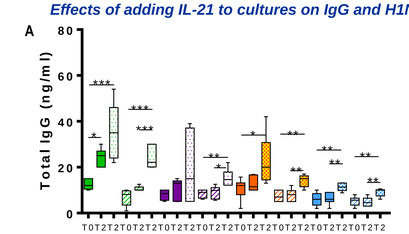
Y-, Y+ and O- VR exhibit H1N1 specific memory B cell responses



Cryopreserved PBMC obtained at T0 and T2 from HIV- and HIV+ young and old subjects were thawed, rested overnight and stained for frequencies of immune activation (CD4+CD38+ HLA DR+), pTfh (CD45RO+CXCR5+), Resting memory (CD20+CD21hiCD10-CD27+) B cells and IL21Receptor expression on RM B cells. PBMC were also stimulated short term (12hr) with H1N1 Ag for intracellular IL-21 cytokine staining or long term (5 days) followed by detection of H1N1 specific memory B cell responses by B cell ELISPOT. Stars indicate the level of significance. ★p < 0.05, ★★p < 0.01, ★★★ p<0.001.

RESULTS -5

Effects of adding IL-21 to cultures on IgG and H1N1 Ab responses



VNR from all groups and VR of most (except Y+) show enhancement in total IgG production at T2 in presence of exogenous IL-21

H1N1 specific memory B cell responses show significant increase from baseline in presence of exogenous IL-21 in VR and VNR of all groups except O+

PBMC at T0 and T2 were stimulated with H1N1 Ag for 5 days in presence or absence of rIL-21 (50 ng/mL). **(A)**, Total IgG was determined by ELISA in culture supernatants and **(B)**, H1N1 specific memory B cell responses by Elispot, expressed as spot forming units (SFU). Stars indicate the level of significance. ★p < 0.05, ★★p < 0.01, ★★★ p<0.001

SUMMARY

- 48 participants 12 each of young (Y) and old (O) HIV+ (+) and HIV neg (-) classified as vaccine responders (VR) and non-responders (VNR) were selected for evaluation of peripheral T folllicular helper (pTfh) and B cells.
- O+ showed highest baseline CD4 T cell immune activation (IA) among all groups; O- showed higher baseline CD4 IA than Y-. Baseline CD4 IA correlated with age and HAI titers in HIV- and HIV+ participants.
- At wk4 post vaccination, pTfh frequency and function were highest in Y- and least in O+; pTfh correlated inversely with age and directly with HAI titers, VR performed better than VNR.
- RM B cell (frequencies, IL21R expression) and H1N1 sp B cell responses at T2 were highest in Y- and correlated inversely with age and directly with HAI titers. O+ showed least expansion post vaccination.
- Exogenous IL-21 enhanced total IgG responses in all VNR at T2 compared to baseline; HINI specific memory B cell responses were enhanced in all VNR except O+ when compared to baseline.

CONCLUSIONS

Antibody responses to seasonal influenza vaccination are independently influenced by aging and HIV infection. These responses are poorest in HIV+ old people despite ART with virologic suppression and CD4 reconstitution implying that both HIV and aging contribute to immune deficiency in HIV+ aging population.

Impaired vaccine responses are associated with underlying immune activation coupled with defects in pTfh and RM B cells. Strategies aimed to decrease immune activation and to augment pTfh responses may be helpful in improving immune responses in the HIV+ aging population.

REFERENCES

1. Deeks, S. G. Top HIV Med 2009;17:118-23
2. Parmigiani, A., Alcaide, M. L., Freguja, R., Pallikkuth, S., et al, PLoS One 2013; 8:e79816
3. Alcaide, M. L. Parmigiani, A. Pallikkuth, S. Roach, M. et al. PLos One 2013;8:e63804
4. Pallikkuth S, Parmigiani A, Silva SY, George V, et al. Blood 2012;120:985-993.

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