POSTER 749
SESSION P-N5

Differences in Adenosine Signaling Pathway among HIV+ Individuals with COPD

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BACKGROUND

- HIV infection has been identified as an independent risk factor for prevalent chronic obstructive pulmonary disease (COPD), radiographic emphysema, incident diagnosis of COPD, and COPD exacerbations. (Fitzpatrick *et al.* 2018)
- The ATP-adenosine (ADO) regulatory pathway modulates the immune-response.
  - Inflamed tissues release ATP into extracellular space that is metabolized to ADO which, in turn regulates immune cells
    - CD73+ expressing CD4+ T cells are decreased in HIV infection regardless of plasma viremia. (Schuler *et al.,* 2013)
  - Regulatory T-cells (Treg) express ectoenzymes CD39/73 that break down extracellular ATP. ADO binds the A2A adenosine receptor on lymphocytes to decrease inflammation.

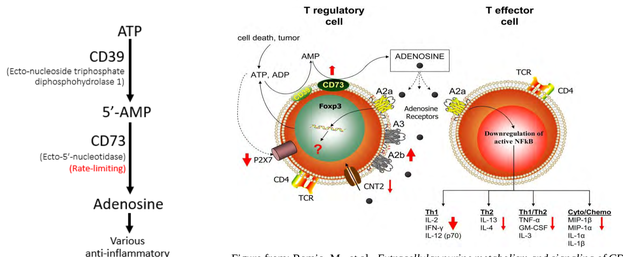
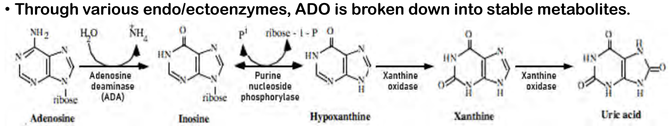


Figure from: Romio, M., et al., *Extracellular purine metabolism and signaling of CD73-derived adenosine in murine Treg and Teff cells.* American Journal of Physiology - Cell Physiology, 2011. **301**(2): p. C530./

- T-cells can also express ectoenzyme CD26 (dipeptidyl-peptidase IV) that can bind adenosine deaminase (ADA), an enzyme that metabolizes ADO.
  - Of note, gp120 can inhibit ADA binding to CD26. (Valuenza *et al.*, 1997)
- Through various endo/ectoenzymes, ADO is broken down into stable metabolites.



STUDY AIM AND METHODS

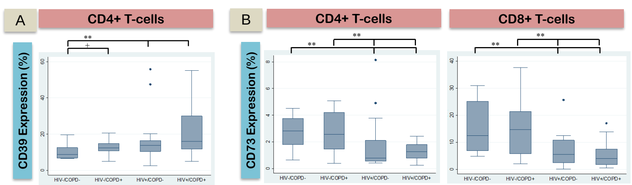
- We conducted a pilot exploratory study to define relationships between the ADO signaling pathway, levels of T cell immune activation and systemic inflammation, and pulmonary function among individuals with HIV compared to HIV- individuals from the Pittsburgh HIV Lung Research Cohort
- **Pulmonary Function Tests (PFT)**: Participants' forced expiratory volume (FEV_1) and forced vital capacity (FVC) before and after albuterol administration, and diffusing capacity (DLCO) measured. COPD defined as: post-bronchodilator FEV_1/FVC <0.70 predicted or DLCO < 80% predicted.
- Peripheral blood specimens were obtained for the following studies:
  - **Multicolor Flow Cytometry**: We determined frequencies of ectoenzymes CD39 and CD73 and dipeptidyl peptidase 4 (CD26) expression on $CD4^+/CD8^+$ T cells and on Treg ($CD4^+$ $CD25^{HI}$ $CD127^{LO/NEG}$ $FOXP3^+$). Levels of T cell activation were evaluated by gating on T cells co-expressing CD38 and HLA-DR.
  - **LC-Mass Spectrometry Analysis**: Plasma samples were processed for adenosine, inosine, 5'-AMP, hypoxanthine, xanthine.
  - **Multiplex Immunoassays**: Plasma samples were processed for sCD14, CRP, and TNF-receptor 1/2.
- **Statistics**: Kruskal-Wallis test by ranks followed by Dunn's pairwise comparison was used to compare frequency/amount of measured variable between HIV-/+ and COPD-/+ patient groups. Spearman correlation was used to evaluate associations between different parameters studied.

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STUDY POPULATION DEMOGRAPHICS

	HIV-/COPD- n = 12	HIV-/COPD+ n = 12	HIV+/COPD- n = 20	HIV+/COPD+ n = 20	Overall P value
Age, median (IQR)	49 (43-57)	57 (53-66)	45 (39-53)	55 (48-61)	0.01
Sex – Male	7 (58%)	7 (58%)	14 (70%)	15 (75%)	0.70
Smoker Pack years, average (IQR)	5 (42%) 0 (0-10.0)	6 (50%) 0.5 (0-22.8)	8 (40%) 0 (0-9.4)	14 (70%) 8.4 (0-28.5)	0.23 0.40
Hepatitis B Hepatitis C	0 0	0 1 (8%)	3 (15%) 3 (15%)	3 (15%) 1 (5%)	0.60 0.40
HIV Status -Viral Load, median (IQR) -CD4 count, median (IQR)	-	-	 0 (0-2147) 525 (448-1044)	 0 (0-136) 598 (389-859)	 0.90 0.60
HIV Treatment -History of treatment -Current treatment	-	-	 19 (95%) 18 (90%)	 19 (95%) 19 (95%)	 >0.9 >0.9

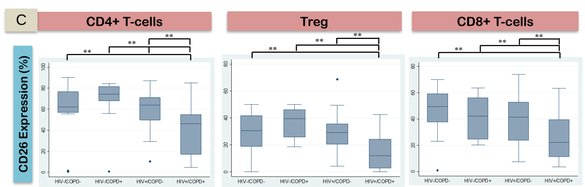
ECTOENZYME EXPRESSION, IMMUNE ACTIVATION AND CORRELATIONS



Legend: + indicates significance of 0.085. **indicates significance of p < 0.05.

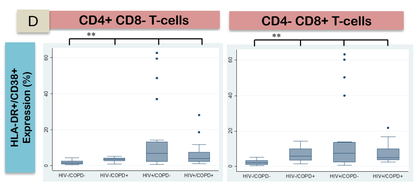
•**A:** In CD4+ T-cells, CD39 expression was increased in HIV+ participants. Among COPD+ participants, CD39 did not correlate with any other immune parameter or PFT result.

•**B:** In CD4+ and CD8+ T-cells, CD73 expression was decreased in the HIV+ groups. Among COPD+ participants %CD4+CD73+ T-cells highly correlated with FEV_1 [Spearman r = 0.45; P = 0.01].



Legend: **indicates significance of p < 0.05.

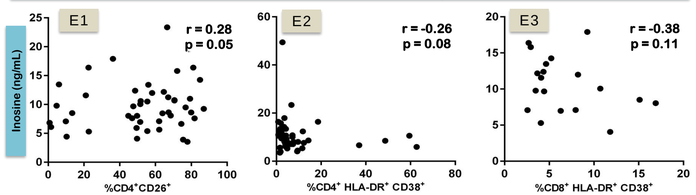
•**C:** HIV+ individuals showed significantly less frequencies CD26 expressing T cells.
- HIV+/COPD+ individuals had the lowest frequencies of T cell CD26 expression compared to the other 3 study groups.
- %CD4+ CD26+ T cells modestly correlated with inosine levels among all 4 study groups. (E1)



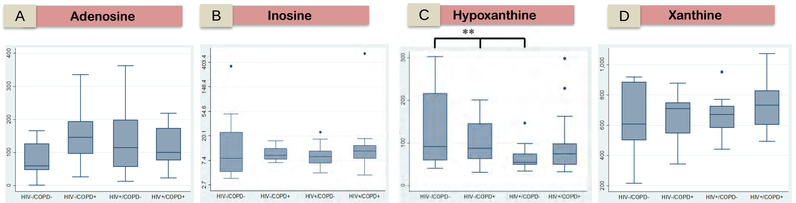
Legend: **indicates significance of p < 0.05.

•**D:** In CD4+ and CD8+, T cell immune activation was increased in HIV+ and/or COPD+ individuals compared to the HIV-/COPD- group.
- In CD4+ T-cells, there was a trend for negative correlation between %CD38+/HLA-DR+ and inosine among all 4 study groups. (E2)
- In the HIV+/COPD+ group, CD8+ T-cells, there was also a trend for a negative correlation between %CD38+/HLA-DR+ and inosine. (E3)

%CD4 CD26 and T cell activation correlations with plasma inosine levels



ADENOSINE PATHWAY METABOLITES

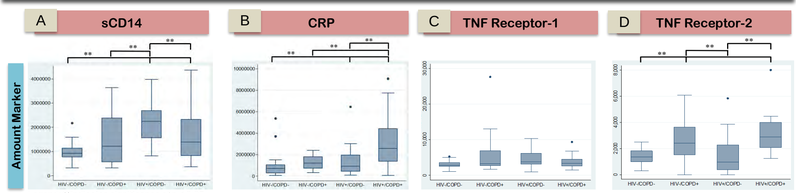


Legend: **indicates significance of p < 0.05.

•**A/C/D:** Adenosine, inosine, and xanthine did not show significant differences between groups.

•**B:** There was a trend for a positive correlation between plasma inosine levels and FEV_1 [r = 0.35, P = 0.06] among COPD(+) participants

SOLUBLE INFLAMMATORY MARKERS AND CORRELATIONS



Legend: **indicates significance of p < 0.05.

		Total (N=52) Spearman [T-value]	HIV- (N=23) Spearman [T-value]	HIV+ (N=29) Spearman [P-value]
1	sCD14 Correlated with			
	Treg CD39+/CD73+	0.22 [0.12]	0.24 [0.27]	0.36 [0.05]
2	CRP Correlated with	Total (N=65) Spearman [T-value]	HIV- (N=22) Spearman [T-value]	HIV+ (N=30) Spearman [P-value]
	Post-albut. FEV_1	-0.25 [0.05]	-0.17 [0.36]	-0.42 [0.01]
	Post-albut. FVC	-0.34 [0.01]	-0.05 [0.76]	-0.57 [<0.01]
	DCLO	-0.30 [0.01]	-0.36 [0.05]	-0.23 [0.18]
	Treg CD26+/39+/73-	0.04 [0.79]	-0.50 [0.02]	0.05 [0.80]
3	TNF Receptor-1 Correlated with	Total (N=66) Spearman [T-value]	HIV- (N=23) Spearman [T-value]	HIV+ (N=30) Spearman [P-value]
	Post-albut. FVC	-0.31 [0.01]	-0.25 [0.17]	-0.29 [0.09]
4	TNF Receptor-2 Correlated with	Total (N=61) Spearman [T-value]	HIV- (N=27) Spearman [T-value]	HIV+ (N=30) Spearman [P-value]
	DLCO	-0.42 [<0.01]	-0.33 (0.10)	-0.43 [0.01]

Legend: albut. = albuterol

•**A/1:** HIV+ individuals showed significantly increased sCD14 levels. The HIV+/COPD+ group had lower sCD14 compared to HIV+/COPD- group. Among all HIV+ participants, sCD14 levels correlated with CD39/73 co-expression on Tregs.

•**B/2:** HIV+/COPD+ showed much greater CRP levels than any other group. In the HIV+ groups, CRP levels highly correlated with post-albuterol levels FEV_1 & FVC.

•**C/3:** TNF-R1 levels were not different between groups. TNF-R1 correlated with FVC readings.

•**D/4:** TNF-R2 showed marked increase in COPD+ groups. TNF-R2 correlated significantly with DLCO.

CONCLUSIONS & FUTURE DIRECTIONS

- Consistent with previous studies, the frequency of CD4+ and CD8+ CD73+ T cells are decreased in HIV infection. We further show that among individuals with COPD, CD73 expression on CD4+ T cells correlates with FEV_1.
- HIV+/COPD+ individuals have the lowest frequencies of CD26+ T cells compared to the other 3 study groups. CD26 expression on CD4+ T cells is lower among HIV+ participants and modestly correlates with inosine levels, which in turn correlate with FEV_1.
- The differences in the components of the adenosine signaling pathway among HIV+ individuals could be a possible mechanism leading to COPD in HIV. Furthermore, given these differences among HIV+/COPD+ and HIV-/COPD+ individuals, strategies targeting the adenosine signaling pathway for COPD treatment may have different results in the HIV-infected population.
- Evaluation of CD26 levels in bronchoalveolar lavage samples are planned.
- Further mechanistic studies should be done to confirm the role of the adenosine signaling pathway in COPD and HIV infection.