

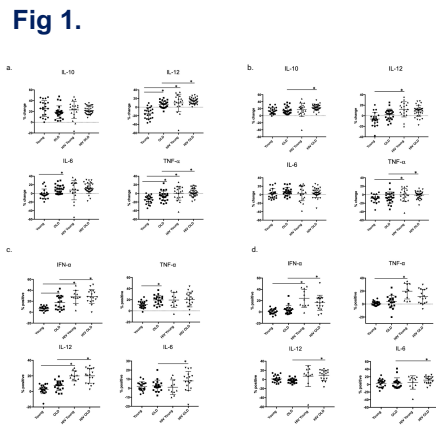
Dectin-1 Stimulation Promotes a Distinct Inflammatory Signature in the Setting of HIV-Infection and Aging



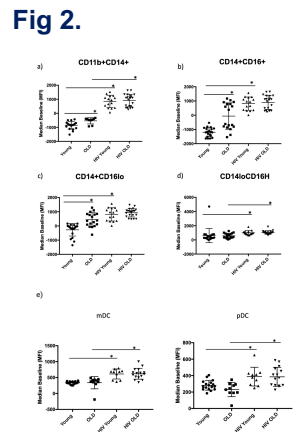
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	Monocytes			Dendritic cells		
	HIV Positive	HIV Negative	p-value	HIV Positive	HIV Negative	p-value
Total	36	45		27	39	
Age Group			0.483			0.215
Younger	14 (38.9)	21 (46.7)		10 (37.0)	21 (53.9)	
Older	22 (61.1)	24 (53.3)		17 (63.0)	18 (46.2)	
Gender			0.214			0.399
Male	21 (58.3)	20 (44.4)		16 (59.3)	19 (48.7)	
Female	15 (41.7)	25 (55.6)		11 (40.7)	20 (51.3)	
Race/Ethnicity			0.014 ¹			0.038 ¹
Hispanic	10 (27.8)	3 (6.7)		5 (18.5)	1 (2.6)	
White	16 (43.9)	33 (73.3)	0.001 ¹	13 (48.1)	32 (82.1)	<.001 ¹
Black	10 (41.7)	6 (13.3)		9 (33.3)	3 (7.7)	
Asian	0 (0)	4 (8.9)		0 (0)	4 (10.3)	
Other	7 (19.4)	2 (4.4)		9 (33.3)	6 (15.4)	
Smoking			0.14 ¹			0.077 ¹
Nonsmoker	11 (30.6)	2 (4.4)		9 (33.3)	1 (2.6)	
Smoker	25 (69.4)	43 (95.6)		18 (66.7)	38 (97.4)	
Recreational Drugs			0.002 ¹			0.777 ¹
None	11 (30.6)	2 (4.4)		9 (33.3)	1 (2.6)	
Cocaine	1 (2.8)	1 (2.2)		1 (3.7)	1 (2.6)	
Heroin	1 (2.8)	1 (2.2)		1 (3.7)	1 (2.6)	
Marijuana	1 (2.8)	1 (2.2)		1 (3.7)	1 (2.6)	
Alcohol	1 (2.8)	1 (2.2)		1 (3.7)	1 (2.6)	
Number of Comorbidities			<.001 ¹			0.024 ¹
0	0 (0)	6 (13.3)		0 (0)	6 (15.4)	
1-3	10 (27.8)	17 (37.8)		10 (37.0)	17 (43.6)	
4-7	19 (52.8)	12 (26.7)		11 (40.7)	11 (28.2)	
>7	7 (19.4)	10 (22.2)		9 (33.3)	5 (12.8)	
History of positive PPD/Quarantiferon			0.131 ¹			0.224 ¹
None	6 (16.7)	2 (4.5)		4 (14.8)	2 (5.3)	
Positive	9 (25.0)	11 (24.4)		6 (22.2)	7 (17.9)	
Diabetes			0.815			0.458
None	9 (25.0)	11 (24.4)		6 (22.2)	7 (17.9)	
Present	5 (13.9)	11 (24.4)	0.272 ¹	4 (14.8)	7 (17.9)	1.000
Cardiovascular Disease			0.911			0.218
None	10 (27.8)	12 (26.7)		10 (37.0)	9 (23.1)	
Present	11 (30.6)	12 (26.7)	0.700	7 (25.9)	9 (23.1)	0.791
CD4 count >200			0.972			0.963
Yes	34 (94.4)	34 (75.6)		23 (85.2)	25 (63.9)	
On ART			0.111			0.74
Yes	4 (11.1)	4 (8.9)		7 (25.9)	2 (5.1)	
HIV VL >100			0.111			0.74
Yes	26 (72.2)	44 (97.8)		34 (124.4)	40 (102.6)	
% life span with HIV			0.111			0.74
<10	26 (72.2)	44 (97.8)		34 (124.4)	40 (102.6)	

¹ Significance assessed using Fisher's Exact Test
² The value of % life span with HIV (since diagnosis) shown in the table represents an interquartile range, with the median (25 percentile, 75 percentile).



Effects of age and HIV-infection on Dectin-1 induced cytokine production in Monocytes and Dendritic cells. HIV-negative young adults (n=21), HIV-negative older adults (n= 24), HIV-positive young adults (n=14), and HIV-positive older adults (n= 22). Dot plots showing percent change in production of IL-10, IL-12 (p70 isoform), IL-6, and TNF-α compared to baseline after stimulation with whole glucan particles (WGP or 1,3/1,6-β-glucan).



Dectin-1 Surface Expression in Monocytes and Dendritic cells. Dot plots of the Mean Fluorescence Intensity (MFI) of Dectin-1 surface expression.

Table 1
Descriptive Statistics stratified by HIV Status (n=81).

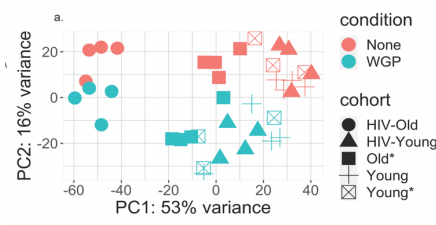


Fig 3. PCA Analysis

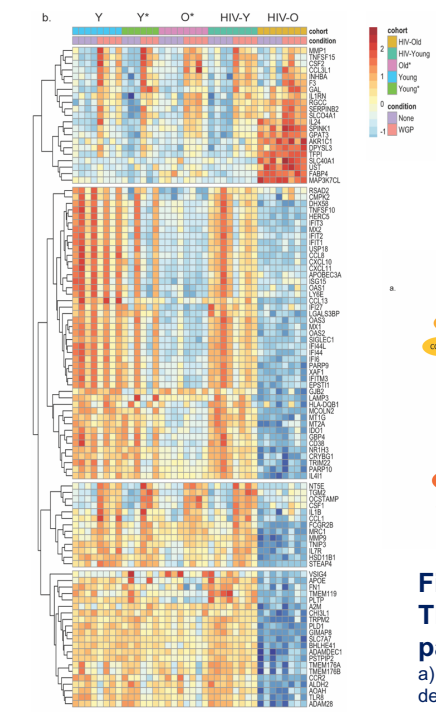


Fig 4. Heat map, Top 100 variable genes. HIV Old vs. all other cohorts, fold change of (FC) 1.2 and FDR < 0.1



Fig 5. Gene Set Enrichment Analysis

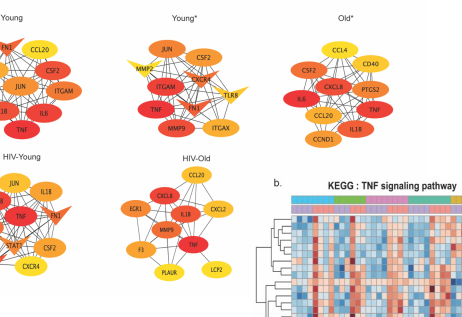


Fig 6. Hub Genes, TNF/Coagulation pathways.
 a) Red color indicates higher degree of interaction, orange/intermediate, yellow/least, Oval = Upregulated DEGs, Triangle = Downregulated DEGs.
 b) Heatmap of KEGG TNF-α and coagulation cascades.

Fig 7. Dectin-1 Stimulation leads to distinct differentiation signatures.

Summary

Dectin-1 is an innate immune receptor that recognizes and binds β-1,3/1,6 glucans on fungi. Dectin-1 function was evaluated in a cohort of HIV-positive and HIV-negative young and older adults. PBMCs were isolated, and stimulated with whole glucan particles (WGP or 1,3/1,6-β-glucan) and used for Multicolor flow cytometry, and sorted to isolate monocytes for RNA seq.

Stimulation with β-D-glucans induced a pro-inflammatory phenotype in monocytes of HIV-infected individuals characterized by increased levels of IL-12, TNF-α, and IL-6, with some age-associated cytokine increases also noted. Dendritic cells showed a striking HIV-associated increase in IFN-α production. Dectin-1 surface expression was increased in the setting of HIV and Aging.

HIV-positive older adults showed a distinct gene signature: Characterized by a robust TNF-α and coagulation response (increased at baseline)– coagulation factors were key hub genes. (Fig 6)

Persistent IFN-α and IFN-γ response (Fig 5)
 Activated dendritic cell signature/M1 macrophage signature upon Dectin-1 stimulation (Fig 7)

Dectin-1 stimulation induced a strong upregulation of MTORC1 signaling in all cohorts, although increased in the HIV-Older cohort (stimulation and baseline). (Fig 5)

The HIV Aging population has a distinct immune signature in response to Dectin-1 stimulation. This signature may contribute to the pro-inflammatory environment that is associated with HIV and Aging.