Understanding NLRP3 Inflammasome Activation at the Intersection of HIV and Aging

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BACKGROUND

With long-term survival of people living with HIV (PLH), increased rates of age-related diseases have been observed. Persistent immune activation and age-related chronic inflammation are hypothesized to be drivers for the development of such diseases. The NLRP3 inflammasome is a multiprotein complex that forms in response to stimulation of pattern recognition receptors by pathogen associated molecular patterns (PAMPs), or by Damage Associated Molecular Patterns (DAMPs). This complex mediates the cleavage of caspase-1, IL-1β and IL-18 to their active forms. Activation of the inflammasome requires a priming step (signal 1) that induces the TLR/NF-κB pathway, and a second step (signal 2), triggered by a PAMP/DAMP and leading to formation of the complex. There is evidence that the development of age-related diseases is related to dysregulated activation of the NLRP3 inflammasome and pathological production of IL-1β. However, significant knowledge gaps in the understanding of human NLRP3 function remain at the interface of HIV and aging. We recruited young (21–40 years) and older (>60 years) HIV-positive and HIV-negative subjects (n = 60). Blood was drawn and Peripheral blood mononuclear cells (PBMCs) were isolated for subsequent experiments. Old*- Older adults with increased co-morbid conditions.

- HIV-positive older adults have increased baseline activation of the inflammasome (↑ Caspase-1 activation, IL-1β)
- HIV-positive older adults have a decreased ability to respond when exposed to PAMPs/DAMPs.
- Differences in Gasdermin expression and formation of the Inflammasome complex demonstrate a dysregulation of Inflammasome machinery in HIV-positive older adults.
- Pathway analysis of isolated monocytes reveals cohort-specific immune and inflammatory signaling that deserve further investigation.

RESULTS

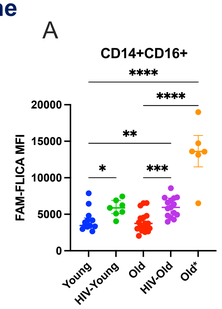
C. Western blots of PBMCs shows increased IL-1β with LPS stimulation in the setting of HIV-infection and Aging, although dampened in HIV+ older adults.

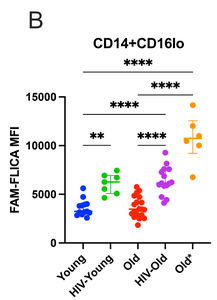
D. Multicolor flow cytometry of monocytes: at baseline increased cytokine levels are seen with increased age, co-morbid conditions and HIV-infection. With LPS/+LPS + ATP stimulation we see a reduced capacity to respond.

E. Gasdermin D transcription is elevated in Older adults and HIV+ Older adults at baseline.

F. Gasdermin D protein levels are increased with increased Age and HIV-infection.

A/B. Caspase-1 activation is increased with HIV and Aging at baseline

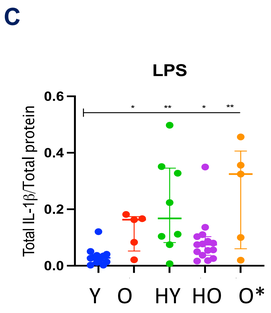




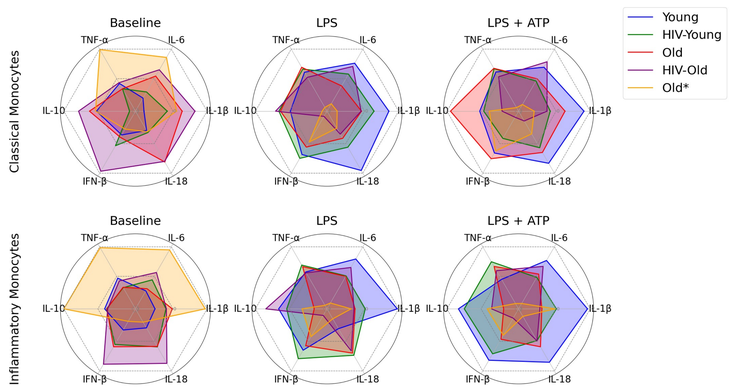
Characteristic Unless otherwise specified values are n (%) tested with a Fisher's exact test	Overall N = 60	HIV- Young N = 14	HIV- Older N = 10	HIV+ Young N = 9	HIV+ Older N = 16	Older* N = 11	p-value per subset: AgexHIV	Older-Only	All Groups
Age[1]	54 (21)	28 (5)	74 (7)	33 (4)	69 (5)	68 (2)	<0.001	0.12	<0.001
Female	26 (48%)	8 (57%)	4 (40%)	1 (11%)	4 (40%)	9 (82%)			
Race	32 (53%)	4 (29%)	1 (10%)	7 (78%)	15 (94%)	5 (45%)	<0.001	<0.001	<0.001
Hispanic Ethnicity	3 (5.0%)	1 (7.1%)	0 (0%)	1 (11%)	0 (0%)	1 (9.1%)	0.4	0.6	0.5
BMI[1]	26.6 (5.1)	25.5 (5.4)	27.4 (5.8)	25.4 (4.0)	26.8 (3.6)	27.8 (6.9)	0.3	0.9	0.5
BMI Categories							0.12	0.2	0.2
BMI 18.5-24.99 kg/m2	23 (44%)	5 (45%)	5 (56%)	6 (75%)	2 (15%)	5 (45%)			
BMI 25-29.99 kg/m2	19 (37%)	4 (36%)	2 (22%)	1 (13%)	9 (69%)	3 (27%)			
BMI >=30 kg/m2	10 (19%)	2 (18%)	2 (22%)	1 (13%)	2 (15%)	3 (27%)			
Diabetes	10 (18%)	0 (0%)	1 (10%)	0 (0%)	6 (46%)	3 (27%)	0.002	0.2	0.006
Hypertension	33 (58%)	0 (0%)	8 (80%)	4 (44%)	12 (92%)	9 (82%)	<0.001	0.7	<0.001
Hyperlipidemia	26 (48%)	0 (0%)	8 (80%)	2 (22%)	8 (80%)	8 (73%)	<0.001	>0.9	<0.001
Metabolic Syndrome	5 (8.3%)	0 (0%)	1 (10%)	0 (0%)	1 (6.3%)	3 (27%)	0.8	0.4	0.14
CAD and/or PVD	4 (6.7%)	0 (0%)	1 (10%)	0 (0%)	3 (19%)	0 (0%)	0.2	0.4	0.2
Asthma and/or COPD	13 (22%)	1 (7.1%)	1 (10%)	2 (22%)	3 (19%)	6 (55%)	0.8	0.074	0.068
Autoimmune or autoinflammatory disease	7 (12%)	1 (7.1%)	2 (20%)	1 (11%)	1 (7.7%)	2 (18%)	0.9	0.7	0.8
Prior history of smoking	14 (26%)	1 (7.1%)	3 (30%)	1 (11%)	5 (50%)	4 (36%)	0.083	0.7	0.12
Current smoker	10 (19%)	0 (0%)	1 (10%)	2 (22%)	2 (20%)	5 (45%)	0.2	0.2	0.04
Recreational drug use in past year	10 (18%)	5 (36%)	0 (0%)	2 (22%)	3 (25%)	0 (0%)	0.2	0.093	0.064
History of positive PPD or Quantiferon	7 (12%)	0 (0%)	0 (0%)	0 (0%)	6 (46%)	1 (9.1%)	<0.001	0.013	<0.001
History of fungal disease	4 (7.0%)	0 (0%)	0 (0%)	1 (11%)	3 (23%)	0 (0%)	0.084	0.1	0.072
CD4 >200	7 (28%)			1 (11%)	6 (38%)	0 (NA%)	0.4	>0.9	0.4
VL >100	0 (0%)			0 (0%)	0 (0%)	0 (NA%)			
On HAART	21 (35%)	0 (0%)	0 (0%)	9 (100%)	12 (75%)	0 (0%)	<0.001	<0.001	<0.001
Percent lifespan with HIV[1]	36 (24)			41 (34)	32 (10)		0.6		0.6
Congenital HIV	2 (8.0%)			2 (22%)	0 (0%)		0.12		0.12

[1] Values are mean (SD) tested with Kruskal-Wallis rank sum test

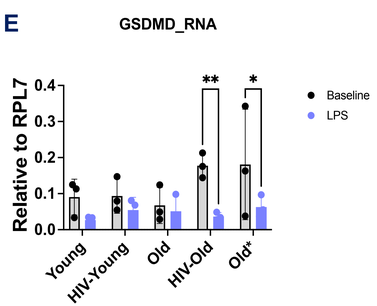
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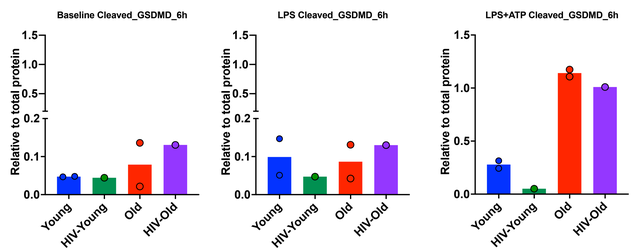
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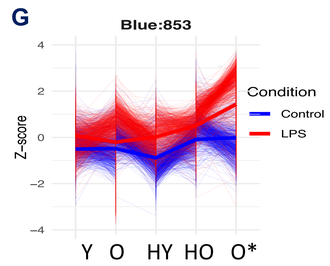
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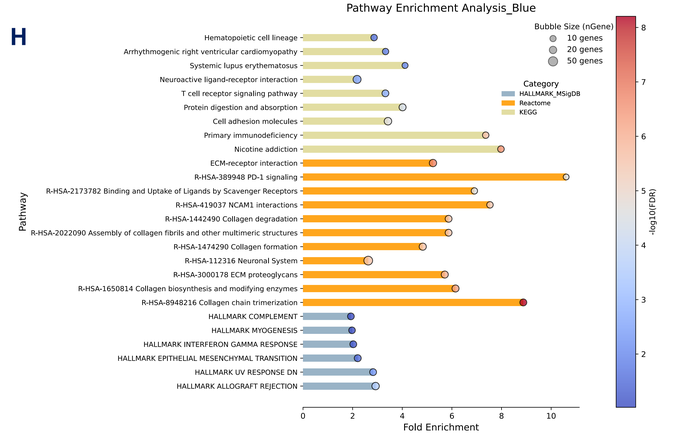
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G/H. Bulk RNA sequencing of Purified Monocytes. WGCNA Analysis shows Cohort-specific transcriptional patterns.

I. Confocal Microscopy showing formation of Inflammasome specks.

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