Healthy HIV-Infected Individuals Harbor HIV in Alveolar Macrophages

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INTRODUCTION

- Despite the advent of HAART, lung infections continue to be a leading cause of death in HIV-infected individuals.
- HIV can persist in monocytic cells and be sequestered in tissue compartments.
- HIV can infect alveolar macrophages, the primary innate immune defense cell in the lung.
- Intrapulmonary HIV burden is maintained by proliferation of macrophages and cell to cell spread of the virus BUT it is unclear whether it is this HIV burden in the lungs that is associated with innate immune dysfunction.
- Alveolar macrophage zinc deficiency impairs phagocytic function in an HIV transgenic rat model, and in alcoholic humans.
- Correction of zinc deficiency in HIV-infected humans delays T-cell loss.

RESEARCH AIMS

1. To quantify HIV viral load within the alveolar macrophage.
2. Determine if alveolar macrophage Proviral DNA is associated with alveolar macrophage phagocytic immune dysfunction.
3. Measure the effect of glutathione and zinc supplementation on alveolar macrophage phagocytosis *in vitro.*

Hypothesis: *Alveolar macrophages are important reservoirs for HIV, contributing to cell-cell spread of the virus within the lung and exhibiting abnormal immune function that contributes to increased risk of lung infections.*

METHODS

Subjects were recruited from Grady Memorial Hospital and the Atlanta community with the following criteria:

Inclusion Criteria:

- HIV subjects with and without HAART therapy
- Age ≥ 25

Exclusion Criteria:

- Liver disease (cirrhosis, bilirubin ≥ 2.0)
- Heart disease (EF < 50%, NYHA Class II-IV, acute MI)
- Renal disease (dialysis, creatinine ≥ 2.0)
- Lung disease (FVC or FEV1 < 80%)
- Diabetes
- Current pregnancy
- Malnutrition (BMI < 17)
- Current tobacco use

Visit 1 Pre-enrollment Evaluation:

H&P, routine blood chemistries, urine pregnancy test, urine cotinine test, spirometry

Visit 2 Intervention:

Blood tests (CD4, HIV viral load), bronchoscopy with BAL

Alveolar macrophage phagocytosis: Alveolar macrophages were isolated from BAL fluid, assessed for viability, and incubated with FITC-labeled *S. aureus*. Phagocytosis assessed with confocal microscopy. Phagocytic index: (% positive cells x mean channel fluorescence)/100.

HIV-1 RNA and DNA quantification: RNA was quantified using Abbott RealTime HIV-1 PCR Assay. Proviral DNA measured using same assay but we removed the reverse transcription stage of amplification protocol.

RESULTS

Table 1: Study Population

VARIABLES	(+) Proviral DNA	(-) Proviral DNA	*p*
N	17	7	
Median Age (IQR)	50 (45-55)	50 (47-53)	0.97
Gender (% male)	47.1	57.1	0.65
Race			0.50
White (n, %)	1 (5.9)	1 (14.3)	
Black (n, %)	16 (94.1)	6 (85.7)	
% HAART	94.1	100	
Median CD4 (IQR)	340 (264-444)	560 (390-730)	0.05
% Undetectable Plasma Viral Load	66.7%	100%	0.08
Median Plasma Viral Load copies/mL (IQR)	0 (0-1506)	0 (0-0)	0.13

Graph 1: HIV Proviral DNA and RNA are Present in Alveolar Macrophages

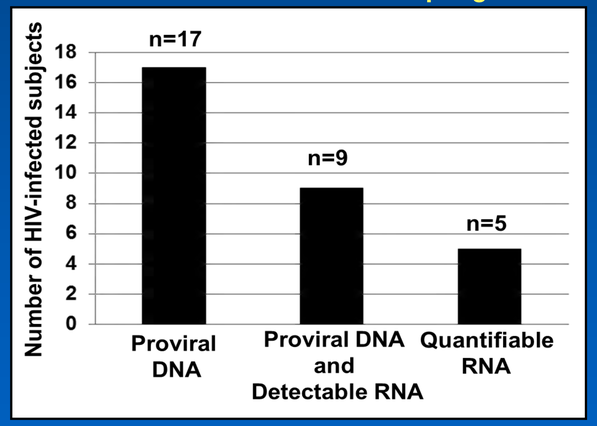


Table 2: Quantifiable HIV RNA in Lung and Plasma

Subjects	Alveolar Macrophage HIV RNA (copies/mL)	Plasma HIV RNA (copies/mL)
1	2305	64,565
2	182	27,542
3	588	undetectable
4	70	undetectable
5	48	undetectable

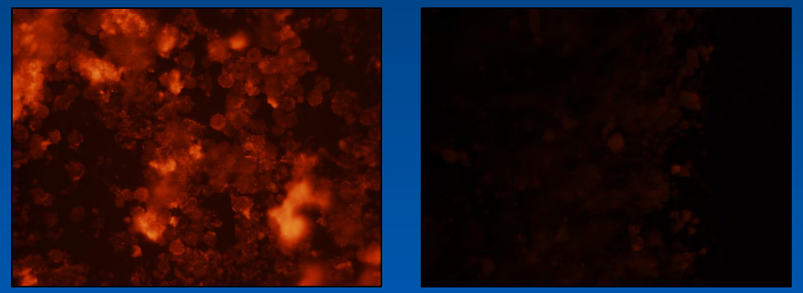
Table 3: Alveolar Macrophage Phagocytic Index in HIV subjects with and without (+) Proviral DNA

Median (IQR)	(+) Proviral DNA	(-) Proviral DNA	*p*
AM Phagocytic Index (% positive x RFU)	10.8 (5.1-31.9)	64.9 (14.0-166.0)	0.04

Table 4: Supplementation of Alveolar Macrophages with 500 µM Glutathione (GSH) and 10 µM Zinc (Zn) Acetate

Median (IQR)	(+) Proviral DNA	(-) Proviral DNA	*p*
Phagocytic Index + GSH	15.9 (11.7-21.2)	55.1 (13.3-91.8)	0.16
Phagocytic Index + Zn	13.0 (10.5-20.8)	71.8 (7.3-94.5)	0.17

Alveolar Macrophage Phagocytosis of *S. Aureus*



(-) Proviral DNA (+) Proviral DNA

CONCLUSIONS

- Alveolar macrophages harbor HIV even in otherwise healthy individuals with undetectable plasma viral loads, representing a potential reservoir for the virus.
- HIV viral replication within alveolar macrophages may impair phagocytosis and other immune functions, leading to an increased risk for lung infections.
- Alveolar macrophage phagocytic function may be improved with GSH and zinc supplementation, but this needs to be corroborated in a clinical trial.
- We speculate that dietary S-adenosylmethionine, a substrate for GSH and cysteine synthesis that is available as a nutritional supplement, and/or zinc could improve host defenses within the lower airways of HIV-infected individuals.

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