HIV INFECTION AND SMOKING DIFFERENTIALLY REGULATE ALVEOLAR MACROPHAGES

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Abstract
Background: HIV infection impacts immune cells in the lung leading to pulmonary complications which persist with antiretroviral therapy (ART). Alveolar macrophages (AM) are principle immune cells in the bronchoalveolar compartment and as such play a pivotal role in host defense against pathogenic microorganisms and tissue remodeling. Examination of the effect of HIV on AM is complicated by the high prevalence of smoking in HIV-infected subjects from the United States. Smoking increases auto-fluorescence of AMs, inhibiting the reliability and resolution of traditional flow cytometry. Cytoimmunity by Time of Flight (CyTOF) utilizes pure metal conjugated antibodies and detection by mass cytometry, which effectively bypasses auto-fluorescence. Here, we utilize CyTOF to comprehensively evaluate the effects of HIV infection and smoking on AMs.

Methods: Bronchoalveolar lavage (BAL) cells from 10 untreated HIV-infected non-smokers, 9 untreated HIV-infected smokers, 10 HIV-seronegative non-smokers and 9 HIV-seronegative smokers was subjected to traditional flow cytometry and CyTOF. Our CyTOF panel consisted of 32 unique markers. Phenotypic analysis using a smaller monoclonal antibody panel was also performed on all samples using traditional flow cytometry.

Results: Compared to those without HIV we found a decrease in CD206 (p = 0.0002), and CD163 (p = 0.002) positive cells, indicating a loss of alternatively activated AMs (M2) caused by HIV infection. The loss of M2 macrophages indicates an increased inflammatory environment. Smoking increased AM expression of CCR5 (p = 0.007) which is a marker of inflammatory macrophages. Together, compared to healthy non-smokers, smoking and HIV increased CXCX4 expression on AM (p = 0.004) demonstrating increased susceptibility to X4 tropic HIV infection.

Conclusions: While the aim of characterizing alveolar macrophages during HIV infection and smoking was our primary goal, this study also demonstrates the sensitivity of mass cytometry, and its ability to detect significant differences between patient groups which would have otherwise been masked by auto-fluorescence. Overall, these findings indicate that HIV and smoking drive alveolar macrophages toward an inflammatory state, leading to an overall more inflammatory environment in the lung.

Background
- We and others have shown that HIV infection profoundly impacts immune cells in the lung1-4.
- Alveolar macrophages (AM) are principal immune cells in the bronchoalveolar compartment and play a pivotal role in host defense against pathogenic microorganisms and tissue remodeling.
- Differential expression of surface markers classifies macrophages to either classically (M1) or alternatively activated (M2).
- M1 macrophages are pro-inflammatory and mediate resistance to pathogens.
- M2 macrophages are anti-inflammatory and promote tissue remodeling.
- High prevalence of cigarette smoking in the HIV-infected population in the United States.
- Smoking increases auto-fluorescence of AM, inhibiting the reliability and resolution of traditional flow cytometry.
- Cytoimmunity by Time of Flight (CyTOF) utilizes pure metal conjugated antibodies and detection by mass cytometry, which effectively eliminates auto-fluorescence.

Methodology
- Bronchoalveolar lavage (BAL) cells and peripheral blood mononuclear cells (PBMC) from 10 untreated HIV-infected non-smokers, 9 untreated HIV-infected smokers, 10 HIV-seronegative non-smokers and 9 HIV-seronegative smokers was subjected to traditional flow cytometry and CyTOF.
- To bypass batch effect, 4 batches of 19 samples (2 BAL and 2 PBMC) plus a PBMC staining control were barcoded, pooled and ran per batch simultaneously.
- The CyTOF BAL panel consisted of 32 markers focused on alveolar macrophages (Fig. 1) while the CyTOF PBMC consisted of 36 markers focused on lymphocytes.
- Phenotypic analysis was performed using traditional flow cytometry methods as well as novel clustering algorithms.
- To evaluate gene expression of AMs, a subset of samples were subjected to RNA-seq. Briefly, AMs were purified using anti-CD14 magnetic microbeads, lysed in TRIzol and RNA was extracted using the RNeasy column. Library construction was made using the Illumina TruSeq mRNA library construction kit. Single-end sequencing was performed using an Illumina HiSeq 4000 for 125 cycles.
- Statistical significance was determined using Mann-Whitney T tests and ANOVA Dunnets multiple comparisons tests.

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Fig 1. BAL CyTOF monoclonal antibody panel. Lineage makers were used to identify immune cell subsets with a focus on AM (left). AMs were then measured for gene expression of 20 unique markers of cytokation, chemokine receptors or pathogen-associated molecular patterns (PAMP) (center and right).

Conclusions
- CyTOF effectively eliminates auto-fluorescence associated with AM and smoking.
- Gene and protein levels of CD163 are correlated with CyTOF but not traditional flow cytometry.
- Levels of anti-inflammatory CD163+CD206+ M2 AM are decreased with HIV infection while expression of HLA-DR is increased, highlighting an inflammatory lung environment.
- Percent of M2 AM is inversely correlated with TNF-α while activated AM are correlated IL-8 and TNF-α.
- Matrix metalloproteinases (MMP), which cleave CD163, are inversely correlated with percent of M2 AM, suggesting a possible mechanism for induced shedding of CD163 in HIV-infected subjects.
- Disease progression in the blood correlates with loss of M2 AM.
- Smoking impacts AM expression of chemokine and PAMP receptors, enhancing the inflammatory environment of the lung.

References