

Dysregulation of Pulmonary Mucosal Immunity During Chronic HIV Infection under suppressive antiretroviral therapy

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Despite antiretroviral therapy (ART), people with HIV (PWH) suffer from high burdens of respiratory infections, lung cancers and chronic lung disease such as emphysema. During ART, residual levels of HIV remain in the lung and smoking further fuels inflammation and immune cell activation. Understanding the unique immune environment within the lungs is critical to unravel the role of the lungs in HIV persistence and the contribution of the lung HIV reservoir to comorbidities. Since 2016, we have screened over 600 PWH attending the McGill Chronic Viral Illness Services clinic in Montreal, Canada, using spirometry and have collected detailed clinical information including tobacco, cannabis and other exposures. These participants are recruited into bronchoscopy studies, along with HIV-negative control participants, in order to obtain bronchoalveolar lavage fluid (BALF) and matched peripheral blood, to compare and contrast HIV burden and perform detailed phenotypic cell characterization. To date, greater than 60 bronchoscopies have been performed. We demonstrated that pulmonary immune restoration is incomplete following ART initiation and even after a decade of ART, HIV-DNA were 13-fold higher in total BAL cells compared to blood. Notably, we characterized CD4+ and double negative CD3+CD4-CD8- T-cells and the main pulmonary mucosal reservoirs while, in alveolar macrophages (AMs), the most abundant cell type within BALF, HIV DNA levels varied in a donor-dependent manner. Pulmonary mucosal CD4+ T cells demonstrated greater levels of immune activation and senescence compared with CD4+ T cells in peripheral blood, and there was robust infiltration of various CCR6+ CD4 T cells subsets—proposed to be cellular reservoirs of HIV, in the lungs than in blood. Notably, PWH frequently develop “CD8 T-cell alveolitis” characterized by accumulation of functionally impaired HIV-specific CD8 T-cells, associated with worse respiratory outcomes. We showed that human pulmonary mucosal CD8 T-cells express low levels of cytotoxic effector molecules, along with significantly less HIV-specific killing capacities than blood CD8 T-cells

due to their perforin deficiency. We showed that smoking and HIV infection could promote cytotoxic CD8 T-cell retention in small airways through different mechanisms: smoking likely increases recruitment and retention of GzmB⁺ CD8 Trm via CXCR6 and CD103, while HIV might be associated with CD8 non-Trm recruitment from the periphery via CX3CR1. Moreover, CD8 effector memory cells were significantly positively correlated with relative abundance of the most abundant bacterial families in lung microbiota including *Prevotellaceae*, *Pasteurellaceae*, *Streptococcaceae*, and *Veillonellaceae*. Higher levels of HIV-DNA in blood associated with decreased abundance of most lung bacteria. Taken together, these findings support the concept that the lungs possess a unique immunological milieu conducive to HIV reservoir persistence despite long-term suppress ART. Approaches aimed at reducing the size of the HIV reservoir will need to account for the unique immunological milieus of organs such as the lungs when developing novel immunotherapies.