



Exploring Alveolar Macrophages as HIV Reservoirs

Professor David M. Guidot

EXPLORING ALVEOLAR MACROPHAGES AS HIV RESERVOIRS

Although pulmonary disorders remain the highest cause of mortality amongst individuals living with chronic infection by the human immunodeficiency virus (HIV), many of the mechanisms underlying their development remain unknown. Professor David Guidot and his team at Emory University are exploring these mechanisms in order to develop novel treatments targeting the alveolar spaces in the lung.

HIV Influence on Pulmonary Disease

Human Immunodeficiency Virus (HIV) is the retrovirus responsible for the development of Acquired Immune Deficiency Syndrome (AIDS). AIDS leaves the host vulnerable to many opportunistic infections that can be fatal. Thanks to the widespread use of anti-retroviral therapies (ART), many individuals infected with HIV can live well for decades with the illness. In spite of this, lung diseases continue to be the primary cause of death amongst individuals living with HIV. As well as being vulnerable to infections, HIV promotes the risk of non-infectious lung diseases such as asthma, chronic obstructive pulmonary disorder, pulmonary hypertension and lung cancer. New therapies for HIV also bring new complications such as sarcoidosis.

So why is lung disease so high in this population? As Professor David Guidot explains: 'We have identified that the localised immune functions within the lung remain severely impaired even when other components of the immune system appear to respond very well to anti-viral drugs. In particular, the small airways are relatively deficient in antioxidants as well as being deficient in zinc, and these deficiencies impair local immune function in the airways.' Professor Guidot has brought together a multidisciplinary team to identify previously unrecognised factors that determine an individual's risk for HIV-related lung disease. 'The goal of our studies is to determine if we can improve the immune function in the lungs of people living with HIV, using dietary supplements of zinc and an antioxidant known as SAME (S-adenosylmethionine), with the hope that this will make them even healthier and significantly decrease their risk of pneumonia and other lung diseases,' he tells us.

Although the CD4+ T-lymphocytes are the primary target of HIV, the virus can also infect the innate immune cells in the airways known as alveolar macrophages. Macrophages are large white blood cells that ingest foreign particles and microorganisms. The team hypothesises that infected macrophages may be acting as reservoirs of HIV infection within the alveolar macrophage pool (alveoli are tiny air sacs in the lung that allow for rapid gaseous exchange). This is a problem because these reservoirs are relatively long lived, less susceptible to ART and stored in membrane-bound compartments that are inaccessible to antibodies and small compounds. Therefore, therapies that enhance macrophage function could potentially decrease both lung infections and chronic airway diseases in individuals living with HIV.

Potential Mechanisms of Impaired Immune Response

Professor Guidot and his team have two primary questions to answer. Firstly, does the alveolar macrophage pool serve as a reservoir of HIV even when peripheral viral suppression has been achieved by ART? Secondly, how does this reservoir alter the environment within the alveolar space and impair alveolar macrophage immune function? To find the answers to these questions, it was necessary to first determine the mechanisms by which HIV infection damages the lungs. Experimental evidence suggests that HIV inhibits antioxidant defences within the alveolar space and causes severe oxidative stress. This occurs through the induction of zinc deficiency in the microenvironment, which prevents the alveolar epithelium and macrophages from generating glutathione and other antioxidants that are critical to maintaining

a healthy redox potential within the alveolar space. By this process, HIV promotes its own ability to infect macrophages and accumulate a large pool of intracellular proviruses, thereby producing a large HIV reservoir. The resulting impaired innate immunity leads to both the increased risk of infections and resistance to clearing the viral reservoir. Research has also shown that the relatively high concentrations of HIV-related viral proteins in the airway lead to lung epithelial barrier dysfunction and are also toxic to alveolar macrophages independently of direct viral infection, which further impairs the immune system.

Professor Guidot explains how he came to work in this field: 'We had been studying the mechanisms by which alcohol abuse renders individuals susceptible to pneumonia and lung injury. We used animal models to identify several novel such mechanisms, including profound depletion of the antioxidant glutathione in the airways and a block in zinc transport into the airways. We translated these findings to clinical studies and have a clinical trial underway in which we are testing if dietary supplementation with zinc and/or S-adenosylmethionine – also known as "SAME", which is converted in the body to glutathione – can improve immune function in the lungs of alcoholics. About eight or nine years ago we started examining HIV transgenic rodents and found that chronic HIV expression causes remarkably similar defects that we had identified in the "alcoholic lung". We published several research papers showing that chronic HIV expression also causes glutathione and zinc deficiency in the airways, and that dietary zinc supplementation can improve lung health in these animals.'

'People living with HIV have a much better prognosis and with current medications can now live for many decades. However, they remain much more susceptible to pneumonia and other lung diseases than people without HIV'



HIV Transgene Expression in Rat Models

A HIV transgene rat model was used to investigate the mechanisms by which HIV infection of the alveolar macrophage alters macrophage activation and impairs immune function. In these HIV rat constructs, the gag and pol genes have been deleted resulting in a pro-virus insertion in which there is no viral replication or infection. However, other HIV

related proteins are expressed with efficient viral gene expression in many organs. The rats develop muscle wasting, cataracts, nephropathies and immune deficiencies, all of which are consistent with an AIDS-like phenotype.

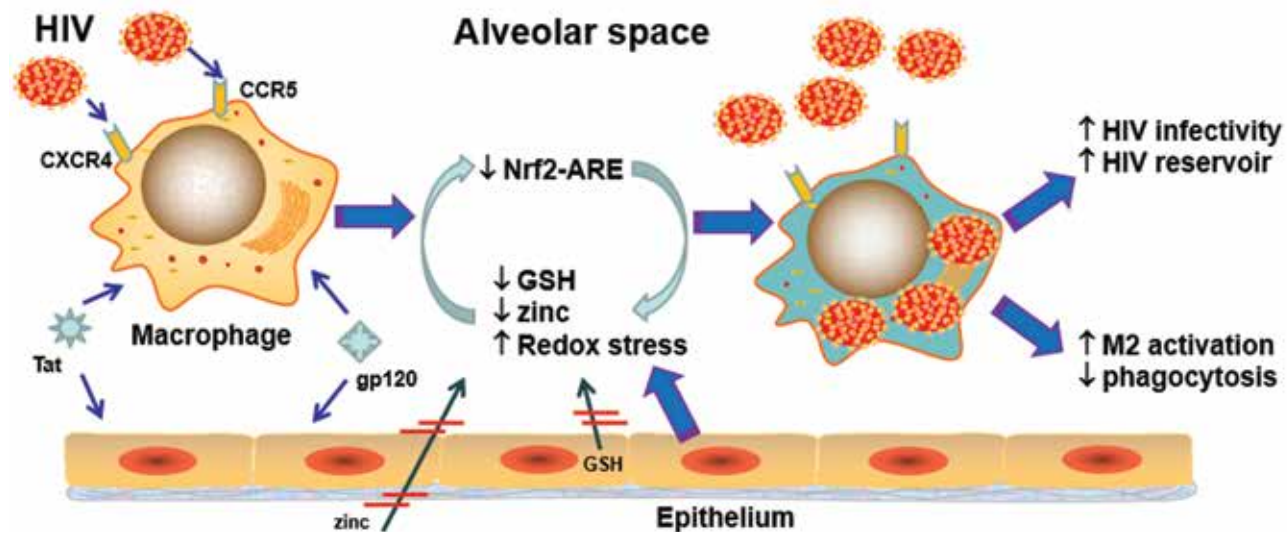
The first study aimed to determine the effects of HIV transgene expression on alveolar epithelial barrier function in rats.

The researchers found that although inflammatory markers were similar in both wild type rats and HIV transgenic rats, oxidation was three times higher in HIV transgenic rats. This oxidative stress, combined with impaired epithelial barrier function and altered expression of critical tight junction proteins, suggests novel mechanisms by which HIV infection renders individuals susceptible to acute and chronic forms of lung disease.

The second study investigated the importance of pulmonary zinc status in maintaining alveolar macrophage immune function. Alveolar macrophage maturation and function depend on a pathway involving granulocyte-macrophage colony-stimulating factor (GM-CSF). Macrophages respond to this through the GM-CSF receptor, which has a binding subunit and signalling subunit. Alveolar macrophages from alcohol-fed rats have significantly fewer receptors and decreased signalling, resulting in decreased immune function. It is hypothesised that a similar process occurs with HIV infection. Professor Guidot and his colleagues found that HIV transgenic expression selectively decreased alveolar macrophage expression of the GM-CSF signalling subunit and impaired bacterial phagocytosis (the ingestion of bacteria by specialised cells). In vitro studies also showed similar findings. This suggests that the capacity of alveolar macrophages to maintain a robust signalling response to GM-CSF is dampened by chronic HIV related protein expression.

This mechanism appears to involve zinc deficiency. Therefore, the study also examined the role of zinc deficiency as a potential mechanism for these detrimental effects. The team found that HIV transgenic rats have significantly lower levels of zinc in the alveolar space and macrophages when compared with wild type rats. In addition, treatment of cell lines with a zinc chelator (which lowers zinc levels) decreased signalling receptor expression and phagocytosis, whereas treatment with zinc restored phagocytic function and zinc levels in alveolar macrophages. This indicates that zinc plays a role in the regulation of cellular glutathione and protects cell membranes from oxidative damage.

They also observed age-related effects on zinc levels, noting a progressive decrease in intracellular zinc levels of alveolar macrophages in rats as they aged. No corresponding decrease was observed in wild



type rats. The increase of AIDS-like pathologies in ageing rats implies a progressive burden of HIV related protein expression and consequent tissue injury over time.

Translating Animal Models into Clinical Trials

In the experimental model, zinc supplementation restored alveolar macrophage innate immune function in HIV transgenic rats. The research team hypothesised that treatment with zinc and a glutathione precursor could improve macrophage function in immunological non-responders (individuals who display a suboptimal response to ART). In order to determine if adjuvant therapy with dietary zinc and SAME decreases the HIV reservoir pool within the alveolar macrophage pool, Professor Guidot and his colleagues first designed a prospective cross-sectional study to quantify the HIV viral load within the alveolar macrophages in a cohort of healthy HIV-infected individuals. Evidence supports the compartmentalisation of HIV between lungs and peripheral blood, suggesting that alveolar macrophages could be a reservoir for HIV. They also wanted to determine if alveolar macrophage pro-viral DNA was associated with immune dysfunction. In this study, alveolar macrophages were found to harbour HIV even in otherwise healthy subjects with undetectable plasma viral loads, supporting the hypothesis of a potential reservoir for the virus. They also found that subjects with positive pro-viral DNA had a significantly lower phagocytic response compared with other subjects. This suggests that supplemental therapies to ART may be necessary to target alveolar macrophage reservoirs and improve lung function.

Professor Guidot and his team followed this study with a pilot clinical trial that treated immunological non-responders with zinc and SAME dietary supplements in order to improve alveolar macrophage function. The treatment appeared to increase peripheral CD4 T-cell count (immune cell count) in infected individuals within just two months. After twelve months, the researchers hope that treatment will continue to increase peripheral CD4 counts in non-responders and decrease the HIV reservoir in the alveolar macrophage pool. They anticipate that this will correspond with improvement in both alveolar and systemic biomarkers of HIV-induced stress. Currently they are following up this promising pilot study with a larger clinical trial in which individuals living with HIV are treated with these dietary supplements for two years to determine if prolonged therapy will restore overall airway health, including zinc bioavailability, anti-oxidant defences, and innate immune capacity.

‘The goal of our studies is to determine if we can improve the immune function in the lungs of people living with HIV, using dietary supplements such as zinc and an antioxidant known as SAME, with the hope that this will make them even healthier and significantly decrease their risk of pneumonia and other lung diseases’

Moving Towards the Future

Guidot and his team are not finished yet: ‘The next steps include analysing the results of our clinical trial to determine if this dietary strategy is effective. In parallel, we are testing other potential therapies including naturally occurring compounds found in plants that can activate multiple anti-oxidant defences simultaneously. Although we are confident that zinc plus SAME will have beneficial effects, there will likely be room for even greater improvement in lung immune function in these individuals.’ At the same time, the team have evidence that HIV affects the functions of the cells lining the airways, and people living with HIV are also at increased risk of emphysema, lung cancer and fibrosis (scarring) of the lung. Therefore, they ‘plan to eventually determine if these strategies that are focused on improving lung immunity will also have benefits for the structural health of the lung.’



Meet the researcher

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Professor David M. Guidot, MD, is Director and Professor of Medicine in the Division of Pulmonary, Allergy, Critical Care and Sleep Medicine in the Emory University Department of Medicine. After obtaining his MD from the University of Michigan, Professor Guidot trained in Internal Medicine at the University of Minnesota and later completed clinical and research fellowship training in Pulmonary and Critical Care Medicine at the University of Colorado. He was recruited to Emory University in 1995 and has served in numerous leadership positions at that institution including Chair of the University Research Committee, Director of the Emory Alcohol and Lung Biology Center and Training Program, and Section Chief at the Atlanta Veterans Affairs (VA) Medical Center. Since starting an independent research laboratory at the Atlanta VA Medical Center in 1995, which later moved to the Emory University campus in 2009 when he assumed his current position, he has been funded by the National Institutes of Health (NIH), the Department of Defense, the American Lung Association and the VA for his research that focuses on the mechanisms by which chronic insults such as alcohol abuse and HIV infection cause oxidative stress and render individuals more susceptible to pneumonia and acute lung injury. In 2010, Professor Guidot was named the Jeffery R. Pine Endowed Chaired Professor of Medicine at Emory University. He continues to lecture medical students, residents, fellows and other faculty as well as mentoring post-doctoral researchers and physicians. He currently chairs the Institutional Training Grants (T32) Review Panel for the National Heart, Lung and Blood Institute (NHLBI) at the NIH.

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