

Project title: Studying the effects of HIV-1 infection of macrophages/DC on immunity to Tuberculosis

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Macrophages and dendritic cells (DC) are sentinel cells of innate immunity involved in recognition of microbial pathogens, initiation of early non-specific host defences and regulation of specific adaptive immune responses. They also function as host defence effector cells involved in killing of intracellular pathogens. The interplay between these cells and T-cell adaptive immune responses underpin our understanding of immunity to *Mycobacterium tuberculosis* (MTb), the success of which is reflected in the observation that only 10% of 2 billion people who become infected with MTb develop active Tuberculosis (TB). Co-infection with Human immunodeficiency virus (HIV)-1 however results in a 50-fold increase in active TB and is a major cause of morbidity and mortality in Africa. As the HIV-1 pandemic extends into South Asia, China and Russia, HIV-1 /MTb co-infection, coupled inevitably with antiretroviral and anti-MTb drug resistance, threatens global health on a colossal scale. Successful vaccination strategies are urgently needed, but have been out of reach for either of these pathogens and remain priorities of global research initiatives. To this end we aim to obtain greater insight into the immunopathology of co-infection to inform novel vaccination and immunotherapeutic strategies.

Intracellular control of MTb is critically dependent on an intercellular paracrine loop in which IL-12 production by infected macrophages /DC drives IFN γ production by MTb-reactive T-cells, that in turn augments intracellular killing of MTb in infected cells. HIV-1 can infect macrophages, DC and activated T-cells, and whilst the consequent depletion of T-cell population is well described, the effect of HIV infection on innate immune macrophage and DC responses to MTb and to IFN γ have not been investigated in detail. We aim to use ex vivo models of HIV-1 /MTb co-infection to study these questions and to assess at a molecular level macrophage /DC and T-cell responses in vivo using tuberculin skin testing of HIV-infected and un-infected patients.

Cultured human macrophages and DC will be derived from primary monocytes. HIV-1, produced from molecular clones or by propagation in peripheral blood mononuclear cells, will be used to infect macrophages and DC. The effect of HIV-1 will be investigated by comparison of HIV infected and uninfected cells following co-infection with MTb, with and without IFN γ stimulation. The host cell response will be investigated by assessment of innate immune intra-cellular signalling events, genomic expression arrays, cytokine protein arrays and specific phenotypic characteristics. The effect of HIV

on internalisation, intracellular trafficking and killing will also be assessed. The mechanism of changes attributable to HIV-1 will be investigated by expression of individual HIV components within macrophages and DC using lentiviral vectors and inhibition of individual HIV components using small inhibitory RNA. The immune response to tuberculin skin testing in HIV-1 infected and un-infected patients under investigation for TB (both in UK and African settings), will be studied by quantitative PCR analysis of gene expression and confocal immunofluorescence microscopy of skin biopsies at the site of inoculation.

This project encompasses the comprehensive range of laboratory techniques with emphases on cell biology, immunology, virology, microbiology, molecular biology and bio-informatics. All the equipment and expertise required for this project are available locally. The project will involve active collaboration with additional groups within Infection & Immunity at UCL, drawing from local expertise at the interface of immunology and virology (Professor Mary Collins, Professor Greg Towers, Dr Richard Jenner & Dr Paul Kellam). Recruitment of clinical samples for in vivo studies is underway through collaboration with clinical researchers at UCL (Professor Rob Miller), London School of Hygiene & Tropical Medicine (Dr Mike Brown) and University of Cape Town (Dr Keertan Dheda). Experience of the clinical context of the immunopathology under study will be acquired in the HIV /TB clinical service at UCLH under the supervision of Dr Noursadeghi and possibly through the collaboration with the University of Cape Town.