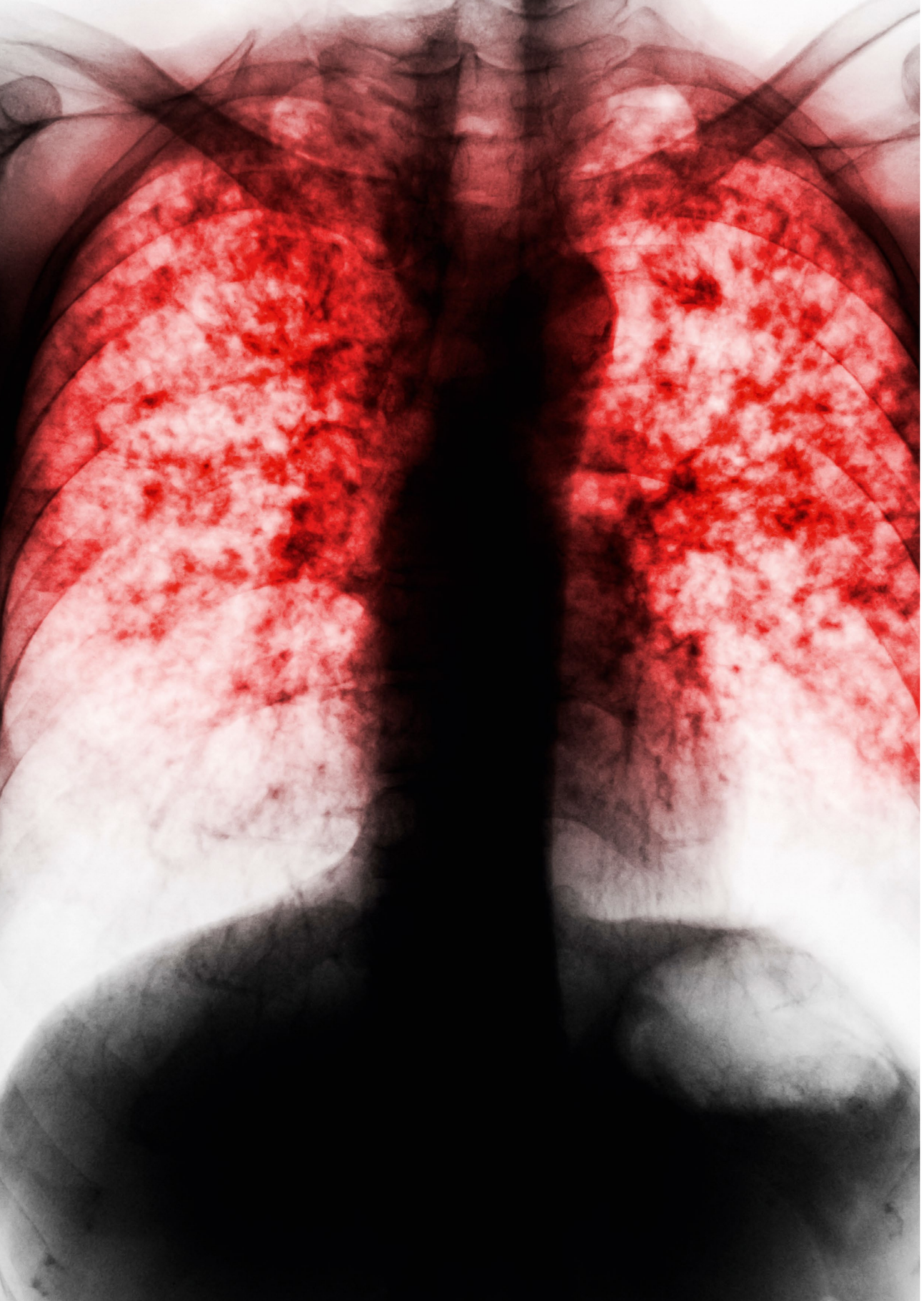


Uncovering new strategies to reduce TB susceptibility in HIV-infected individuals

Dr Henry Mwandumba is a Clinician Scientist at the Malawi-Liverpool-Wellcome Trust Clinical Research Programme, and Consultant Physician at Queen Elizabeth Central Hospital, Blantyre, Malawi in Southeast Africa. He leads a group of researchers with two main goals; to understand how humans develop immunity to TB in the lungs, and why the risk of TB is greater during HIV infection.

Tuberculosis (TB), caused by the bacterium *Mycobacterium tuberculosis*, is a serious cause of disease and mortality worldwide, especially in developing countries. Co-infection with the human immunodeficiency virus (HIV) aggravates this situation, and puts intense pressure on national healthcare services in sub-Saharan Africa, where up to 80% of TB patients are HIV-infected, and where TB is now the leading cause of death in HIV-infected individuals.

Dr Mwandumba believes that a better understanding of the mechanisms underlying resistance and susceptibility to TB is essential to reduce the incidence of HIV-associated TB, and to develop new therapies for TB in general.

ANTIRETROVIRAL THERAPY IS NOT ENOUGH

Antiretroviral therapy (ART) is the standard treatment for HIV, and it usually involves a combination of drugs that work together to slow down viral replication. $CD4^+$ T cells are a subset of white blood cells that are targeted by HIV. Successful ART therapy leads to an increase in this cell population, thus boosting the body's immune defences.

While ART has significantly reduced the rates of HIV and HIV-associated TB worldwide, TB is still 5-10 times more prevalent in HIV-infected adults on ART than in HIV-uninfected individuals. The risk of developing active TB is increased in HIV-infected individuals, even before significant $CD4^+$ T cell depletion occurs. These facts indicate that $CD4^+$ T cells are not the only determinant of TB risk, and that increasing their numbers alone is not enough to maintain full protection against TB.

The increased risk of TB in HIV sufferers also suggests that HIV infection alters the immune environment in the lung, since TB most often infects the lung. The important question of how HIV impacts lung immunity and susceptibility to TB has dominated Dr Mwandumba's research for over a decade.

ALVEOLAR MACROPHAGES – THE BIG EATERS

Alveolar macrophages (AM) are highly differentiated immune cells that occupy the interface of the external environment and the alveolar tissue of the lungs. They are often the first professional phagocytes encountered during TB infection, and like all phagocytes, they are capable of phagocytosing (eating), internalising and delivering pathogen-derived material to acidic, pathogen-degrading lysosomes inside the cell.

The endosomal-lysosomal system is an extremely effective barrier against bacterial infection, comprising three major activities: phagocytosis, endocytosis, and endosomal acidification. During phagocytosis, AM engulf bacteria, which then become enclosed into intracellular organelles called phagosomes. During endocytosis, phagosomes are trafficked through the AM in compartments called endosomes, eventually fusing with lysosomes, which are specialised organelles containing degradative enzymes. Lysosomal enzymes function under acidic conditions, and progressive endosomal acidification is therefore a prerequisite for a fully functioning endosomal-lysosomal system.

ANSWERS FROM HUMAN LUNGS

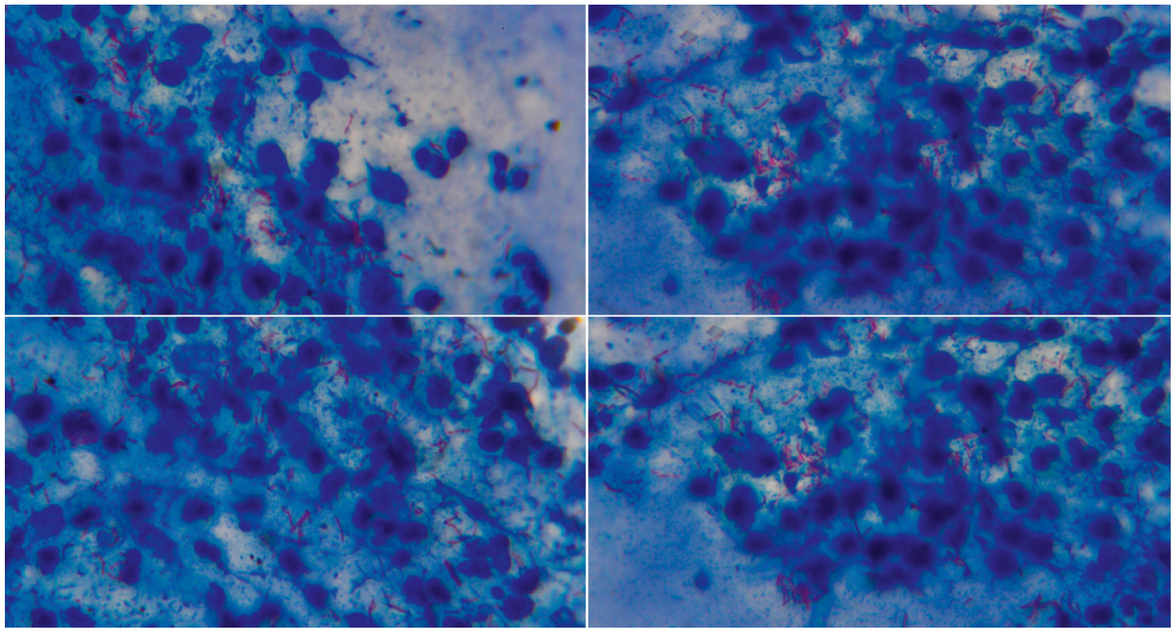
As part of a long-standing research programme at Queen Elizabeth Central Hospital, Dr Mwandumba's group has access to lung samples from HIV-infected, TB-infected, co-infected, and healthy human volunteers. The group employs a combination of microscopy, flow cytometry (cell staining and counting), and molecular biology techniques to visualise immune cells in these lung specimens, to assess their function, and to detect infection-induced changes in their behaviour.

TB INFECTION ARRESTS AM PHAGOSOMES

It is well documented that HIV and TB can synergise and provoke immune responses that exacerbate both infections. However, despite the importance of AM in lung immunity, very few researchers have investigated their physiology in TB patients. Dr Mwandumba's research addressed this deficit in a study examining the properties of human AM obtained from the lungs of patients infected with TB.

The study revealed that the ability of AM to phagocytose synthetic beads was not affected by HIV or TB infection. Furthermore,

My vision is to see a substantial reduction in this high burden of HIV-associated TB by undertaking relevant clinical research



the endosomal/lysosomal activities of AM were equally intact in cells derived from HIV- or TB-infected individuals, when using synthetic markers for endocytosis and phagosomal acidification. However, *Mycobacterium tuberculosis*-containing phagosomes in TB-infected AM failed to acidify and fuse with lysosomes, thus halting the progression of the endosomal-lysosomal system, in agreement with earlier cell culture-based studies.

Dr Mwandumba and his colleagues were the first to demonstrate that *Mycobacterium tuberculosis* arrests the endosomal-lysosomal system in this way during human TB infection. This suggests that the lungs of patients with TB and HIV might be low in critical anti-microbial cytokines (immune signalling molecules), which are produced upon macrophage activation and phagosome acidification.

IMPACT OF HIV ON ALVEOLAR MACROPHAGES

The role of AM in immunity against respiratory pathogens is undisputable. It is also well accepted that HIV infection increases susceptibility to lower respiratory tract infections such as TB. What is less clear however, is the exact consequence of HIV infection on the physiological functions of AM.

Within the past decade, evidence has emerged for two distinct AM populations, small and large. This discovery prompted Dr Mwandumba's group to examine the impact of HIV infection on AM function in a highly controlled manner, accounting for both small and large AM. The study included AM from healthy, asymptomatic, antiretroviral therapy naive HIV-1-infected and HIV-1-uninfected adults, in order to identify specific defects in AM function that might explain the increased susceptibility to lower respiratory pathogens in HIV-infected individuals.

I believe that public health interventions, informed by relevant data on mechanisms underlying increased susceptibility to TB, are required for effective TB control

The group developed novel assays to detect HIV-infected AM by combining flow cytometry with a technique known as fluorescence *in situ* hybridisation (FISH). In simple terms, they used detectable fluorescent probes to bind HIV-specific messenger RNA, allowing the group to detect and track HIV in different cell populations. They also measured AM phagocytic capacity and proteolysis (enzymatic degradation of proteins) using reporter beads.

They found that HIV imparts differential effects on key AM functions. Small AM were more likely to be infected by HIV, resulting in impaired phagocytosis. The effects of HIV on proteolysis, which does not require direct viral infection, were more generalised. These findings provide new insights into how HIV alters lung immunity and highlights the small AM as an important reservoir for HIV infection. The study also highlights small AM as a potential therapeutic target to improve pulmonary immunity and reduce the susceptibility to TB and other lower respiratory tract infections in HIV-infected individuals.

WHAT HAPPENS DURING ANTIRETROVIRAL THERAPY?

Successful ART halts HIV replication in host immune cells, and should promote immune recovery. However, TB rates in HIV-infected individuals remain significantly higher than in healthy individuals, even after three years on ART. Dr Mwandumba's group set out to understand this by investigating both AM and CD4$^+$ T cell functions in lung samples from healthy vs. HIV-infected adults.

Reassuringly, HIV viral load was lower and CD4$^+$ T cells were more abundant in HIV-infected adults on ART therapy. TB-induced AM responses in individuals on ART for at least four years were similar to non-infected adults. However, those on ART for less than four years exhibited impaired AM responses. Influenza-specific CD4+ T cell responses were intact in all individuals on ART, while mycobacterium-specific CD4+ T cell responses were impaired in individuals receiving ART, regardless of therapy duration. These findings highlight the impairment of TB-specific AM and CD4$^+$ T cell responses in the lungs of HIV-infected individuals, and may explain why the risk of TB is higher during HIV infection, despite apparently successful ART.

PAVING THE WAY FOR NEW THERAPIES

The work carried out by Dr Mwandumba's group to date has uncovered some important aspects of HIV- and TB-induced effects on lung immunity. It has helped to fill the knowledge gaps surrounding what constitutes protective immunity against TB in human lungs, and why the risk of TB is greater in HIV-infected individuals, despite long-term ART. Ultimately, this and future work will further advance this area, and will contribute to novel therapeutic strategies and public health interventions aimed at reducing the incidence of HIV and TB co-infection, and TB incidence more generally.

Q&A

What has been the biggest technical challenge with your research to date?
We conduct internationally competitive cutting-edge biomedical research in an area with a high disease burden but limited resources for research. We are able to do so because we have invested a significant amount of time and resources into establishing appropriate infrastructure, developing unique and novel assays to enhance our work, as well as developing and retaining highly skilled and competent young researchers. Reaching and maintaining all these goals have been the biggest technical challenges of my research career so far.

Does TB also coincide with other infections, as it does with HIV?
Co-infection with TB and infections other than HIV has been reported in some HIV-infected and HIV-uninfected individuals but there is lack of a direct link or synergy between these infections as we see with HIV and TB.

Your research revealed that it takes at least four years on ART before HIV-infected individuals achieve similar TB-specific immune responses to HIV-uninfected individuals. Why do you think this takes such a long time?
This likely reflects the time it takes for ART to reverse the direct (killing of HIV-infected CD4$^+$ T cells) and indirect (activation and accelerated death of immune cells) effects of HIV in various parts of the body in order to create an environment that allows repopulation of the lung with new TB-specific and non-specific immune cells.

Is it worthwhile vaccinating against TB in areas with high incidence rates of HIV infection?
BCG vaccine, the only TB vaccine currently available, is not recommended for use in HIV-infected individuals. The vaccine contains an attenuated (reduced virulence) strain of bovine TB, which can cause disseminated TB in individuals with weakened immune systems, including those infected with HIV. It is therefore not worthwhile giving this vaccine to HIV-infected individuals in areas with high incidence of HIV infection.

How effective is antibiotic treatment against TB in HIV-infected individuals?
Antibiotics currently available for TB are effective for the treatment of TB both in HIV-uninfected and HIV-infected individuals provided they are taken for the recommended period of time, treatment doses are not missed, and the TB is not resistant to the antibiotics used.

Detail

RESEARCH OBJECTIVES

Dr Henry Mwandumba's aim with his research is to gain a greater understanding of how humans develop immunity to TB in the lung and why the risk of TB is increased with HIV.

FUNDING

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COLLABORATORS

Key partners

- University of Malawi College of Medicine, Malawi
- Liverpool School of Tropical Medicine, UK
- Queen Elizabeth Central Hospital, Malawi
- Cornell University, USA
- University of Massachusetts, USA

Collaborators

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- Prof Bertie Squire, Liverpool School of Tropical Medicine
- Prof Paul Clapham, University of Massachusetts

BIO

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