

Immune responses

Tuberculosis remains a global epidemic to rival any other. **Dr Gilla Kaplan** describes her illuminating research into how a comprehensive understanding of tuberculosis as a multiplicity of strains and host-pathogen interactions could contribute to control of the epidemic



Firstly, could you outline the primary objectives of your research and its intended outcomes?

In general, I am interested in the host immune response to tuberculosis (TB) and leprosy, and in gaining an understanding of both what regulates pathogenesis and of how to modify the immune response to reduce pathogenesis, so the long-term damage from the infection is minimised.

Diseases like leprosy and TB are caused by microbacteria: leprosy by *Mycobacterium leprae* and TB by *Mycobacterium tuberculosis* (MTB). After infection, the major clinical symptoms are not merely manifestations of the infection itself, but also of the host immune response attempting to contain or control it. To understand what is happening, you have to look at the interaction between the bacterium, or the pathogen, and the host. In that interaction lies the explanation as to how the immune response regulates the severity of disease, the

rate of progression of disease, and/or the extent of tissue damage.

Could you describe the current state of global TB infection and treatment: how widespread is the disease now and how urgent is the case to tackle it?

TB is at critical levels: there is probably more disease now than ever before. There is no real indication that the control programmes that have been in place since the discovery of antibiotics have recently had a major protective or preventive effect on the disease burden. We try to understand why this is happening and I think there are three reasons. One is that TB is simply very difficult to control, even with the multiplicity of antibiotics that we have. Second is that the HIV epidemic in many countries overlaps with the TB epidemic and the immune suppression of HIV weakens the immune response which would protect against TB. That has created a significant increase in susceptibility and progression from infection to active disease.

Finally, all the years of antibiotic treatment – sometimes haphazard and discontinuous treatment which was not well controlled – has given rise to multi-drug resistance. Although antibiotics were expected to cure infectious diseases and solve the problem, we are losing that weapon as the bacteria become resistant to the drugs. And they are doing it faster than we can develop new drugs. So we're going back to a time when, in some cases, we cannot treat TB: for these individuals we're back where we were before we had antibiotics. That combination of factors has essentially created a significant escalation in the number of cases and the difficulty of treating TB and the urgent

need to do something about it.

Does your research have any potential in elucidating and potentially treating any other diseases? If so, which diseases are these and how might the research be applied to them?

Since we work on immunity, we have some interest in the cross-talk between HIV immune suppression and protection against TB. Some of the concepts in the host pathogen interaction and the cross-talk between the infecting agents and the immune response would probably relate to other infectious diseases too, including parasitic infections, chronic viral infections and other bacterial infections.

Where does your research receive its funding and how does this affect your work?

Most of my funding comes from the NIH: either individual grants to myself, or being part of bigger contracts such as the Tuberculosis Research Unit contract the NIH holds with a group of TB investigators. Most of my immune modulatory work is funded by the Bill and Melinda Gates Foundation and I have also had small amounts of funding from other sources.

Central to the research effort in my lab is a training grant from Fogarty International, a component of the NIH, which supports training for foreign students and postdocs. The grant is specifically targeted for training South African investigators working in TB research. This activity keeps us very heavily involved in TB researchers in South Africa. Candidates come through the lab, spending anything from three to six months here, returning repeatedly. We help them learn techniques from investigators in our own, or other, laboratories.

Beyond antibiotics

Each year, tuberculosis claims millions of victims worldwide. NIH-funded research is increasing our understanding of the complex immunological processes which direct the virulence of this disease – and many others

ACCORDING TO THE WHO's statistics, there were 9.4 million new cases of tuberculosis (TB) in 2009. Even this huge number could be a conservative estimate, according to Dr Gilla Kaplan, Head of Laboratory, Mycobacterial Immunity and Pathogenesis at the University of Medicine and Dentistry of New Jersey, whose first-hand research experience has given her an insight into the scale of this epidemic. Efforts to contain TB are hindered by a web of factors: the virulence with which the organism causing TB – *Mycobacterium tuberculosis* (MTB) – infects its hosts; infections brought about through the lowered immune responses in those with HIV; and finally the often careless use of antibiotics, which has led in some cases to strains of MTB which are resistant to one or more drugs (multi drug-resistant).

Treating TB is a lengthy, complex, costly and potentially painful process, which can take from six to 18 months to clear the infection. Chemotherapy – with its widely-known and pronounced side effects – is still not definitively known to be aided by host immunity which is expected to damage bacilli in the lungs; indeed, immunity may even drive MTB into a dormant state, making the bacilli non-responsive to antibiotics. Through a better understanding of MTB and its complex interactions with host factors, Kaplan's laboratory is endeavouring to work towards more effective treatment in this and other major infectious diseases.

VARIED VIRULENCE

Due to the variations in severity, progression and extent of the disease in different patients, Kaplan's lab hypothesised that this diversity might be contributed to, not only

by the nature of the host immune response, but also, by characteristics of the bacterium. After gathering clinical isolates from a range of patients, the Kaplan lab began testing the extent of strain virulence in the mouse infection model. The investigators infected mice with the same number of bacilli of different clinical TB isolates, closely observing their responses: "What we could show was that some strains killed the mice faster than others; that the strains that killed the mice more efficiently induced a weak or delayed immune response," she elucidates. Those strains which gained a foothold in the host before the immune response could fully react – therefore killing the mice faster – were also those shown recently to be spreading more efficiently in humans: especially in the HIV-infected population.

MTB isolates, through the improvement in sequencing technology, have recently been shown to be much more diverse than previously thought. While not hugely pronounced, this diversity is enough to tip the balance in the host-pathogen interaction and therefore induction of immunity and survival of the mice. Kaplan has recently been mostly working with two MTB isolates, CDC1551 and HN878, for specific reasons, as she explains: "They have become the hallmark of the less-

virulent strain versus the more-virulent strain in the animal models; or the one that induces a strong immune response versus the other which induces a weak immune response".

Understanding host responses to these clinical isolates – particularly when HN878 is known to be spreading clinically – could have a direct effect on understanding and better controlling the epidemic in humans. At the core of this understanding are the activity levels in key genes regulating the Th1 protective host immune response, much higher in CDC1551 than HN878, rendering the progression and pathogenesis of the disease much more harmful in the later.

'FILLING IN THE GAPS'

The laboratory uses mouse and rabbit models of central nervous system (CNS) and pulmonary infection. There are practical benefits to the widely-used mouse model – such as minimal space taken up in expensive containment facilities – but also drawbacks, Kaplan explains: "To understand the immune response, the mouse is wonderful because we have all the tools to do so. There's a 'catch-22' though: it's the easiest and best immunologic model available, but not the most suitable when it comes to mimicking the clinical picture in humans".



INTELLIGENCE

ANALYSIS OF XDR-TB AND MDR-TB STRAINS: SAFETY, DIAGNOSIS AND PATHOGENESIS

OBJECTIVES

TB control is increasingly compromised by the global rise in HIV-induced immune suppression and in M/XDR-TB. Effective M/XDR-TB epidemiologic surveys and case management, as well as the development of new diagnostics and anti-TB drugs, require strong laboratory capabilities. For example, defining drug resistance profiles in patients undergoing TB therapy is an area which Dr Kaplan is seeking to address.

KEY COLLABORATORS

Clifton E. Barry III, PhD, National Institute of Health, Maryland, USA • **Henry Boom, MD**, Case Western Reserve University, Ohio, USA • **David Russell, PhD**, Cornell University, New York, USA • **Linda-Gail Bekker-Wood, MD, PhD**, Desmond Tutu HIV Centre, University of Cape Town, Cape Town, South Africa • **Gerrit Coetzee, MD**, National Health Laboratory Services, Johannesburg, South Africa • **Clive Gray, MD**, University of Cape Town, Cape Town, South Africa • **Willem Hanekom, MD**, University of Cape Town, Cape Town, South Africa

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The rabbit model differs in that the animals are larger and more expensive to house, but this model displays a disease process which is much more like human TB. MTB infected rabbits develop granulomas in the lung. These structures are aggregates of uninfected as well as MTB infected cells that become centrally necrotic and can develop cavities. These structures are the hallmark of human TB. Taking the best from each model to gain the most comprehensive understanding of host-pathogen interaction is crucial: "From a pathology point of view, the rabbit is much more like the human, but assessing the immune response in rabbits is much more difficult. Ultimately," Kaplan continues, "we do both, so that we can fill in the gaps and know that we are looking at a picture that adds up to something more like human disease".

Alongside these *in vivo* animal models, Kaplan's New Jersey laboratory conducts basic *in vitro* analyses of cell responses to MTB infection. Members of the lab study the activation of the monocytes at the early stages of infection, assessing the response to different strains, and how they are regulated at the cellular level. Of course, to obtain a full understanding of host-pathogen interactions, paying attention to the MTB organism itself is vital, Kaplan tells us: "We also do quite a lot of work on understanding the differences between the bacterial strains, which properties cause disease, how the different strains are spreading in the population, how they acquired drug resistance and so on," she outlines.

THALIDOMIDE ANALOGUES

In studying a condition called Erythema Nodosum Leprosum (ENL) in leprosy patients, Kaplan's earlier work led her to investigate thalidomide – a sedative introduced in the 1950s and withdrawn in the 60s after it was found to cause birth defects if used by pregnant women. The drug was later found to be useful in treating patients with ENL. Through exploring which aspects of the immune response were responding to thalidomide, Kaplan made a crucial observation: "We discovered that thalidomide inhibited or reduced – but not ablated – the production of one of the soluble hormones which drives and regulates the immune response – a molecule known as tumor necrosis factor-alpha (TNF- α)". This molecule plays a central role in the immune response to many diseases but too much TNF- α causes severe pathology. So being able to down-regulate, or modify the TNF- α response could provide a groundbreaking tool for alleviating disease/pathology in conditions as diverse as cancers, serious chronic inflammatory disorders and infectious diseases.

Overcoming the challenges of gaining permission from the FDA, as well as seeking someone to synthesise the drug and get it approved for use in the U.S. – which she found in the partner company Celgene Corporation – Kaplan,

together with Celgene scientists, developed two classes of novel, synthetic analogues of thalidomide that reduce TNF- α production by up to 50,000 times more than the parent drug and with fewer deleterious side effects. Some of these molecules are already extensively used to treat haematologic cancers and others are being examined for efficacy in chronic inflammatory and autoimmune diseases. This, Kaplan believes, has been a major achievement in their work: "The development of thalidomide and thalidomide analogues for clinical indications in humans has been a fantastic journey. It has made not only a major contribution clinically, but has also been a major economic success for the company". Kaplan is now turning her attention to the use of these drugs in infectious disease such as TB. "All of that is based on work done in our lab," she enthuses.

TB VACCINES AND OTHER PATIENT STUDIES IN SOUTH AFRICA

In addition to Kaplan's studies at the Public Health Research Institute, she has a number of off shore collaborative studies in human populations being conducted at the University of Cape Town, Cape Town, South Africa. The pathogenic process that occurs in the lung of TB patients is being analysed together with Dr David Russell, and the effect of antiretroviral treatment of HIV infected individuals on TB disease and MTB transmission are being studied in collaboration with Dr Linda-Gail Bekker Wood. In addition, immunologic correlates of BCG vaccination-induced protective immunity against TB disease in infants are being defined in collaboration with Dr Willem Hanekom and Dr Henry Boom. Kaplan has recently begun a study to explore host and pathogen factors that contribute to the failure of treatment of MDR TB, ultimately leading to emergence of XDR TB strains in HIV-infected and non-HIV-infected patients in collaboration with Drs Gerrit Coetzee and Clive Gray of the National Health Laboratory Services and the University Cape Town, South Africa. Similar studies have also been initiated in collaboration with Dr Cliff Barry in China.

PUBLISH OR PERISH

For the purpose of seeking partners and funding in her research ventures, Kaplan has no doubt that dissemination of research results is vital: "You don't get funded if you don't publish: it's 'publish or perish'. Also getting your results into the public domain is very important to inform other scientist about your studies".

Encouragingly, through her research and that of others, Kaplan believes we are reaching a point of really understanding the 'cross-talk' in host-pathogen interaction, and opening up new insight into how 'resetting' the immune system and establishing a new and advantageous balance could contribute to controlling TB.