THE HOST FIGHTS BACK: INNATE AND ADAPTIVE IMMUNE MECHANISMS AGAINST *PNEUMOCYSTIS MURINA*

Nontobeko F. Mthembu[1], Patricia Otieno-Odhiambo[1], Muazzam Jacobs[1], Frank Brombacher[1, 2], Jay Kolls[4] Gordon D. Brown[3], J. Claire Hoving[1]

[1]*Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Cape Town, South Africa.*
[2]*International Centre for Genetic Engineering and Biotechnology Cape Town, South Africa.*
[3]*Aberdeen Fungal Group, Institute of Medical Sciences, University of Aberdeen, United Kingdom.*
[4]*The Richard King Mellon Foundation, Institute of Paediatric Research, University of Pittsburgh, School of Medicine.*

Background
Fungi are opportunistic pathogens which kill an estimated 1.5 million people annually. Pneumocystis jirovecii is an opportunistic fungal pathogen causing severe pneumonia in immunocompromised and immunosuppressed individuals. Pneumocystis pneumonia, (PJP) is an AIDS defining-illness causing morbidity and mortality among HIV infected patients if left untreated AIDS is characterized by the gradual depletion of CD4+ T cells, shown to be crucial in early recognition and clearance of Pneumocystis (PC). In Sub-Saharan Africa, the accurate diagnosis of Pneumocystis is a challenge due to resource poor settings. Therefore data on the prevalence of Pneumocystis jirovecii is very limited. Using an animal model of disease, we propose to dissect various components of both the innate and adaptive immune response to Pneumocystis to understand host immune mechanisms in disease. Innate immunity to fungi is mostly characterised by pattern recognition receptors (PRRs) such as C-type lectin receptors (CLRs) and Toll-like receptors (TLRs), shown to induce the acquired immune response. Firstly, we will investigate a role for the newly described CLR, dendritic cell immunoactivating receptor (DCAR2) in PC recognition. DCAR2 is an FcRγ-coupled CLR belonging to the same cluster as Mincle, Dectin-1 and Dectin-2 all associated with binding PC. A role for DCAR2 during Pneumocystis has not been investigated. Secondly, we will also determine if the inflammatory cytokine TNFα is required for clearance.

Methods
Using the previously established Pneumocystis mouse model we will interrogate DCAR2- and TNFα-deficient mice. Mice will be infected with Pneumocystis cysts propagated in immunocompromised RAG1-deficient mice (T and B cell deficient). Disease progression will be measured at week 1, 2 and 3 post infection using qPCR. Host immune responses disease severity will be determined using different parameters such as, serum antibody levels, cellular inflammation, cytokine production, and Pneumocystis burden.

Results
Our preliminary data suggests that DCAR2-deficient mice clear infection more efficiently compared to wild type mice when performing qPCR. Further analysis will be required. Despite the fact that patients on anti-TNFα therapy are susceptible to PJP, our preliminary data indicate no differences in PC lung burden between the TNFα-deficient mice and TNFα+/+ mice. However, TNFα-deficient mice had reduced mucus production and inflammatory cells suggesting a reduced immune response to PC.

Conclusion
DCAR2 may be an interesting CLR as it seems to delay PC clearance in contrast to Mincle and other CLRs. TNF does not seem to be essential in clearing PC despite its apparent role in patients.