ImmunoTools *special* Award 2014

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Investigating the Interaction between the pathogenic fungus *Cryptococcus neoformans* and host dendritic cells

In this new study our lab has set out to study the role of dendritic cells (DCs) in the control of *C. neoformans* infection. DCs form an important link between the innate and adaptive immune response during infection – they are the only cells in the immune system capable of activating naive T cells and thus are vital in the generation of adaptive immunity during *C. neoformans* infection.

Cryptococccus neoformans is a human fungal pathogen that causes the often fatal disease cryptococcosis. In the first few years of life, most individuals come into contact with *C. neoformans* - it is a ubiquitous environmental fungus - however only those with existing immune deficiencies become seriously ill; this is because the immune system is able to clear infection before symptoms develop. Historically immune deficiencies have been relatively rare; however with the rise of HIV AIDs in the late 20th century, and the increasing use of immune suppression to treat cancer and transplant rejection, cryptococcosis has become an important health concern. In Sub Saharan Africa, where HIV is endemic, *C. neoformans* is thought to causes up to 600,000 deaths per annum (60% of all HIV related deaths in this area). In addition to *C. neoformans* infection, a closely related Cryptoccous species, *Cryptococcus gattii* has been reported in the last decade to have caused a cluster of fatal cryptococcosis cases in the North American Pacific Northwest area. Most worrying about this outbreak is that the majority of those infected did not have an existing immune deficiency. The biological basis for the differences in virulence between *C. neoformans* and *C. gattii* is still largely unknown.

Cryptococcal infection begins in the lungs following inhalation of infectious spores; once inhaled the spores germinate, and then grow as budding yeast in the alveolar space. It is during this growth phase that *C. neoformans* comes into contact with the host immune system; at this point the immune response is innate and mediated largely by macrophages and neutrophils. The main function of these two cell types is to phagocytose and destroy invading pathogens. Phagocytosis by host macrophages however is not initially effective at clearing infection as *C. neoformans* can survive and replicate within the macrophage all the while hiding from the innate immune response. If respiratory infection by *C. neoformans* is left unchecked the fungus eventually disseminates to the central nervous system (via the blood stream) leading to fatal meningoencephalitis.

Although the initial host innate immune response is not always effective at containing *C. neoformans*, in immune competent individuals Cryptococcus rarely disseminates to the central nervous system. This is because the innate immune system is augmented by the adaptive immune response. Specifically a Th1 adaptive immune response mediated by $CD4^+$ T helper cells has been shown to be protective against Cryptococcosis, release of IFN-y and IL-12 by $CD4^+$ T helper cells activates macrophages and neutrophils making them more effective at killing Cryptococcus and thus more able to clear infection. The importance of $CD4^+$ helper T cells to the control of infection is underscored by the close association of *C. neoformans* with HIV which depletes host $CD4^+$ T cells.

As a model system for dendritic cell infection we will use monocyte derived dendritic cells isolated from human peripheral blood samples and differentiated *in vitro*. Monocytes isolated via Ficoll separate will be cultured in the presence of ImmunoTools human recombinant GM-CSF and IL-4 for ~ 10 days. Differentiation into dendritic cells will be confirmed via flow cytometry using ImmunoTools anti human antibodies, Positive differentiation: CD11c, HLA-DR, CD86, Negative differentiation: CD14 – a monocyte maker lost during DC differentiation.

Utilising our extensive *Cryptococcus* knockout collection we will examine how the presence and absence of various virulence factors affect the maturation of human iDC during *in vitro* infection. DC surface markers related to dendritic cell maturation state will be stained with ImmunoTools anti human FITC conjugated antibodies - CD80, CD86, ICAM-1, HLA-DR, and CD1a, to examine for relative expression level before and after contact with various

Cryptococcus strains via flow cytometry. Using ImmunoTools pre conjugated antibodies a large number of surface markers can be assessed in a single experiment quickly and easily. Results from these experiments will inform future work and help with the targeting of new therapies towards treating cryptococcosis.

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FITC - conjugated anti-human CD1a, CD14, CD38, CD40, CD45, CD80, CD86, CD54, HLA-DR, Control IgG1, Control IgG2a, Control IgG2b,

PE - conjugated anti-human CD3, CD11c, CD14, Annexin V,

PerCP - conjugated anti-human CD4,

Multicolour combinations anti-human:
CD4 FITC / CD8 PE

human IFN-gamma ELISA-set for 96 wells, (3 reagents),

recombinant human cytokines: rh GM-CSF, rh IL-4, rh MCP1 / CCL2, rh TNF-a

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