C. neoformans is an opportunistic fungal infection spread through airborne means. It affects immune compromised individuals and increases their susceptibility to the disease. Previous studies showed that dendritic cells (DCs) can inhibit C. neoformans through phagocytosis and lysosomal killing from within the DC. The lyosomal extract from these DCs has antifungal activity, and we now have more quantification data identifying its components. We hypothesized that DC lysosomal proteins, nostrin, calmodulin, and coronin I are key antifungal against C. neoformans. For these studies, we inoculated lyosomal extract or those individual proteins with C. neoformans to measure anti-fungal activity. Our results showed nostrin and coronin I had significant antifungal activity while calmodulin significantly increased cryptococcal growth. Cystatin B was toxic to mammalian cells. Because calmodulin increased cryptococcal growth, we hypothesized that following incubation with C. neoformans, the media contained growth-enhancing nutrients. For this, we examined the media for macronutrients, amino acids, and metals following incubation of C. neoformans with calmodulin and other growth-enhancing compounds 3H-EA, cystatin B, calnexin, nostrin, and C. neoformans. We found that inoculation of C. neoformans with calmodulin, cystatin B, and C. neoformans led to increased bacteria in the media, while treatment including Cd, Fe, Mn were also increasing following incubation of C. neoformans with calmodulin. Interestingly, some compounds alone increased trace metals, but inoculation with C. neoformans brought these levels back to baseline amounts. Future studies will compare these components in the media to those from infections with anti-cryptococcal molecules.

Introduction

C. neoformans is an airborne fungal pathogen, which left untreated causes meningitis in immune compromised individuals and can be fatal. There are many species of Cryptococcus in the wild, but C. gattii and C. neoformans are two of the major pathogenic species. Tests and the disease in rare matter and will cause someone to come in contact with C. gattii and become infected. Infection with C. neoformans begins with small birds such as pigeons, and their feces carry the fungal organism which can then be inhaled into the lungs. And from there the organism can make its way into the brain and cause deadly meningitis. C. neoformans mainly causes disease in immune compromised individuals, but other species of Cryptococcus such as C. gattii can cause disease in healthy people. Most forms of Cryptococcus can be transmitted through airborne means, and this organism causes disease in about 250,000 individuals worldwide each year.

When C. neoformans enters the lungs, macrophages and dendritic cells (DCs) recognize the fungus and can work to fight the infection. Once inside a macrophage or DC, the fungus undergoes a gradual acidification as the phagosome becomes a phagolysosome where the lysosome releases its proteins and enzymes out onto the fungal organism. However, C. neoformans can survive and replicate in the phagolysosome of some types of macrophages. The lysosomes from DCs have been shown to have anti-cryptococcal activity. There are more than 3,000 different proteins found in the DC lysosome that we identified by mass spectrometry. We have observed that some of the lysosomal proteins inhibit the growth of C. neoformans, while others actually enhance C. neoformans growth. Therefore, we were interested in understanding the mechanisms of this enhanced growth and identifying which lysosomal proteins may be more available to C. neoformans that can enhance its growth. These findings may be important to understand how the organism can replicate within the phagolysosome of some macrophages.

The goal and purpose in studying cryptococcal interactions with macrophages and dendritic cells is to understand how immune cells can kill the fungus or how the fungus may use host factors to promote its growth, potentially creating new targets for therapies. By developing methods that function in aiding mechanisms already in place to destroy Cryptococcus or down-regulating host factors that the fungus may use for growth, we could create effective, non-toxic drugs that could potentially save many people.

Methods

Cryptococcal strains and media: C. neoformans strain H99 (serotype A) was grown for 15 hours at 37°C with shaking at 100 rpm. Then cells were collected by centrifugation and washed three times with sterile phosphate buffered saline (PBS). Yeast levels were quantified using trypan blue dye exclusion in a hemocytometer. The yeast used for the inoculation was selected from the hemocytometer.

Inoculation of DC lysosomal extracts with Cryptococcus: Lysosomal extracts were incubated with C. neoformans. In the killing studies, extracts were grown at 37°C, harvested at 6 h, and aged for 1 h. At 1 h, extracts were aged for 1 h. At 1 h, extracts were added to the C. neoformans. After 30 minutes, fungal viability was assessed using a trypan blue exclusion assay.

Conclusions/Future Directions

Conclusion: Several of the lysosomal proteins enhanced the growth of C. neoformans. This information suggests that some of the proteins may break down the media to make nutrients more available to aid in C. neoformans growth.

Figure 5. Trace Metals in Supernatants after Incubation of C. neoformans and Lysosomal Proteins

Figure 1. Effect of Calmodulin on C. neoformans Growth

Figure 2. Effect of Coronin on C. neoformans Growth

Figure 3. Effect of Nostrin on C. neoformans Growth

Figure 4. C. neoformans Enhanced Growth by Lysosomal Proteins

Funding: OSU Startup; NIGMS-MH F08GM013646; Oklahoma Center for Respiratory and Infectious Diseases (OCRIP) pilot grant.