Review

**Histoplasma capsulatum: Master Evader of Innate Immunity**

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**Introduction:**

*Histoplasma capsulatum* (var. *capsulatum*) is a thermally dimorphic fungal pathogen with worldwide distribution (Fig. 1) and is the causative agent of histoplasmosis. In Japan *H. capsulatum* is considered an imported pathogen with only 75 cases over time. Infection is established by inhalation of 2-6 microns micro-conidia from the soil inhabiting saprophytic mycelial stage. In the lungs, conidia transform into the parasitic yeast form, causing disease that ranges from benign, to chronic cavitating, or to disseminated infection depending on the immunocompetence of the host. Reactivation of previously controlled histoplasmosis has become a serious problem in patients with acquired immunodeficiency syndrome (AIDS). This review will concentrate on the inherent properties of *H. capsulatum* that are used to combat the antimicrobial mechanisms of macrophages. Information about the interaction of *H. capsulatum* with various macrophages: human monocytes, monocyte derived macrophages, alveolar macrophages, and macrophage cell lines is surveyed. Data from mouse macrophage experiments vs. *H. capsulatum* will also be examined. The most relevant information, as far as human histoplasmosis in the pulmonary compartment is concerned, will be emphasized. However, this data is limited because of the difficulty in obtaining permission from institutional human research boards for lavage of lungs to obtain normal human alveolar macrophages.

**H. capsulatum. The Evasive One**

**Cell Wall.** *H. capsulatum*’s first tool for evasion is the composition of the cell wall. Using anti-alpha-(1,3)-glucan antibody-gold transmission electron microscopy (TEM) alpha-(1,3)-glucan was found to dot the surface of virulent *H. capsulatum*. Furthermore, fluorescent antibody techniques demonstrated alpha-(1,3)-glucan was prominent on the surface on the cell wall surface of budding yeast cells. In other reports the cell wall surface of avirulent variants of *H. capsulatum* consisted mainly of beta-(1,3)-glucan rather than alpha-(1,3)-glucan. alpha-(1,3) glucan on pathogenic *H. capsulatum* enables it to evade a number of different receptors on the surface of macrophages. For example, such microconidia do not engage macrophage toll like receptors (TRs) or Dectin-1 and therefore thwart innate immunity and the induction of important inflammatory cytokines.

**What goes on in the pulmonary compartment?** Inhaled microconidia reaching the alveoli are confronted with low level complement components, surfactant proteins, 37°C, elevated CO₂ levels, and most importantly alveolar macrophages. Binding of microconidia with surfactant proteins (SP) A and D remains to be substantiated, and they are not opsonized because of the low levels of complement, but to
their advantage they are ingested by alveolar macrophages. It has been a puzzle for some time how un-opsonized microconidia could be ingested by alveolar macrophages using the C3R receptor complex \(^{12}\). A recent report identified a heat shock protein (hsp 60) on \textit{H. capsulatum} that binds CD11b/CD18 in the C3R complex and initiates ingestion \(^{13}\).

**Sneaking inside: bypassing the oxidative burst.**

\textit{H. capsulatum} microconidia slip into alveolar macrophages using the C3R complex for phagocytosis. Macrophage ingestion using the C3R, as does un-opsonized microconidia, fails to trigger the oxidative burst. Moreover, opsonized yeast cells ingested by alveolar macrophages failed to trigger detectable products of the oxidative burst \(^{14}\). In other reports, phagocytosis by macrophages of materials covered with deposits of C3b and C3bi using the C3R for ingestion, fail to trigger the oxidative burst \(^{15}\). On the other hand, \textit{H. capsulatum} yeast cells opsonized by specific antibody for such cells, ingested by the antibody receptor (FcR) on mouse macrophages, induced a robust oxidative burst \(^{16}\). Interestingly, when opsonized \textit{H. capsulatum} yeast cells were ingested by way of C3R, it had no effect of the oxidative burst generated by concurrent ingestion of zymosan using macrophage mannose receptors \(^{17}\). Therefore, \textit{H. capsulatum} takes advantage of the unique feature of macrophage ingestion by the C3R complex and slips into the macrophage evading the oxidative burst.

**The easy life in the phagosome.**

After ingestion by alveolar macrophage, \textit{H. capsulatum} microconidia transform to the yeast form and start to adapt to the new environment. Yeast cells ingested by macrophages start to make changes within one hour by protein synthesis of several novel proteins, increased synthesis of other proteins and decreased synthesis of some constitutive proteins (Table 1) \(^{18}\). This could be a reaction the acidic (pH 5.0) of the phagosome and alter protein synthesis of yeast cells within 1 h \(^{19}\). \textit{H. capsulatum} yeast cells ingested by human macrophages can buffer the pH 5.0 acidic phagosome by still undefined mechanism, changing the pH to around 6.4 to 6.5 \(^{20}\). In vitro experiments show that cultures of yeast cells starting at pH 5.0 can alter the pH of the medium and the medium comes close to neutral \(^{21}\). This phenomenon is critical for survival and proliferation of yeast cells in the phagosome.

**Watch out for those dangerous lysosomes.**

The cytosol of macrophages contain numerous lysosomes, small vesicles 1 to 2 microns in size, containing several types of acidic hydrolases. Under ordinary conditions lysosomes fuse with the phagosome membrane and dump acidic hydrolases into the phagosome for digestion phagocytosed material. It has been reported that lysosomal extracts from alveolar macrophages, under acidic in vitro conditions, inhibited growth of \textit{H. capsulatum} yeast cells \(^{22}\). When macrophages ingest \textit{H. capsulatum} yeast cells, there
is ample evidence that lysosomes fuse with the phagosomes\textsuperscript{23}. By buffering the phagosome pH, \textit{H. capsulatum} is one step ahead of dangerous contents of lysosomes, the acid hydrolases from lysosomes are rendered impotent.

**Nutritional immunity: getting iron to \textit{H. capsulatum}**

Iron privation is of form of nutritional immunity against pathogens\textsuperscript{24}. Growth of \textit{H. capsulatum} yeast cells in culture medium is inhibited 50\% by 40\% serum\textsuperscript{25}. Serum transferrin sequesters iron (Fe III) from the surroundings and delivers it to cells. Transferrin receptors on macrophages\textsuperscript{30} transports Fe III to acidic endosomes or acidic phagosomes; however, \textit{H. capsulatum} yeast cells in phagosomes have changed the pH to 6.5, under these conditions one atom of iron from transferrin is freed making iron available for yeast cell metabolism\textsuperscript{27}. The importance of iron for \textit{H. capsulatum} yeast cells growth in the phagosome has been demonstrated by the use of an iron chelator (deferoxamine) or an inhibitor of iron release from transferrin (chloroquine), both of which can lead to macrophage killing of ingested \textit{H. capsulatum} yeast cells\textsuperscript{28}.

**Cellular Immunity**

Can't hide any longer.

\textit{H. capsulatum} yeast cells proliferate in the permissive macrophage phagosome reaching a limit which results in demise of the macrophage\textsuperscript{29}. Release of yeast cells from macrophages into the surroundings and material from damaged or disintegrated yeast cells is ingested by macrophages or dendritic cells and presented as antigen to T-cells. In particular, CD4\textsuperscript{+} T\textsubscript{h} -cells respond to \textit{H. capsulatum} antigens by proliferation and release of cytokines and chemokines. One of the very important cytokines produced by T-cells is interferon-gamma (IFN-\textgamma). IFN-\textgamma receptors on macrophages\textsuperscript{30} bind IFN-\textgamma and start a signaling pathway that results in activation of macrophages for enhanced anti-fungal activity\textsuperscript{31} and anti-microbial activity\textsuperscript{32}.

Macrophages activated by IFN-\gamma respond to ingestion of microbes by a robust oxidative burst due to up-regulation of components in the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex\textsuperscript{33}. Killing of \textit{H. capsulatum} yeast cells by IFN-\gamma activated human monocyte derived macrophages was first reported in 1991\textsuperscript{34}. Monocytes cultured for 3 days in presence of IFN-\gamma morphed into macrophages. Activated macrophages challenged with \textit{H. capsulatum} yeast cells killed 58\% of yeast cells as determined reduction in inoculum colony forming units (cfu) in a 2 hr challenge period (Fig. 2). It can be noted that yeast cells that were not killed by activated macrophages, multiplied at the same rate as yeast.
cells in controls (Fig. 2). The monolayer of IFN-γ treated monocytes was not homogenous, many macrophages displayed spindle morphology, others were more rounded. Killing by activated macrophages was completely abrogated by superoxide dismutase indicating that superoxide generated by an oxidative burst was necessary for the fungicidal activity.

It is possible that IFN-γ is not the only cytokine or chemokine that can activate macrophages for killing H. capsulatum yeast cells. It was reported that the cytokine, macrophage colony stimulating factor (MCSF), could transform human monocytes into macrophages that were activated for killing of H. capsulatum yeast cells. Monocytes cultured for 5 days in MCSF (10,000 U/ml) resulted in macrophages that killed 73% of yeast cells, as measured in reduction of cfu in the challenging inoculum. Killing of yeast cells by MCSF activated macrophages was reduced to 33% in presence of SOD, indicating an important role for superoxide anion in the killing process.

**Inflammation**

_Danger from outside and inside: PMNs._

As cellular immune responses to H. capsulatum unfolds in the lungs, epithelial cells secret the chemokine IL-8. Polymorphonuclear neutrophils (PMNs) respond to secretion of the IL-8 by migrating to the source of the chemoattractant. Although PMNs ingest H. capsulatum yeast cells, PMNs are short-lived and don’t provide suitable place for H. capsulatum yeast cells to proliferate. Moreover, PMNs are dangerous for pathogens because they are loaded vesicles containing toxic granules. Azurophilic and specific granules are released by PMNs to the outside by degranulation, spilling toxic contents onto pathogens. Azurophilic granules contain, among other materials, defensins and myeloperoxidase; whereas, specific granules contain lysozyme and NADPH oxidase. By isolation of subcellular fractions of PMNs it was found that azurophil granules contained fungistatic activity.

PMNs readily ingest serum opsonized H. capsulatum yeast cells by binding to the PMNs C3R complex. As measured by luminol-enhanced chemiluminescence, PMNs generate an oxidative burst upon phagocytosis of H. capsulatum yeast cells, comparable to the oxidative burst produced by ingestion of Candida albicans yeast cells. Even though H. capsulatum yeast cells were killed in a cell free system (KI + HPO + H2 O2), yeast cells were not killed by the oxidative burst of PMNs, as measured by reduction of inoculum cfu. Electron microscopy illustrated phagosome-lysosome fusion in PMNs with ingested yeast cells and lysosome material seen around ingested yeast cells (Fig. 3). These data indicate that H. capsulatum yeast cell are resistant to killing by PMNs even though phagocytosis of yeast cells generate an oxidative burst and there is no inhibition of phagosome-lysosome fusion.

On the other hand, PMNs were fungistatic for H. capsulatum yeast cells in 24h to 72h co-cultures. At high PMN to yeast cell ratio (500 to 1) there was no increase in cfu compared to inoculum cfu at 24h–72h in co-cultures (Fig. 4). Even at a PMN to yeast ratio 5 to 1, there was significant inhibition.
of yeast cell proliferation in co-cultures (Fig. 4).

Humoral Immunity

Final outcome.

In most cases of histoplasmosis, the primary lesions in the lungs resolve in 10 to 20 days. By this time, antibody production to *H. capsulatum* antigens has taken place and delayed-type hypersensitivity to histoplasmin is measurable. In highly endemic areas of histoplasmosis, there is 90% positivity to skin testing with histoplasmin. Moreover, following resolution of primary infection with *H. capsulatum*, radiological examination revealed multiple focal lesions in the lungs and granulomas that form are often calcified. In an autopsy series of cases, as many as 85% of all patients had pathological evidence of previous histoplasmosis.

The development of histoplasmin was very important for the study of histoplasmosis. Both the yeast and mycelial phase produced exoantigens when grown in broth culture and these exoantigens were the primary constituents of histoplasmin. Upon purification of crude preparations it was found that two fractions contain the active material and were labeled H and M antigens. Histoplasmin, besides being used for skin testing, is also valuable for serodiagnosis of histoplasmosis.

References


