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Real-time visualization of immune cell clearance of *Aspergillus fumigatus* spores and hyphae

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Abstract

Invasive aspergillosis (IA) is a disease of the immunocompromised host and generally caused by the opportunistic fungal pathogen *Aspergillus fumigatus*. While both host and fungal factors contribute to disease severity and outcome, there are fundamental features of IA development including fungal morphological transition from infectious conidia to tissue-penetrating hyphae as well as host defenses rooted in mechanisms of innate phagocyte function. Here we address recent advances in the field and use real-time *in vivo* imaging in the larval zebrafish to visually highlight conserved vertebrate innate immune behaviors including macrophage phagocytosis of conidia and neutrophil responses post-germination.

Keywords

Aspergillus; Macrophage; Neutrophil

1. Introduction

Aspergillus fumigatus is a globally ubiquitous filamentous fungus and the most frequently encountered opportunistic mold pathogen. While most notorious for causing the often fatal disease invasive aspergillosis (IA) in severely immunocompromised patients, *A. fumigatus* can cause a range of diseases as a function of host immunity. In all cases, disease initiation occurs with the inhalation of conidia. Inhaled conidia can evade or subvert host immunity to germinate into tissue-penetrating hyphae making immune mechanisms that prevent germination or kill invading hyphae essential for preventing, stalling, or clearing infection. At-risk populations for developing IA are an immunologically diverse group and increasing in number as medical technologies reliant upon immunomodulatory procedures become

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.fgb.2017.05.005>.

more widespread. This, coupled with significant strain heterogeneity in *A. fumigatus* (Keller, 2017), compounds a unified etiologic model of the host-fungal interface. Nonetheless, clinical and research data have solidified the necessity of innate immune mechanisms as inherited genetic disorders or other disturbances within innate phagocyte function are significant risk factors in developing IA. Here we will review some recent studies that have utilized imaging-based methods, among others, that expand the toolkit for studying *A. fumigatus* pathogenesis and touch on the broader implications and impacts of these observations.

2. Review

2.1. Macrophages represent the first phagocytic line of defense against conidia

Following introduction of dormant *A. fumigatus* conidia into a vertebrate host, macrophages represent the first line of defense among professional phagocytes. Indeed, phagocytosis by macrophages *in vivo* is a rapid and efficient process (Video 1). While still dormant, there are inherent virulence determinants that play a role in conidial-macrophage interaction. The outermost surface of *A. fumigatus* conidia is comprised of a proteinaceous rodlet layer, which confers hydrophobicity and immunological inertness (Aimanianda et al., 2009). Removal of this rodlet layer exposes underlying carbohydrates eliciting a much stronger immune activation with a concomitant increase in susceptibility to host defenses (Carrion et al., 2013). Playing a more immunoinhibitory role in conidial defense is the structural pigment melanin, which can aid in scavenging reactive oxygen species and dampen the initial immune response to dormant conidia (Chai et al., 2010). Post phagocytosis, melanin inhibits acidification of the phagolysosome by interfering with the translocation of the NADPH oxidase subunit p22^{phox}, contributing to conidial survival within the phagolysosome (Akoumianaki et al., 2016). Shedding light on macrophage-based mechanisms post-germination in the phagosome, it was shown that *A. fumigatus* germlings can be passed to a new macrophage to continue inhibition of growth and disease progression (Shah et al., 2016) furthering our understanding of dynamic macrophage interactions with *A. fumigatus*.

2.2. Germination recruits neutrophils and activates robust extracellular fungicidal responses

Following the morphologic transition from conidia to hyphae (Fig. 1), *A. fumigatus* presents the immune system with a new challenge requiring extracellular killing mechanisms as the filamentous form is often too large to phagocytose. It is this hyphal form that penetrates host tissue, preceding dissemination and lethality, making containment of hyphal growth essential for host survival. While demonstrating ability to kill both conidial and hyphal forms (Gazendam et al., 2016), neutrophils are widely regarded as the dominant innate immune cell for hyphal killing because of their ability to mount a successful extra-cellular defense (Ellett et al., 2017; Knox et al., 2014). Indeed, neutrophils efficiently migrate towards and are able to kill *A. fumigatus* hyphae *in vivo* (Video 2). Despite efficient neutrophil trafficking towards hyphae, *A. fumigatus* possesses galactosaminogalactan (GAG), an extracellular matrix component that partially masks pro-inflammatory carbohydrates and mediates adherence and penetration into host tissue and full virulence (Gravelat et al., 2013).

However, after physical contact with neutrophils, *A. fumigatus* can switch to an evasive hyper- branching phenotype (Ellett et al., 2017).

3. Conclusion

While the development of IA has classically been associated with host neutropenia, it is becoming increasingly appreciated that *A. fumigatus* poses a health threat to a broader population comprised of diverse immune states. As a consequence, it is important to understand the relative contributions and behaviors of immune cells in controlling *A. fumigatus* across morphological development. A useful approach for addressing these needs are live, visual observations of fungal/phagocyte interactions. Through the use of these methods, dynamic interactions between innate phagocytes across *A. fumigatus* morphological development are coming into focus and will allow opportunities to address some outstanding questions in the field.

For example, persistence of live *A. fumigatus* conidia for more than several days in both mouse (Tanaka et al., 2015) and zebrafish (Knox et al., 2014) models supports the efficacy of fungal spore defences (Aimanianda et al., 2009; Akoumianaki et al., 2016) conferring a tolerance to host inflicted stress. Furthermore, as a certain percentage of spores persist *in vivo* it is still unclear the spatial and temporal factors surrounding fungal killing. Are non-phagocytosed spores the ones that germinate? Or does germination occur post-phagocytosis and precede killing, aided by lateral transfer (Shah et al., 2016)? Even though conidia are immunologically inert (Aimanianda et al., 2009) and killed by both macrophages and neutrophils (Gazendam et al., 2016) there are nonetheless dynamic interactions with macrophages that still need to be explored.

While neutrophils are the dominant phagocyte against hyphae, the recent observation that *A. fumigatus* can undergo a hyperbranching phenotype upon neutrophil stimulation (Ellett et al., 2017) shows that this interaction is more complex than recruitment towards and extracellular killing of vulnerable hyphae. While the benefit of hyperbranching is dependent upon neutrophil number and function (Ellett et al., 2017), *in vivo* observations at the neutrophil-hyphal interface (Vid. 2) (Knox et al., 2014) show that macrophages are also tightly associated at this junction. Is there a synergistic fungicidal response between these innate phagocytes? If so, what are the mechanisms of crosstalk, and how do distinct predisposing conditions for IA influence this interaction?

Taken together, the importance of innate phagocytes in host defense against *A. fumigatus* is clear. However, the interplay between fungal and host factors that determine pathogenic outcome remain to be fully elucidated. Through advances in imaging-based research methods we are offered an exciting window of observation into the dynamics of both host and pathogen driving new questions, approaches, and an additional means of assessing the efficacy of new therapeutic approaches that modify fungal behavior, phagocyte antifungal activity, or both.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Cartoon model of innate phagocyte responses across *A. fumigatus* morphological development. Throughout the transition from conidia to hyphae (black arrows) *A. fumigatus* conidia lose the protective rodlet and melanin components (grey arrow), exposing underlying carbohydrates, including galactosaminogalactan (GAG), on the hyphal surface. Innate phagocyte recruitment patterns (blue arrows) are influenced by fungal morphology with conidia being largely phagocytosed by macrophages while hyphal development activates neutrophil recruitment with concomitant extracellular killing, creating a tight association of phagocytes around hyphal structures. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)