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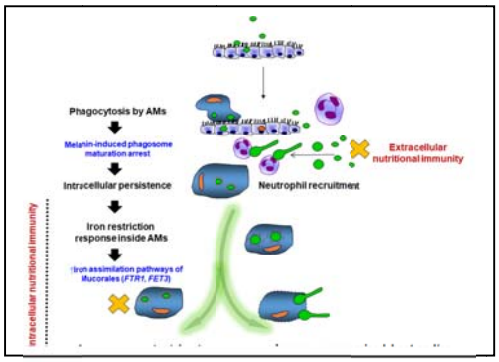
PRESS RELEASE

IMBB Researchers Identify a Critical Role of Iron Restriction inside Macrophages in Host Defense against Human Pathogenic Fungi



The PhD student Angeliki Andrianaki, working together with the IMBB Researcher Irene Kyrmizi, the head of the research team George Chamilos and collaborators, uncover the essential role of iron restriction inside macrophages in host defense against Mucorales, an emerging group of human pathogenic fungi.

The findings of this study have important implications in pathogenesis and therapeutics of human fungal diseases and are published today in *Nature Communications*

Airborne filamentous fungi (molds) are ubiquitous saprophytic organisms that are harmful for humans. However, certain molds, including *Aspergillus* and Mucorales, are major causes of life-threatening infections in severely immunocompromised patients. In particular, patients with defects in numbers, chemotaxis or effector functions of phagocytes are at substantial risk for development of invasive mold pneumonia. In addition, abnormalities in iron metabolism result in unique predisposition for infections by Mucorales. In view of the high mortality rates (50% to 70%) of invasive mold infections despite antifungal therapy, there is a dire need for better understanding the molecular aspects of disease immunopathogenesis in order to design new therapeutic strategies.

Protective immunity against molds is largely mediated by professional phagocytes. Physiologically, hundreds to thousands of inhaled fungal conidia (spores) are efficiently phagocytosed and killed by alveolar macrophages (AMs) on a daily basis without causing disease. Germinating spores that escape surveillance from AMs trigger inflammation and are rapidly eliminated by recruited neutrophils. The molecular events leading to elimination of fungal conidia inside AMs are incompletely understood. Recent studies from our group and others demonstrated the important role of a specialized autophagy pathway termed LC3 associated phagocytosis (LAP) in phagosome maturation and killing of fungal conidia by macrophages. Importantly, *Aspergillus* cell wall melanin targets LAP to protect fungal conidia from killing and promote pathogenicity. However, apart from LAP, additional antifungal effector mechanisms in macrophages have not been well characterized.

In the present study the investigators performed immunology studies in mice infected with Aspergillus vs. Mucorales, and validated the findings in patients with Mucorales infection. The investigators employed molecular biology and immunology techniques to demonstrate the selective ability of Mucorales, as opposite to other filamentous fungi, to remain in dormancy inside AMs. Mechanistically, intracellular "persistence" of Mucorales conidia is related to their ability to retain melanin on the fungal cell wall surface and induce phagosome maturation arrest via inhibiting LAP. Intracellular inhibition of Mucorales by macrophages is an important effector mechanism, as infection of immunocompetent mice with swollen conidia, which evade phagocytosis, results in acute lethality within hours. Concordantly, AM depletion via clodronate liposome treatment or targeted ablation with diphtheria toxin (DT) in transgenic DTR mice

markedly increases susceptibility to mucormycosis. Host and pathogen transcriptomics, iron supplementation studies, and genetic manipulation of iron assimilation of fungal pathways demonstrate that, in the absence of LAP, iron restriction inside macrophages becomes a critical effector mechanism against Mucorales.

The results of this work reveal the essential role of Mucorales–macrophage interplay for infection outcome and introduce evidence that a central pathogenetic event in development of infection is related to the prolonged intracellular survival of the fungus inside these immune cells. In particular, intracellular persistence of Mucorales might compromise the activity of existing antifungal agents and explain the high rate of relapse of the infection following cessation of antifungal therapy. More importantly, this study leads to a pathogenetic model of mucormycosis, which places nutritional immunity inside macrophages in the forefront of antifungal immunity in the lung and explains how abnormalities in iron metabolism lead to development of immunodeficiency. Further understanding of the molecular mechanisms of iron homeostasis in macrophages during Mucorales infection will pave the way for design of novel therapeutics against this devastating human disease.

Reference:

Andrianaki AM, Kyrmizi I, Thanopoulou K, Baldin C, Drakos E, Soliman SSM, Shetty AC, McCracken C, Akoumianaki T, Stylianou K, Ioannou P, Pontikoglou C, Papadaki HA, Tzardi M, Belle V, Ettiene E, Beauvais A, Samonis G, KontoyiannisDP, Andreakos E, Bruno VM, Ibrahim AS, Chamilos G. Iron restriction inside macrophages regulates pulmonary host defense against Rhizopus species. Nat Commun. 2018 Aug 20;9(1):3333. doi: 10.1038/s41467-018-05820-2.

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Intracellular Persistence of fungal conidia inside Alveolar Macrophages (AMs). Representative confocal image of AMs sorted from the lungs of immunocompetent mice 3 days after infection with conidia of Mucorales fungi. There is evidence of intracellular conidia and the early stages of phagocytosis of a conidium by AMs. Mucorales cell wall surface polysaccharides are stained in green and alveolar macrophages are stained in red for the lysosomal protein marker cathepsin D.