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EDITORIAL

Immune responses to Mucorales growth forms: Do we know everything?

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Fungal infections constitute a major health challenge producing considerable morbidity and mortality especially among immunocompromised patients.[1] Both innate and adaptive immune responses are extremely important in combating challenges by yeasts or filamentous fungi causing life-threatening infections to immunocompromised and even to immunocompetent hosts with risk factors. Immune responses to *Candida* spp. and *Aspergillus* spp have been well studied and reviewed.[2,3] By comparison, much less is known about the immune responses against members of Mucorales order.[4]

Both mononuclear and polymorphonuclear phagocytes (PMNs) have the capacity to destroy fungal forms by the generation of toxic metabolites, fungicidal peptides and proteases. Mononuclear cells are able to phagocytose and kill spores of various filamentous fungi including spores of Mucorales species, whereas PMNs attach and extracellularly destroy the hyphal forms created by fungal spores that escape destruction by the macrophages. In addition to their pro-inflammatory role, activated phagocytes through antigen presentation and induction of T-helper cell responses provide a cross-link between innate and adaptive immunity.

However, within Mucorales order, different zygomycetes may have variable susceptibility to effector cells. For example, *Cunninghamella bertholletiae* exhibits decreased susceptibility to PMNs as compared with *Rhizopus arrhizus* or *Rhizopus microsporus*.[5] Human PMNs have also been shown to exhibit reduced capacity to induce oxidative damage against hyphae of *R. arrhizus* compared with *Aspergillus fumigatus*, which may explain the high pathogenicity profile of zygomycetes.[6] Although PMNs respond to *R. arrhizus* hyphae by up-regulating TLR2 mRNA and showing an increased gene expression profile for cytokines and chemokines, including IL-1β and TNF-α, there is no link as yet established between increased inflammatory response and attenuated production of reactive oxygen species by PMNs exposed to *R. arrhizus* hyphae.[6] Nevertheless, pro-inflammatory cytokines have been shown to play an important role activating innate immune cells in their struggle against fungal forms. TNF-α, IFN-γ, G-CSF and GM-CSF are among the most important agents tackling this struggle. IFN-γ and GM-CSF have been shown to augment antifungal activity of PMNs against *R. arrhizus, R. microsporus and Lichthemia corymbifera*.[7] Animal models such as *Drosophila melanogaster*, immunosuppressed mice and persistently neutropenic rabbits have been used to study the immunopathogenesis of invasive mucormycosis more thoroughly.[8-10]

In a further attempt to evaluate the cellular responses against Mucorales, Schmidt et al enriched and cultivated anti-*Rhizopus arrhizus* T cells from healthy individuals. These cells proliferated upon re-stimulation, exhibited cross-reactivity to some but not all Mucorales tested, and increased the activity of phagocytes.[11] In addition, natural killer (NK) cells were shown to damage a wide spectrum of Mucorales, but the antifungal effect was higher if NK cells were administered at an early time point of infection.[12]

What is the reason of more difficulty to prevent and manage mucormycosis than other mycoses? Is it just because Mucorales are more resistant to commonly used antifungal agents like voriconazole? Challenging host immune response is another possible reason that may play a role.[13] There are challenging obstacles that lead to difficulties in the management of invasive mucormycosis by amphotericin B. These include unique host-based risk factors for mucormycosis, the fungus' resistance to innate host defenses and distinctive features of its

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immunopathogenesis, such as extensive angioinvasion, increased virulence and use of chelators by the fungus as siderophores. In addition to these obstacles, the difficulties in early diagnosis, including nonspecific clinical manifestations, lack of serological methods, as well as limitations of culture and molecular methods, lead to delay in initiation of antifungal therapy. Finally, the variability of susceptibility to amphotericin B and resistance to most other conventional antifungal agents leads to major limitations in successful treatment of this devastating infection.

As mucormycosis is the fourth most common, after candidiasis, aspergillosis and cryptococcosis, and highly lethal invasive fungal infection, Wurster et al in their article in this issue of the Journal[14] investigated the cytokine response of human mononuclear cells to conidia and germ tubes of different Mucorales and Ascomycota. Comparative analysis of cytokine expression showed that peripheral blood mononuclear cells (PBMCs) respond to Mucorales species by an increased pro-inflammatory response in contrast to *A. fumigatus* and *F. solani*, Ascomycota that possess immunoprotective hydrophobins on their cell wall. The authors showed that dormant *Rhizopus* spores induce early and strong inflammatory cytokine (IL1β and TNF-α) gene expression in human mononuclear cells as well as elevated secretion of TNF-α, IL1β, IL6, IL8, GM-CSF and MCP-1 by mononuclear cells incubated with spores. The immunogenicity of dormant spores was observed for various Mucorales species, such as *R. arrhizus, R. microsporus, R. circinelloides, C. bertholletiae, L. corymbifera* and other species. Resting spores of *R. arrhizus* induced the upregulation of co-stimulatory molecules on dendritic cells. In another study, data that came also from a comparative analysis performed between *R. arrhizus* spores and *Aspergillus* conidia for their ability to induce the release of TNF-α and IL-6 by human monocytes demonstrated that *R. arrhizus* was not able to induce significantly higher levels of the above mentioned cytokines compared with *Aspergillus* spp[15] In contrast, Wurster et al in their Fig. 3 showed that TNF-α and IL-1β mRNA levels were significantly higher in human monocytes exposed to *R. arrhizus* as compared with *Aspergillus*, but the comparison of these cytokines between the filamentous fungi was not followed through the protein level; therefore, one cannot draw firm conclusions on differences in the cytokine profile of TNF-α induced by *R. arrhizus* and *Aspergillus*, as transcriptional and translational processes do not necessarily coincide. Although the robust pro-inflammatory protein profile shown in the Fig. 2 of the article against *R. oryzae* spores is impressive and undeniable, the comparative analysis between *R. arrhizus* vs. *Aspergillus* spp or other filamentous fungi being based on mRNA data for 2 cytokines does not let us draw firm conclusions about the probable differences in the induced immune responses. It is not known whether any evasion mechanisms increase the pathogenicity of some species of Mucorales over other Mucorales or hyphomycetes.

The authors also found that T-helper cells specifically responding to *Rhizopus* spores can be detected in healthy subjects. They offered the hypothesis that the increased immune response to Mucorales maybe due to the absence of hydrophobins from their cell wall surface, but the proof for such an explanation comes from descriptive studies obtained through scanning electron microscopy and in silico data. They used hydrofluoric acid, a strong chemical that can hydrolyze, apart from hydrophobins, other complex polysaccharide components present on the cell wall of hydrophobin-negative fungal organisms. Scanning electron microscopy image analysis showed altered cell wall morphology, indicating that the chemical agent did in fact act upon the cell wall surface of *R. arrhizus*.

Is the immune response against Mucorales known enough? Yes, if we compare what we know today to what we knew 20 y ago. However, plenty of questions are still there and require their answers. A big question is whether these in vitro phenomena also occur in vivo with so many complex systems in action. What is the role of different classes of antifungal agents on the host immune responses? All 3 classes of antifungal agents, ie azoles, polyenes and echinocandins, have been found to have differential effects on immune responses in the case of *Aspergillus* spp potentially modulating the fungus-host interplay.[4,16] By learning these interactions better we will be able to treat mucormycosis and other serious fungal infections more easily. This is a major task of today's medicine.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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