Mucorcin is a Ricin-Like Toxin that is Critical for the Pathogenesis of Mucormycosis

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Abstract

Fungi of the order Mucorales cause mucormycosis, a lethal infection with an incompletely understood pathogenesis. We now demonstrate that Mucorales fungi produce a cell-associated toxin that plays a central role in virulence. Polyclonal antibodies against this toxin inhibit its ability to damage human cells in vitro, and prevent hypovolemic shock, organ necrosis, and death in mice with mucormycosis. Inhibiting the expression of the toxin in Rhizopus delamarei, the most common cause of mucormycosis, by RNAi also compromises the capacity of the fungus to damage host cells and attenuates virulence in mice. Surprisingly, this 17 kDa toxin has structural and functional features of the plant toxin, ricin, including the ability to inactivate ribosomes, the existence of a motif that mediates vascular leakage, and a lectin receptor binding sequence. Antibodies against the toxin react with ricin by ELISA and Western blotting. Therefore, we propose the name ‘mucorcin’ for this newly identified toxin. Our findings demonstrate the importance of mucorcin in the pathogenesis of mucormycosis and provide evidence that a ricin-like toxin is produced by organisms beyond the plant and bacterial kingdoms. They also suggest that mucorcin is a promising therapeutic target to treat or prevent lethal mucormycosis.

Background and Objectives

- Mucormycosis is a lethal fungal infection that usually affects immunocompromised hosts with overall mortality rate of ~40 to 100%
- A characteristic feature of mucormycosis is the propensity of Mucorales fungi to invade blood vessels, resulting in thrombosis and subsequent tissue necrosis
- Mucorales express CtohK protein, which facilitates invasion of human umbilical vein endothelial cells (HUVECs) via binding to glucose regulated protein 78 kDa (GRP78) receptor during hemagglutination and endocytosis
- Mucorales-mediated damage of HUVECs remain an unidentified mechanism
- Killed hyphae of Rhizopus delamarei (most common cause of mucormycosis) and other Mucorales cause considerable damage to host cells. Thus, fungal-derived toxins are likely involved in the host tissue damage
- The objective of this study was to isolate and characterize the hyphal associated toxin in R. delamarei that cause host tissue damage.

Results

Figure 2. R. delamarei toxin is sufficient to cause damage in vitro and in vivo. 
(a) The effect of the toxin on different cell lines (n=7/group). (b) Damage of extracted or recombinant toxin on epithelial cells at compared time points (n=6/group). Data in a and b = median ± interquartile range from two experiments. 
(c) Mouse weight loss and (d) survival (n=3/group) intravenously injected with 0.1 mg/ml toxin q.d. Jx. (e) Representative mouse organ H&E histomorphographs showing the effects of the toxin. Livers showed necrosis (white arrow), infiltration and calcification of PMNs (black arrow) due to inflammation. Lungs showed hemorrhage and megakaryocytes (arrows).

Figure 3. Inhibition of R. delamarei toxin attenuates virulence of R. delamarei. 
(a) Representative Western blot and densitometry analyses (n=4) of the wild-type empty plasmid, or RNAi toxin strains. (b) Confocal images showing reduced expression of the toxin in the RNAi toxin mutant. (c) RNAi toxin inhibition and (d) anti-toxin antibodies reduced R. delamarei-induced injury of AS49 cells (n=6/group from two experiments, data are median ± interquartile range). (e) RNAi toxin inhibition prolonged survival of mice. (f) Anti-toxin IgG prolonged survival of mice. N=17-18 mice/group for (e) and 20 mice/group for (f) and from two experiments.

Figure 4. Histology of organs showing involvement of the toxin in tissue damage. Histopathological sections of lungs of uninfected mice (a), mice infected with the RNAi empty plasmid R. delamarei strain (b) showed hyphae and granulocyte infiltration (left panel, arrows) and angioinvasion (right panel, arrow), vs. mild signs of inflammation and no angioinvasion for mice infected with RNAi toxin (c). Anti-toxin IgG group showed regular lung tissue (d). Damaged lung tissues (brown color) of mice infected with R. delamarei transfected with RNAi empty plasmid or RNAi toxin (e) or those infected with wild-type R. delamarei and treated with either an isotype-matched IgG or anti-toxin IgG (f) were quantified by ApoTag kit.

Figure 5. R. delamarei toxin is expressed in lung tissue collected from a mucormycosis patient. Staining of a mucormycosis patient lung using anti-toxin IgG (green color). Mucorales hyphae and nuclei shown in yellow and magenta, respectively. R. delamarei toxin staining shown in association with hyphae and released in the tissue (white arrow).

Figure 6. R. delamarei toxin and ricin share biological features. (a) R. delamarei toxin has 29% homology with ricin chain A and two domains of chain B. (b) 3D structural model of R. delamarei toxin shows similarity with ricin B chain. (c) Anti-R. delamarei toxin IgG and anti-ricin IgG bind to ricin-coated or R. delamarei toxin IgG coated ELISA plates. (d) Anti-R. delamarei toxin IgG, anti-ricin IgG or galactose inhibit ricin-mediated AS49 cell damage (n=6/group from 2 experiments, data = median ± interquartile range).

Conclusions

- We identified and characterized a hyphal-associated toxin in Mucorales.
- This toxin damages host cells in vitro and is required for the pathogenesis of mucormycosis in mice.
- Suppression of toxin production in R. delamarei attenuates virulence in mice and anti-toxin antibodies protect mice from Mucormycosis.
- This toxin has structural and functional similarities with ricin toxin produced by the caster bean plant. Thus, ricin-like toxins are produced by organisms beyond the plant and bacterial kingdoms.

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