Response of neutrophil granulocytes to *Curvularia lunata*

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Introduction

Members of the genus *Curvularia* are melanin producing filamentous fungi. Some species have been recovered from opportunistic human infections. The human pathogenic species typically cause phaeohyphomycoses, i.e. mould infections caused by melanized fungi, which can manifest as invasive mycoses with frequent involvement of the central nervous system in immunocompromised patients or as local infections (e.g. keratitis, sinusitis, and cutaneous lesions) in immunocompetent people. Although the number of cases is increasing, only little information is available about the human pathogenic feature of these fungi.

Neutrophil granulocytes play important role in elimination of fungal invaders through their effector functions: phagocytoses, ROS production and NET formation.

In the present study, we compared the activation and ROS production of neutrophils in the presence of *Curvularia lunata* and *Aspergillus fumigatus*.

Results

Figure 1.: Activation of neutrophil granulocytes: myeloperoxidase (MPO) activity was determined with 3,3',5,5'-tetramethylbenzidine (TMB, Sigma-Aldrich) after co-incubation.

Figure 2.: Extracellular release of hydrogen peroxide: release was measured with Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit (Invitrogen) 30 and 60 minutes after infection.

Figure 3.: Cell death of neutrophil granulocytes following the co-incubation was measured with LDH Cytotoxicity Detection Kit (Takara Bio).

Methods

In our experiments, *C. lunata* SZMC 23759 and *A. fumigatus* SZMC 23245, both isolated from human eye infection were examined.

Harvested conidia were germinated in modified KRPB (145mM NaCl, 5.7mM Na₂HPO₄, 4.86mM KCl, 5.5mM glucose, pH 7.35) buffer for 4 (*C. lunata*) or 8 (*A. fumigatus*) hours. Serum opsonisation was maintained in the last hour of germination with +10% serum. Before the experiment, in case of samples signed as „w/o supernatant”, the germination supernatant was changed to fresh KRPB.

Neutrophil granulocytes were isolated from heparinized venous blood of healthy donors with dextran sedimentation followed by centrifugation over Ficoll and hypotonic lysis of erythrocytes. Viability and purity of the cells were checked with trypan blue and Wright staining, respectively. Infection of neutrophils was carried out with germinated conidia in a ratio of 5:1.

Conclusions

- Activation of neutrophil granulocytes was detected in case of both fungal strains when serum opsonisation was performed. However, *C. lunata* could induce activation without opsonisation as well and the supernatant of the germinating conidia was also able to induce the activation. This may assume a soluble factor produced by *C. lunata* that can be recognised by neutrophils.
- Cell death was much higher in the presence of *A. fumigatus* than that of *C. lunata*, when the strains were serum opsonised.
- Release of H₂O₂ was detected only when the fungi were opsonised with serum. However *C. lunata* induced the release of a higher amount of H₂O₂ than *A. fumigatus*. After 60 minutes, we could not detect any production while in case of *Aspergillus*, there was still a detectable amount.

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