In vitro coagulation triggers anti-*Aspergillus fumigatus* neutrophil response

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Background: The association of *in vitro* activation of platelets with decrease of galactomannan release, impaired elongation and loss of fungal cell wall integrity^{1,2} indicates that platelets are involved in anti-*Aspergillus* defense. Furthermore, the ability of *Aspergillus* to induce platelet aggregation *in vitro* and frequent association of severe disseminated aspergillosis with disseminated intravascular coagulation^{3,4} suggest interactions of circulating *Aspergillus* with blood platelets could play a role during aspergillosis by triggering coagulopathy.

Objective: To gain a better understanding of *Aspergillus* interactions with platelets in the blood, especially during clot formation

In vitro model by inoculating either conidia or hyphae into blood samples

Strains: *A. fumigatus* CBS 144.89 or ATCC 204305

Fungal forms: resting conidia, swollen conidia, germings or hyphae

Figure 1. Spiking of blood samples with *A. fumigatus* conidia or hyphae

Anatomopathological and ultrastructural examination of the clot

- Addition of *A. fumigatus* conidia into blood without additive resulted in a **co-localization of resting or swollen conidia and germings with platelet aggregates**, and an increase in the number of surrounding neutrophils (Fig 2B-2D).
- Co-localization with platelet aggregates was also observed for **hyphae**, but **leukocytes were not recruited around aggregates**, and some hyphae were also randomly distributed within the clot (Fig 2E).
- Neutrophil activation in the clot was supported by the observation of **neutrophil-derived cytoplasmic structures surrounding conidia or hyphae** using TEM (Fig 2F-2H).

Figure 2. *A. fumigatus* interactions with blood cells within the clot visualized by microscopy (A-E) and TEM (F-H).

Interactions of *A. fumigatus* with blood cells in the other blood compartments

Interactions of *A. fumigatus* with platelets and/or neutrophils were observed in all the compartments, except the serum. **Conidia phagocytosed by neutrophils were observed in the residual blood clot (Fig. 3C).**

Figure 3. *A. fumigatus* interactions with blood cells within the whole blood (A-B), residual blood clot (C), PRP (D-E)

Conclusion: These data suggest that the **interaction of platelets with *A. fumigatus* during clot formation triggers neutrophil mobilization and activation**, thus establishing a link between inflammation and coagulation. A more systematic **investigation of coagulation disorders** would be needed to assess the role of this phenomenon during aspergillosis.

Chemokine levels determined in the serum and PRP supernatant

- Increased CCL5** and, at a lesser extent, **CXCL4** levels, were determined **in the serum after resting or swollen conidia and germings**, but not hyphae, had been spiked into blood samples.
- Addition of **all fungal forms** resulted in significant increase in **CXCL1 in the PRP** (mainly for strain CBS 144.89), but not in the serum.
- Activation with **all fungal forms** amplified **CXCL8 secretion in the PRP**, and at a lesser extent **in the serum**.

Figure 4. Quantification of CXCL4, CCL5, CXCL1 and CXCL8 in PRP and serum after inoculation of resting conidia (RC), swollen conidia (SC), germings (G) or hyphae (H)

Références: 1. Perkhofer S et al. Human Platelets Attenuate *Aspergillus* Species via Granule-Dependent Mechanisms. J. Infect. Dis. 198;1243–6 (2008). 2. Rambach G et al. Identification of *Aspergillus fumigatus* Surface Components That Mediate Interaction of Conidia and Hyphae With Human Platelets. J. Infect. Dis. 212;1140–9 (2015). 3. Lai CC et al. Invasive pulmonary aspergillosis: high incidence of disseminated intravascular coagulation in fatal cases. J. Microbiol. Immunol. Infect. 40, 141–7 (2007). 4. Balsitis M et al. Primary invasive aspergillosis with disseminated intravascular coagulation as a presenting feature of non-Hodgkin's lymphoma. Med. Mycol. Case Rep. 9, 15–7 (2015).