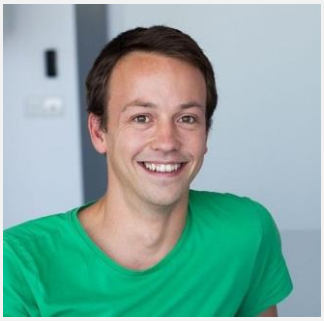
ImmunoTools *special* Award 2015



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"Killing mechanisms of *Candida albicans* and *Aspergillus fumigatus* by human neutrophils; evidence from innate immunity defects."

The occurrence of fungal infections such as those with *Candida* and *Aspergillus* spp. has progressively increased over the last decades as a consequence of the extensive use of immunosuppressive therapies (1;2). Invasive fungal infections, in particular, are associated with high mortality rates of 40-50% (3;4).

Neutrophils are the most important effector immune cells in fungal killing. It appears that adaptive immunity is primarily responsible for controlling *Candida* infection at the mucosal level by interleukin-17 (IL-17) producing lymphocytes, whereas cells of the innate immune system are critical for preventing systemic candidiasis (5-8). The latter is illustrated by the increased prevalence of invasive fungal infections in patients with chemotherapy-induced neutropenia or with neutrophil functional defects, such as genetic deficiencies of the NADPH oxidase as seen in Chronic Granulomatous Disease (CGD) patients (9).

The neutrophil mechanism of fungal killing is poorly understood. First, it is not known which pathogen recognition receptors and their downstream signaling pathways are involved in the killing of *Candida albicans* and *Aspergillus fumigatus*. Both C-type lectin receptors (CLRs), including dectin-1, dectin-2 and mincle, and also integrins, especially complement receptor 3 (CR3, CD11b/CD18, αMβ2), have been demonstrated to recognize the β-glucans and mannans exposed on the *C. albicans* and *A. fumigatus* cell wall (1;10;11). However, two studies performed with dectin-1 knockout mice contradict each other, with one showing that dectin-1 was required for the control of systemic candidiasis, whereas the other claimed that this receptor was not relevant (12;13). In mouse models, both dectin-1 and CR3 have been described to trigger spleen-tyrosine kinase (Syk) and phosphatidylinositol-3-kinase (PI3K) to elicit a cytotoxic response and to activate the signaling adaptor protein CARD9 (14-

16). Recently, human patients with CARD9 deficiency have been characterized to suffer, typically, from Candida meningitis (17), and this was found to be associated with an impaired killing capacity of CARD9-deficient neutrophils towards Candida (28). Although neutrophils are essential in the host defense against *Candida* and *Aspergillus* spp. little is known about recognition and signaling cascades involved in the cytotoxic response. To unravel these mechanisms we used blocking antibodies and chemical compounds, apart from the natural knock-out models that patient cells represent.

In the project we investigated the neutrophils from healthy controls and patients with various primary immunodeficiencies. To assess the condition and purity of the isolated neutrophils we used the following markers for flow cytometry: FITC - conjugated anti-human CD11b, CD14, CD16, CD18, CD62L, Control-IgG1, Control-IgG2a, Control-IgG2b, Annexin V.

One of the important antimicrobial functions of the neutrophils is the mobilization of the azurophilic and specific granules upon stimulation. Therefore we stimulated the neutrophils with PAF/fMLP and CytoB/fMLP and measured the expression of CD63 (azurophilic granule marker) and CD66b (specific granule marker) by flow cytometry.

Furthermore, we determined the pro-inflammatory status of the neutrophils and monocytes from the immunodeficient patients. Therefore we stimulated the neutrophils and monocytes with TLR and dectin-1 ligands overnight, and we measured in the supernatant the IL-6 and IL-8 production by an ELISA-kit.

One of the results is that we found that human neutrophils use two distinct and independent mechanisms to kill *Candida albicans*, governed by the presence of opsonins on the yeast. The first mechanism, for the phagolysosomal killing of unopsonized *Candida albicans* depends on CR3 (CD11b/CD18) recognition and signaling via Syk, PI3K and CARD9, but is completely independent of NADPH oxidase activity. The second mechanism, for phagolysosomal killing of serum-opsonized Candida albicans, is strictly dependent on the Fc-gamma receptors and Reactive Oxygen Species (ROS) production by the NADPH oxidase system, in which the tyrosine kinase Syk does have a role but PI3K does not. The study clearly demonstrates the presence of two independent pathways for fungal killing that may contribute to the identification of novel therapeutic targets and provide an explanation for the clinical phenotype observed in dectin-1 deficiency, CARD9 deficiency and CGD (19).

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