The role of neutrophil-derived EVs in promoting *Candida albicans* escape from phagocytosis

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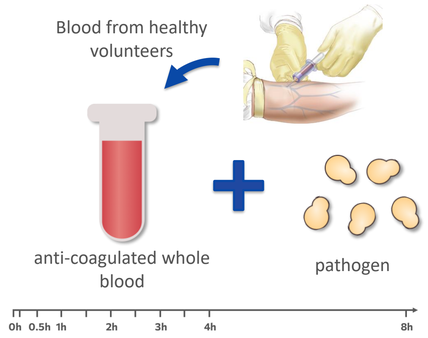
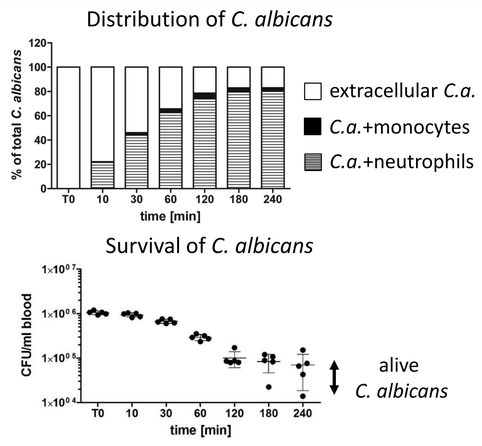
Abstract

- Opportunistic fungus ***Candida albicans*** is one of the leading causes of **bloodstream infections**, especially in immunocompromised patients
- A substantial proportion of fungal cells remain **extracellular** in the human whole-blood infection assay, indicating effective **immune evasion strategies**
- Extracellular *C. albicans* cells acquire **host-cell molecules** through interactions with **neutrophil-derived extracellular vesicles (EVs)**
- **Mathematical modeling** is used **to unravel the mechanism** how EV-decoration of the pathogen surface modulates its interaction dynamics with the host
- **EV-decorated *C. albicans*** are **phagocytosed about 60% less efficiently** than cells lacking host-derived surface features
- These results uncover a previously **unrecognized role of neutrophil-derived EVs in promoting immune evasion** of *C. albicans* by modulating its susceptibility to phagocytosis

Human whole-blood infection assay

- **mimics the *in vivo* scenario** of the innate immune response [1]
- allows monitoring **host-pathogen interactions**
- many variables are accessible to **direct experimental quantification**

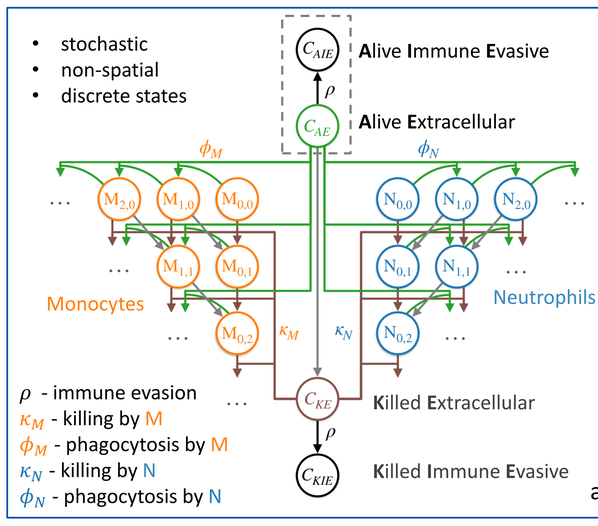


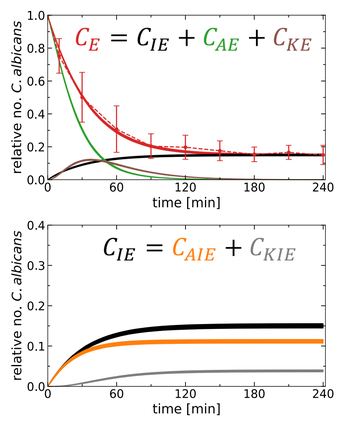


➢ Some fungal cells remain **extracellular**

State-based model [1,2]

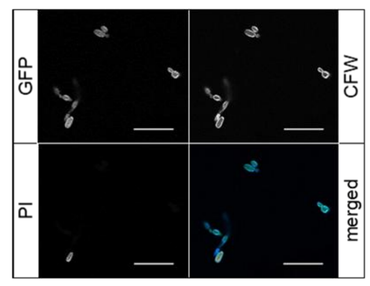
- stochastic
- non-spatial
- discrete states





Virtual infection model prediction: almost all extracellular *C. albicans* stay alive

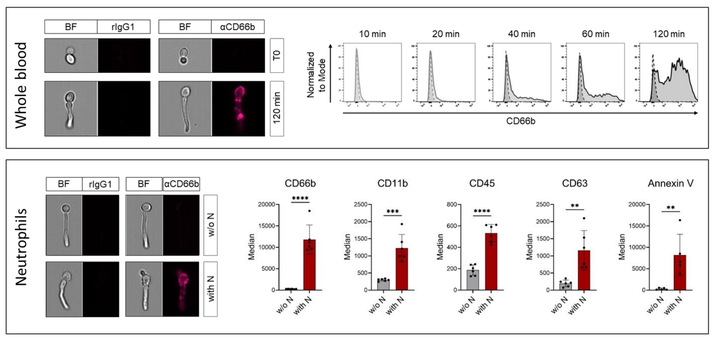
Experimental validation:



confirmed experimentally

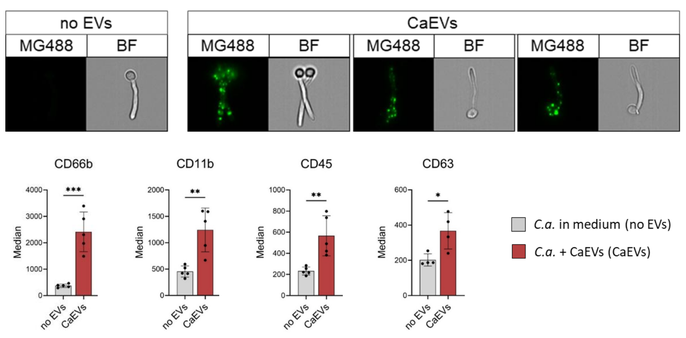
Experimental characterization of EVs

Neutrophils coat *C. albicans* with host molecules



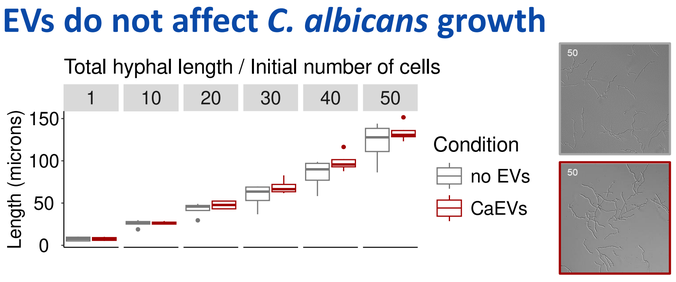
Imaging flow cytometry and flow cytometry analyses of the surface of extracellular *C. albicans* during whole-blood and neutrophil infection revealed the deposition of neutrophil proteins.

Isolated EVs associate with *C. albicans*



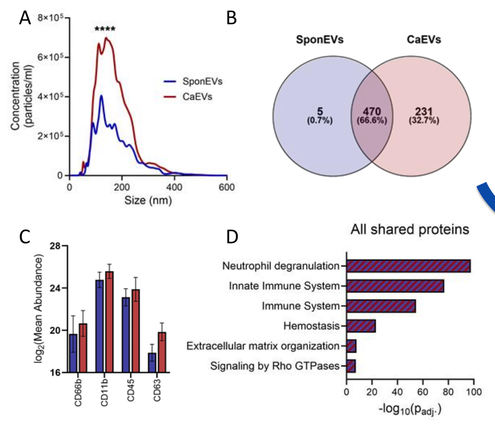
Neutrophil-derived EVs bind to *C. albicans* and mediate the transfer of neutrophil surface markers, as demonstrated by flow cytometry.

EVs do not affect *C. albicans* growth



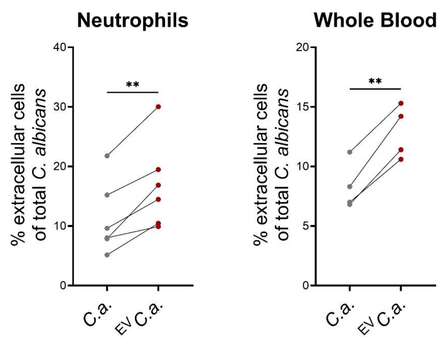
Live-cell imaging of *C. albicans* with and without CaEVs; fungal growth assessed by hyphal length. Numbers indicate the respective time frame.

Characterization of neutrophil-derived EVs



EVs isolated from neutrophils exposed to *C. albicans* (CaEVs, red) or mock-infected (SponEVs, blue). (A) Size and concentration of isolated EVs. (B) Proteins shared and unique to SponEVs and CaEVs. (C) Abundance of deposited neutrophil markers in EVs. (D) Significantly enriched categories among shared proteins.

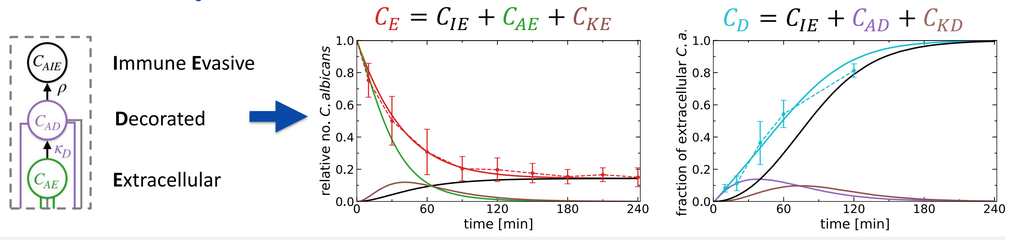
EVs reduce phagocytosis of *C. albicans* by neutrophils



Extracellular *C. albicans* (%) during infection of neutrophils and whole blood. *C. albicans* were pre-incubated with CaEVs ($^{EV}C.a.$) or in medium without EVs as control (*C.a.*).

Hypothesis-driven virtual models

Decoration precedes immune evasion



Decoration rate:

$$\overline{\kappa_D}(t = n\Delta t) = \kappa_{D0} + \kappa_D \sum_{m=0}^{n} \frac{N_{GP}(t = m\Delta t)}{G_{0,0}(0)}$$

κ_{D0}: decoration by **SponEVs**

κ_D: decoration by **CaEVs**

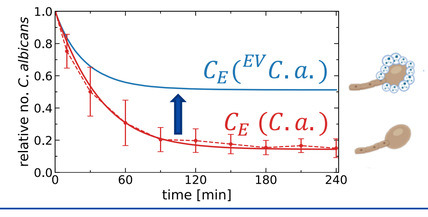
$N_{GP}(t = m\Delta t)$: # neutrophils that phagocytosed for the first time

$G_{0,0}(0)$: total # neutrophils

Model prediction:

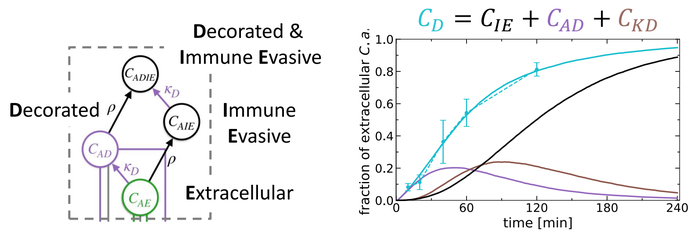
Phagocytosis assay

confirmed experimentally



Virtual infection model predicts an increase in extracellular population of pathogen when pre-treated with neutrophil-derived EVs

Decoration and immune evasion are independent

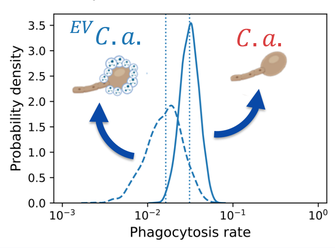


Model quantifies rates:

- *n*-dimensional mode of ABC posteriors to estimate parameters
- phagocytosis rates by neutrophils:
  - non-decorated *C. a.* : $\phi_N \approx 0.031\ s^{-1}$
  - decorated *C. a.*: $\phi_{ND} \approx 0.015\ s^{-1}$

➢ EV-decoration of *C. a.* **slows down** phagocytosis by neutrophils ~ **2 times**

ABC posterior distributions



Both models predict **different extracellular population dynamics** for pre-decorated *C. albicans* at later time points $C_E(^{EV}C.a.)$

Suggestion for a new experiment!

Conclusion and outlook

- **Neutrophil-derived EVs** are released upon *C. albicans* exposure and bind to fungal cells
- EV binding does not affect fungal growth
- Mathematical modeling predicts that **EV binding reduces phagocytosis efficiency** of neutrophils; this result was confirmed experimentally
- Mathematical modeling does not exclude **an alternative pathway** for immune evasion **without prior decoration**
- Quantification shows that **decorated pathogens are phagocytosed ~ 2× slower**
- To distinguish between competing hypotheses, we suggest an **additional experiment**

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References
[1] Hünniger *et al.* 2014. *PLOS Comput Biol.* 10(2)
[2] Lehnert *et al.* 2015. *Front Microbiol.* 6:608

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