Neutralization of the *Candida albicans* toxin, candidalysin, blocks epithelial damage and dampens inflammatory responses associated with vulvovaginal candidiasis immunopathogenesis

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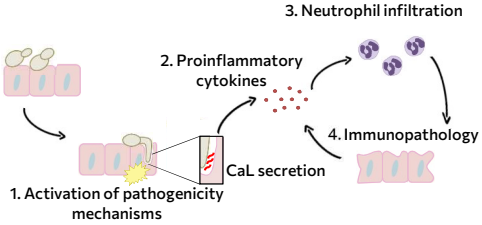
Abstract:

The fungus ***Candida albicans*** is typically a harmless member of the human microbiota, but can cause **vulvovaginal candidiasis (VVC)**[1]. *C. albicans* **secretes a toxin candidalysin (CaL)**, which causes host damage and elicits an immune response[2]. However during VVC, recruited **neutrophils exacerbate inflammation** leading to symptoms. Unknown causes of infection, recurrence, and antifungal resistance complicate VVC treatment[3]. Therefore, as a therapeutic strategy, **we evaluated using nanobodies to neutralize candidalysin** to prevent epithelial damage and hyperinflammation.

What is vulvovaginal candidiasis (VVC)?



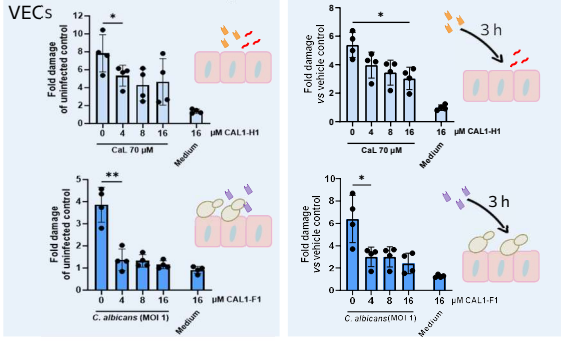
- 75% of women have at least one infection[3]
- 5% of women have recurrent (R)VVC and have at least 4 infections per year
- VVC is treated with:
  - ❖ Probiotics containing lactobacilli that normally antagonize *C. albicans* pathogenicity in healthy women
  - ❖ Antifungals, specifically azoles

Testing anti-candidalysin nanobodies on vaginal epithelial cells (VECs):

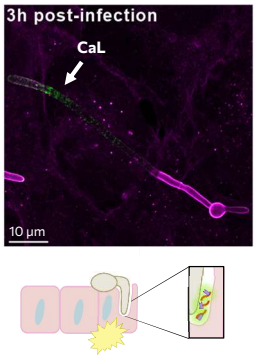
Measuring host damage – lactate dehydrogenase (LDH) release:

Simultaneous:
Add CaL/ *C. albicans* and nanobodies simultaneously on VECs

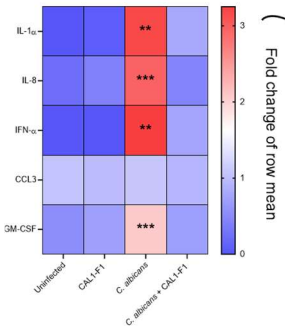
3 h treatment:
Treat/ infect VECs for 3 h before adding nanobodies



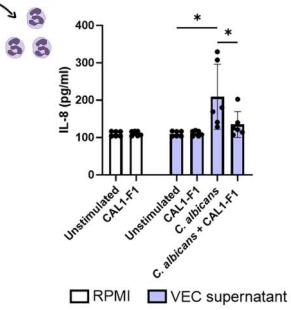
Nanobodies bind CaL inside of the invasion pocket:



Cytokines released by VECs:



IL-8 release by neutrophils stimulated with VEC supernatants:



- Nanobody decreased cytokine secretion by VECs
- Neutrophils stimulated with supernatants of VECs infected in the presence of nanobody secrete less IL-8

In silico modelling of anti-candidalysin nanobody and candidalysin interaction on VECs:

Objectives:

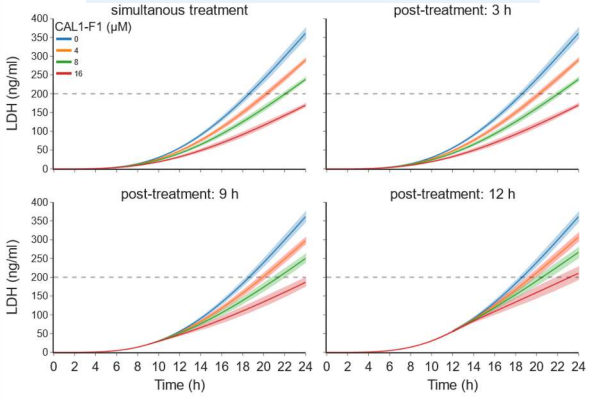
Use *in vitro* data to model the neutralization of CaL

- Test and verify findings using *in vitro* infection models
- Predict optimal dosing: concentration and time

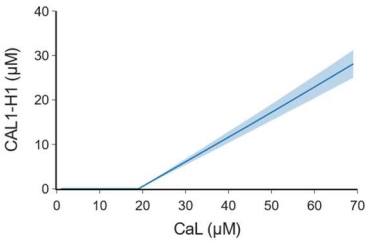
Two ordinary differential equation (ODE) models[4]:

1. Interaction between synthetic CaL and CAL1-H1
2. Interaction between *C. albicans*-secreted CaL and CAL1-F1

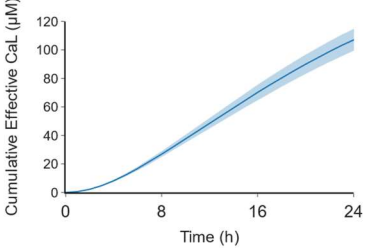
Treatment strategies for *C. albicans*–secreted CaL:



Optimal dosing strategy:



C. albicans secretion of effective CaL:



Measure:
- LDH (lactate dehydrogenase) after certain time points

Threshold for neutralization:
- 10% represents the proportion of uninfected VEC damage to total VEC damage after 24 h
- ca 200 ng/ml LDH

Results from modelling synthetic CaL:
- Optimal dosing strategy: How much CAL1-H1 is needed to reduce VEC damage to below 10%?
- Linear relationship between nanobody and CaL
- The neutralization ratio is *ca.* 2 CaL : 1 nanobody

Results from modelling *C. albicans*-secreted CaL:
- With no nanobody treatment VEC damage will get higher than 10% after 18 h
- 16 µM nanobody is sufficient to reduce damage below 10% damaged VECs after 24 h
- Post-treatment can be conducted after 9 h with only a minor change in host damage
- Model quantifies the amount of "effective" CaL that needs to be neutralized over time
- After 24 hours around 107 µM is released by 20,000 cells

→ At maximum around *ca.* 53.5 µM is needed for neutralization

Conclusion & Outlook:

- Anti-candidalysin nanobodies neutralize candidalysin, thereby inhibiting VEC damage and subsequent immune responses that drive VVC pathogenesis
- By combining *in vitro* data with *in silico* modelling, we provide a preclinical proof-of-principle
- Forms the basis for future development and application of anti-candidalysin nanobodies as VVC treatment *in vivo*

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References
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