Effectiveness of polyclonal antibody anti-CR3-RPAb directed against surface antigen CR3-RP in the biofilm development of *Candida albicans* and *Candida dubliniensis*

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INTRODUCTION

The formation of fungal biofilms remains a serious medical problem. In particular, once biofilms are formed, they display high levels of resistance against most common antifungals. *Candida albicans* and *Candida dubliniensis* are closely related organisms with respect to morphology and phylogeny, but differ in their expression of virulence-associated proteins involved in adherence and biofilm development. Complement receptor 3-related protein (CR3-RP) is one of the key surface antigens expressed by many pathogenic *Candida* species associated with adherence and biofilm formation.

The main objective of this research was to investigate the role of the polyclonal antibody (Ab) directed against antigen CR3-RP in the inhibition of biofilm formation of *C. albicans* and *C. dubliniensis* using different *in vitro*, *ex vivo* and *in vivo* models, and to assess a potential effect of Ab treatment on preformed biofilm of both species.

MATERIAL & METHODS

RESULTS

(All results are illustrated in the figures 1 – 5)

Fig. 1 *In vitro* biofilm assay. Determination of metabolic activity of biofilm cells (XTT reduction assay) and quantifying of biomass (Crystal violet staining).

- A) Anti-CR3-RP Ab added at the beginning biofilm formation – reduction of about 75% and 46% for *C. albicans* SC5314 and *C. dubliniensis* CBS7987, respectively.
B) Anti-CR3-RP Ab added after 24 h to pre-formed biofilm – reduction of about 26% and 20% for *C. albicans* SC5314 and *C. dubliniensis* CBS7987, respectively.

Fig. 3 CLSM microscopy illustrates structure, quantity and thickness of 48-h biofilm formed by both yeast spp.: A) SC 5314+PBS - 28 µm, B) CBS 7987+PBS - 20 µm - C) SC 5314+anti-CR3-RP Ab - 18 µm, D) CBS 7987+anti-CR3-RP Ab - 12 µm

Fig. 4 Reduction in biofilm development on mouse tongues *ex vivo*: A) SC 5314+PBS, B) CBS 7987+PBS, C) SC 5314+anti-CR3-RP Ab, D) CBS 7987+anti-CR3-RP Ab. Figures A-B (control suspension incubated with 1xPBS) illustrate the massive biofilm on tissue with enormous invasion to the inner layers of the tongues. The biofilm formed by suspension pre-treated with the anti-CR3-RP Ab (figures C-D) was incompact and thinner. Adhesion and invasion to the tissue was markedly reduced.

Fig. 2 Brightfield microscopy of biofilm. Effect of anti-CR3-RP Ab on biofilm formed by the standard strain *C. albicans* SC5314 (a-c) and *C. dubliniensis* CBS7987 (d-f) on polystyrene surface *in vitro*; control 48 h biofilm without antibody – a, d; biofilm formed in the presence of anti-CR3-RP Ab added in the adherence phase (t = 0 h) – b, e; biofilm formed in the presence of anti-CR3-RP Ab added to pre-formed biofilm (t = 24 h) – c, f. Bars represent 100 µm. All samples were stained with 0.6% crystal violet.

Fig. 5 *Galleria mellonella* „killing assay“ illustrates survival of larvae after administration of anti-CR3-RP Ab *in vivo*. The differences in the number of survivals were observed in both strains A) *C. albicans* SC 5314 and B) *C. dubliniensis* CBS 7987. The increase of survival in treated group was up to 40 % after 72 h for both strains compared to the control group in which only 1xPBS was applied. The larvae were considered death (figures 1 and 3) when they were black and did not response to the touch. The survivals (figures 2 and 4), treated with the anti-CR3-RP Ab, were considered alive when they were viable and reactive to the external stimuli.

SUMMARY

- ❑ The CR3-RP protein is important surface protein associated with adhesion and biofilm development of *C. albicans* and *C. dubliniensis*.
- ❑ Results demonstrate decreased metabolic activity and biomass of biofilms exposed to Ab in both tested *Candida* species. When antibody was added at the beginning biofilm formation. Additionally, the effectiveness of Ab on the pre-formed 24h biofilms of both species was confirmed.
- ❑ The presence of antibody decreased an invasion and biofilm formed on mouse tongues and increased survival of *G. mellonella*.
- ❑ Data suggest a potential activity of the anti-CR3-RP Ab against biofilm-associated *Candida* infections.