Haemocyte responses in *Galleria mellonella* infected by *Candida glabrata*, *Candida nivariensis* and *Candida bracarensis*

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Introduction

Candida glabrata is the second or third leading cause of invasive candidiasis in many European countries. This species has acquired a relevant clinical significance because of its low sensitivity to current azole antifungal drugs. *Candida glabrata* has two related species, *Candida nivariensis* and *Candida bracarensis*. The animal model in *Galleria mellonella* allows us to study the response of the innate immune system through the production of hemocytes in response to a possible infection.

Objectives

To determine the haemocyte responses during invasive infections caused by *Candida glabrata*, *Candida nivariensis* and *Candida bracarensis* in a *Galleria mellonella* model.

Methods

Galleria mellonella larvae were distributed in groups of five and infected with each of the following reference strains: *Candida glabrata* ATCC 90030, *Candida glabrata* NCPF 3203, *Candida nivariensis* CBS 9984, *Candida nivariensis* CECT 11998, *Candida bracarensis* NCYC 3397 and *Candida bracarensis* NCYC 3133.

Larvae were inoculated with 10 μ l of 1×10^5 , 1×10^6 and 1×10^7 yeast/ml in the last left pro-leg with a precision syringe. Two control groups were used: untouched larvae and larvae inoculated with PBS buffer supplemented with ampicillin (20 μ g/ml). Larvae were incubated for 3 h at 37 °C. Haemolymph of each larva was collected and was mixed in the same volume with IPS buffer (Insect

Physiological Saline buffer) to avoid melanisation. The haemocyte response was determined by counting cells using a haemocytometer. Each assay was repeated at least three times. The results obtained with each inoculum were analysed using one-way ANOVA to evaluate the differences between strains ($p < 0.05$ was considered as statistically significant).

Results

Larvae infected with both *Candida glabrata* strains presented the same behaviour: statistically significant differences in the response of haemocytes were observed between the untouched larvae and those infected with the 1×10^5 and 1×10^6 inocula, but not differences were detected with 1×10^7 cells/ml inoculum (Figure 1).

Figure 2: Haemocyte production in larvae infected with *Candida nivariensis* CBS 9984 and CECT 11998. (* statistically significant differences with Untouched larvae)

Larvae infected with *Candida nivariensis* CECT 11998 presented statistically significant differences between the untouched larvae and those infected with the 1×10^5 and 1×10^6 inocula, but not with CBS 9984 strain, in which differences were detected with 1×10^7 and 1×10^5 cells/ml but not with 1×10^6 cells/ml (Figure 2).

Figure 1: Haemocyte production in larvae infected with *Candida glabrata* ATCC 90030 and NCPF 3203. (* statistically significant differences with Untouched larvae)

Figure 3: Haemocyte production in larvae infected with *Candida bracarensis* NCYC 3397 and NCYC 3133. (* statistically significant differences with Untouched larvae)

Larvae infected with *Candida bracarensis* strains presented differences with untouched larvae. No significant differences between larvae infected with all the assayed inocula were showed (Figure 3).

Conclusion

Galleria mellonella is a useful model to study invasive candidiasis throughout analysis of haemocyte responses to the infections caused by *Candida glabrata*, *Candida nivariensis* and *Candida bracarensis*. The haemocytes responses in *Galleria mellonella* larvae infected with *Candida glabrata* and *Candida nivariensis* was similar but differences between the inocula injected were observed. However, there were no differences in the haemocytes production in larvae infected with *Candida bracarensis*. The most adequate inoculum to study the phagocytic capacity was 1×10^5 cells/ml since with higher inoculums the larvae died and there was no time to generate a clear immune response.

Funding

This study was partially financed by GIC15/78 IT-990-16 (Gobierno Vasco-Eusko Jaurlaritza). AH has a grant from the UPV/EHU.