

SERUM INTERLEUKIN-6 LEVELS IN MURINE MODELS OF *CANDIDA ALBICANS* INFECTION

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Two Balb/C mouse models of *Candida* infection were used to detect serum interleukin-6 (IL-6) responses. The first model used systemic infection by *Candida albicans* ATCC 10231 strain infected through the lateral tail vein of mice without any specific pretreatment. The median *Candida* burdens of the kidneys were 1.5×10^6 CFU/ml 24 h postinoculation (p.i.) and 1.2×10^7 CFU/ml 72 h p.i., while median serum IL-6 levels were 479.3 pg/ml and 934.5 pg/ml, respectively. The *Candida* burden showed significant correlation with serum IL-6 24 h p.i. ($R^2 = 0.6358$; $P = 0.0082$) but not 72 h p.i.

The second model was a mouse vaginitis model applying intravaginal inoculation of mice pretreated with subcutaneous estradiol-valerate (10 mg/ml) 3 days before infection. *Candida* cell count in vaginal lavage fluid was 2.8×10^6 CFU/ml 24 h p.i. and 1.4×10^8 CFU/ml 72 h p.i. Serum IL-6 response was detected in 4 of 15 mice 24 h p.i. and 9 of 15 mice 72 h p.i. Even the responders had low IL-6 serum levels (mean values 29.9 pg/ml and 60.1 pg/ml, respectively) not correlating with *Candida* cell count in vaginal lavage fluid.

In conclusion, serum IL-6 had strong relationship with systemic *C. albicans* infection while the local *C. albicans* infection of the vagina led to partial, prolonged and limited serum IL-6 response.

Keywords: interleukin-6, vulvovaginitis, systemic candidiasis, mouse, *Candida albicans*

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Introduction

Candida albicans (*C. albicans*) is a permanent member of the human microbiome and colonizes the human gastrointestinal, respiratory and reproductive tracts. However, when the host physiology is damaged (i.e. immunosuppressant therapy, diabetes mellitus) this fungal-host interrelationship can degenerate and may lead to opportunistic fungal infections, which emerge as mucosal or invasive fungal disease [1, 2].

Candida species are the fourth most frequent cause of healthcare-associated bloodstream infections in the hospital environment, which is associated with high mortality (30–50%) [3]. On the basis of the ARTEMIS DISK 1997–2003 surveillance program *C. albicans* is the most common species causing disseminated candidiasis worldwide (66% of all *Candida* species) [3, 4]. According to last published epidemiology data, comparable rate was observed at University of Debrecen and at University of Szeged, *C. albicans* was identified in 58% and 64% of *Candida*-positive blood culture, respectively [5, 6]. The major risk factors of *Candida*-caused bloodstream infections are immunosuppressant therapy, broad-spectrum antibacterial agents, prolonged use of catheters in intensive care unit as well as different surgery interventions [4].

Vulvovaginal candidiasis (VVC) is a common mucosal infection caused by different *Candida* species including *C. albicans*. Approximately 70–75% of healthy women suffer at least one VVC episode during their childbearing age. In the USA VVC is the second most common type of vaginal infections after bacterial vaginitis. The most typical predisposing factors of VVC are disturbance in hormone levels owing to pregnancy, antibiotic treatment, high-estrogen contraceptive usage and uncontrolled diabetes mellitus [7, 8].

During the acute phase of infection, IL-6, a 22–30 kDa glycoprotein is secreted into the bloodstream and induces both B cell and T cell differentiation as well as production of antibodies and acute phase proteins [9–11]. Since host cytokine response against *Candida* infection is poorly understood, a systemic and a vaginal candidiasis mouse model were used to measure serum IL-6 responses.

Materials and Methods

In the course of experiments the strain *C. albicans* 10231 ATCC was used. Balb/C immunocompetent female mice (19–22 g) were infected in our *in vivo* models. The animals were maintained in accordance with the Guidelines for the

Care and Use of Laboratory Animals. The experiments were approved by the Animal Care Committee of the University of Debrecen (permission no.: 12/2008).

During the examination two models were used (vaginal and systemic infection mouse model). Animals were divided into 3 major groups per models. In vaginal infection model, 50 μ l subcutaneous estradiol-valerate (10 mg/ml) was given to all mice three days prior to infection [11–13].

Animals were infected intravenously through the lateral tail vein (200 μ l of infective inoculum) in case of systemic model [14]. In the systemic model the infectious dose of *C. albicans* was 3×10^5 CFU/mouse based on our preliminary investigations. In the vaginal model, mice were infected intravaginally with 3×10^5 stationary-phase *C. albicans* cells in 50 μ l volumes. Inoculum density was confirmed by plating serial dilution on Sabouraud agar plates. In case of systemic mouse model the animals were sacrificed (cervical dislocation) after 24 h and 72 h, kidney pairs were removed and homogenized aseptically. Fungal tissue burden was determined by quantitative culturing. The homogenates were diluted by 1 ml sterile saline and serial tenfold dilutions were made in saline, subsequently plated onto Sabouraud dextrose agar and the colony count was determined after 48 h. The control mice did not receive infective inoculation [14].

At 24 h p.i. the first group of the mice of vaginal model was sacrificed and flushed their vagina with sterile physiological saline (100 μ l). Thereafter 50 μ l volumes was taken out from lavage fluid, serially diluted tenfold and plated onto Sabouraud dextrose agar. This method was repeated after 72 h at the second group. The third group (control mice) received just hormone treatment. Control mice were investigated both at 24 h p.i. and 72 h p.i. All colony counts were determined after 48 h culture on Sabouraud plates.

In case of both models, blood was taken in way of retro-orbital with Pasteur pipette (0 h, 24 h and 72 h p.i.). The samples were centrifuged at 24 h after the collection (5 minute $6000 \times g$). The serum was isolated from each sample and put into sterile Eppendorf tubes. The serum samples were stored at -20°C .

The serum samples were measured by Luminex xMAP (Biomedica) with mouse cytokine panel. The differences between groups were analyzed by Mann–Whitney U-test. Chi-square for trend test was used to test increasing number of responders by time. The level of significance was set at $P < 0.05$.

Results

IL-6 levels and systemic infection

At 24 h after intravenous *C. albicans* ATCC 10231 inoculation, the median of cell count was 1.5×10^6 CFU/ml. The distribution of the *Candida* burden at 24 h was characterized by 2 mice with kidneys remained sterile and 13 mice with *Candida* cell count ranging 6×10^5 – 8.6×10^6 CFU/ml. An average 7.6-fold increase was found in the infected kidneys compared with the starting inoculum (Fig. 1).

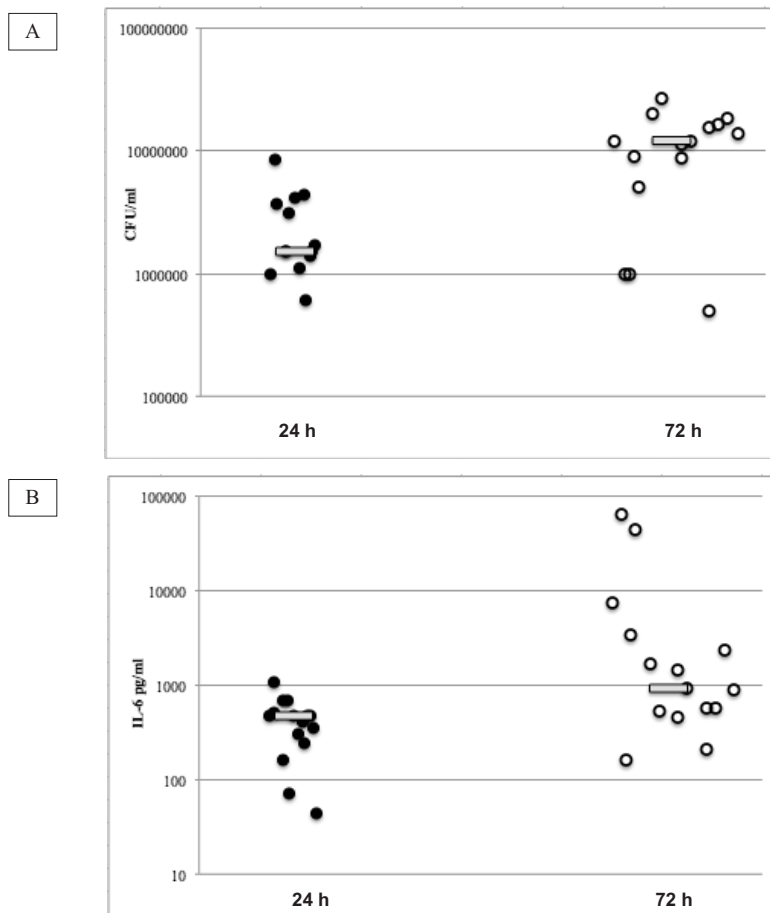
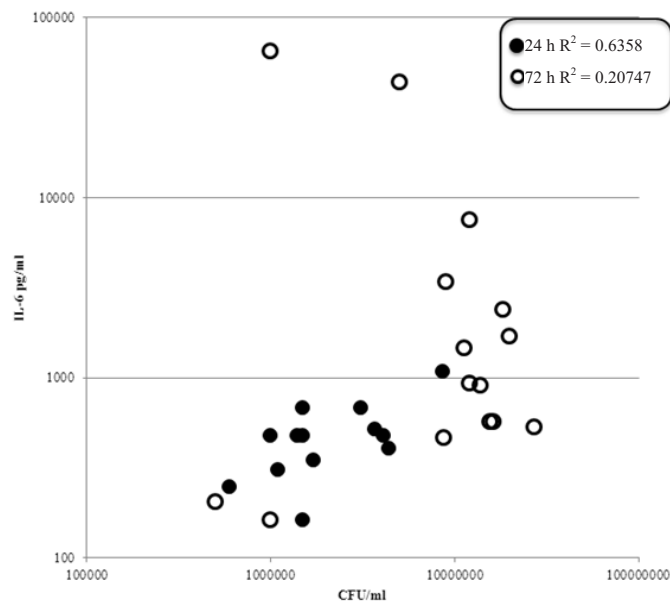


Figure 1. *Candida* cell burden of mice kidneys (A) and IL-6 levels of serum (B) in systemic candidiasis. At 24 h the two lowest IL-6 values (<100 pg/ml) belong to mice with kidneys remained sterile

At 72 h after intravenous *C. albicans* ATCC 10231 inoculation, the median of cell count was 1.2×10^7 CFU/ml. All 15 mice revealed *Candida* infection in the kidneys, the *Candida* cell count ranged from 5×10^5 CFU/ml to 2.7×10^7 CFU/ml. An average 38-fold increase was found in the infected kidneys compared with the starting inoculums. The increase in the *Candida* burden of the kidneys between days 1 and 3 was significant ($P = 0.002$) (Fig. 1).

On day 0, the baseline serum IL-6 was below the detection level in all tested mice as well as serum IL-6 levels of control mice remained below detection level after either 24 h or 72 h observation. In mice inoculated with *C. albicans*, the serum IL-6 level were markedly above the detection threshold both 24 h and 72 h p.i. (44.2–1083.6 pg/ml at 24 h; 162.4–64841 pg/ml at 72 h). Nevertheless, the two mice with sterile kidneys at 24 h p.i. had outlyingly low serum IL-6 levels. Between days 1 and 3, the IL-6 level of the serum had an average 20-fold increase ($P = 0.004$) (Fig. 1). At 24 h correlation was observed between the *Candida* cell count and IL-6 level ($R^2 = 0.6358$, $P = 0.0082$). After 3 days of the infection, the correlation disappeared in spite of the increasing trend of both markers ($R^2 = 0.20747$) (Fig. 2).



IL-6 levels and vaginal infection

At 24 h after intravaginal *C. albicans* ATCC 10231 inoculation, all 15 mice were suffering from marked vaginal discharge, the median *Candida* cell count was 2.8×10^6 CFU/ml vaginal lavage fluid (range: 1×10^5 – 3×10^7 CFU/ml). An average 19.6-fold increase was detected in the lavage fluid compared with the starting inoculum. At 72 h after vaginal *C. albicans* inoculation, the median of cell count was 1.4×10^8 CFU/ml. All 15 mice revealed *Candida* infection in their vagina, the *Candida* cell count ranged from 9.4×10^5 CFU/ml to 6.5×10^8 CFU/ml. On day 0, the baseline serum IL-6 was below the detection level in all tested mice. At 24 h

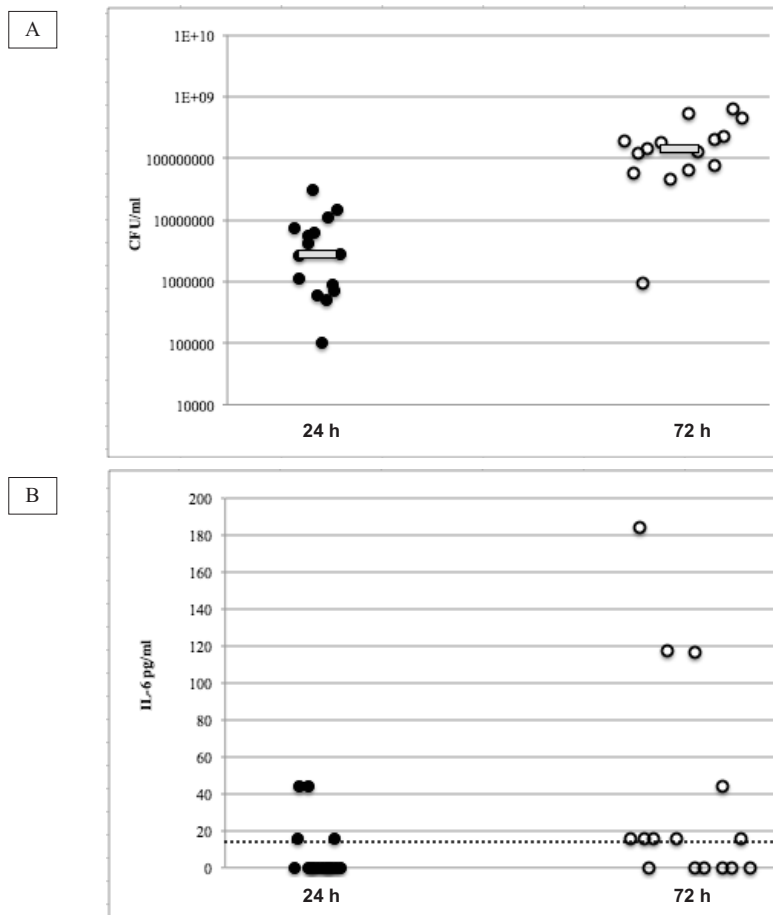


Figure 3. *Candida* cell burden of lavage fluid (A) and IL-6 levels of serum (B) in vulvovaginal candidiasis

detectable IL-6 level was observed above the threshold in 4 of 15 mice. At 72 h this ratio was 9 out of 15. At 24 h and 72 h detectable IL-6 levels were not noticed in control mice. The proportion of IL-6 responder mice significantly increased by time of infection ($P = 0.01$). The ranges serum IL-6 levels of the responders to vaginal infection (15.6–44.2 pg/ml at 24 h p.i. and 15.6–184 pg/ml at 72 h p.i.) were much lower than the levels measured in systemic infections (Fig. 3).

Discussion

C. albicans is one of the most common inhabitants of our microbiome. This *Candida* species caused infections can emerge from mucocutaneous candidiasis to life-threatening candidaemia [2, 3, 15].

During our investigations proinflammatory IL-6 cytokine response was determined in case of systemic candidiasis and in *Candida* vaginitis mouse model. In the course of *C. albicans* infection, some proinflammatory cytokines (IL-6, TNF- α , IL-12) are essential for the efficient control of infection [7]. IL-6 induces acute phase proteins increased secretion therefore it has a pivotal role for development on proper innate immune response [7]. Romani et al. observed that IL-6 deficient mice are more susceptible to disseminated candidiasis than wild-type mice [16] suggesting that IL-6 production is fundamental in terms of protection [17].

In the background of two mice with sterile kidneys 24 h after intravenous inoculation of *C. albicans*, we assume the role of time factor, i.e. the systemic infection has not completely resulted in colonization of kidneys by this time in the whole inoculated population. Systemic proliferation of *C. albicans* might have been delayed in these two mice as indicated by serum IL-6 levels exceeding those in single organ infected but scoring under the IL-6 levels of mice with kidney colonization. In the disseminated candidiasis model at 24 h the measured IL-6 level correlated with the *Candida* cell count. By 72 h p.i., the serum IL-6 levels increased further, however lost correlation with microbial burden. An explanation for this phenomenon is that strong defence mechanisms might have interfered with the microbial burden more efficiently by this time. Another possible explanation is that the serum IL-6 levels by this time might be close to the individual maximum value. Steinshamn et al. investigating serum IL-6 level from 3h to 72 h p.i. in their mouse model observed its peak at 24 h [18]. Comparable IL-6 level was observed at 24 h in case of our disseminated candidiasis model. However, at 72 h our measured IL-6 value was significantly higher compared with at 24 h measured value. Presumably the reason of this phenomenon that our mice were infected in-

travenously while Steinshamn et al. give the infected inoculum intraperitoneally to mice [18].

In our VVC mouse model, significant IL-6 level increase was not measured at 24 h. Presumably the reason of this phenomenon that the infection remained local, therefore it could not trigger systemic immune response against *C. albicans*. By 72 h the fungal vaginal burden and the measured IL-6 level further increased, but the rates of increase were slower and lower compared with those in our systemic mouse model.

In conclusion our VVC model was adjusted successfully. Serum IL-6 had strong relationship with systemic *C. albicans* infection, while the local *C. albicans* infection of the vagina led to partial, prolonged and limited serum IL-6 response. Further *in vivo* experiments are needed to assess the role of different cytokines in the course of mucocutaneous and systemic candidiasis.

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