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Non-lethal *Candida albicans cph1/cph1 efg1/efg1* mutant partially protects mice from systemic infections by lethal wild-type cells

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ABSTRACT

Although *Candida albicans cph1/cph1 efg1/efg1* mutant cells are not lethal to mice, they proliferated in infected mice instead of simply being cleared by the host immune system. Here, we have shown that the *cph1/cph1 efg1/efg1* mutant partially protects mice from systemic infections by the lethal wild-type *Candida albicans* cells. Our results further indicate that a second dose of the *cph1/cph1 efg1/efg1* mutant did not boost the degree of protection.

Introduction

The prevalence of fungal infections has increased significantly due to alterations in immune status associated with the acquired immunodeficiency syndrome (AIDS) epidemic, cancer chemotherapy, organ and bone marrow transplantation, and invasive hospital procedures (White *et al.* 1998; Yang & Lo 2001). Among fungal pathogens, *Candida albicans* is the most frequent cause of disease, ranging from minor infections in immunocompetent individuals to lethal systemic infections in immunocompromised individuals (Pappas *et al.* 2003).

Candida albicans can switch from a unicellular yeast form into filamentous forms having pseudohyphae and hyphae. Under laboratory culture conditions, such as in media containing sera, where the wild-type cells are induced to form hyphae, the *cph1/cph1 efg1/efg1* mutant cells failed to form either pseudohyphae or hyphae (Lo *et al.* 1997). In a mouse model of systemic infections, the wild-type cells cause death of the injected mice, most likely due to tubular necrosis leading to renal failure. Recently, we have investigated the *in vivo* proliferation and invasion of *C. albicans* cells in infected mouse kidneys in order to elucidate why the wild-type cells, but not the

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cph1/cph1 efg1/efg1 mutant cells, are lethal to the mice. Our results indicated that although the *cph1/cph1 efg1/efg1* mutant cells were not lethal, they were capable of establishing zones of infection in restricted areas and colonizing areas near the renal pelvis instead of simply being cleared by the mouse immune system (Chen *et al.* 2006).

The effect on hosts infected with an attenuated strain of *C. albicans* and subsequently challenged with a virulent strain has been reported in previous studies (Bistoni *et al.* 1986; Fernandez-Arenas *et al.* 2004). In this study, we investigate whether a low dose of viable, non-lethal *cph1/cph1 efg1/efg1* mutant cells can protect mice from infection, and whether an additional second injection of these mutant cells can boost the degree of protection.

Materials and methods

Strains and media

The strains used in this study were the wild-type strain SC5314 (Gillum *et al.* 1984) and the mutant strain HLC54, *ura3::* λ *imm434/ura3::* λ *imm434 cph1::hisG/cph1::hisG efg1::hisG/efg1::hisG-URA3-hisG* (Lo *et al.* 1997). Yeast–peptone–dextrose (YPD; 1 % yeast extract, 2 % peptone, 2 % dextrose), and synthetic dextrose media (SD; 0.67 % yeast nitrogen base without amino acid, 2 % dextrose) were prepared as described (Sherman 2002).

The mouse model for virulence

The mouse model for virulence was modified from a previous report (Chen *et al.* 2006). BALB/c mice (white, male; weight 25–30 g; age 10–12 weeks) from the National Laboratory Animal Centre in Taiwan were used. An experiment was initiated by growing *Candida* cells on YPD at 30 °C overnight. Cells were then suspended in saline and adjusted to the desired concentrations according to the measurement of the optical density at 600 nm. The actual concentrations were verified by scoring the cells in a haemocytometer and by plating to determine the viable counts. Each *Candida* strain was tested for virulence by injecting 0.5 ml of the cell suspension into the tail vein of a mouse. This study was carried out in accordance with the Guide for the Care and Use of Laboratory Animals from the National Research Council. The animal study protocol for experimental procedures used in this study was approved by the Institutional Animal Care and Use Committee at the National Health Research Institutes.

Statistical methods

All analyses were performed using the SAS program (version 8.2, SAS Institute, Gary, NC). Survival curves were estimated using the Kaplan–Meier method. Two-sided log-rank tests were performed to compare the overall survival between the groups. $P < 0.05$ was considered to be statistically significant.

Results

A saline suspension of *Candida* cells or saline alone was injected into the tail veins of mice and the outcomes of all treatments are summarized in Table 1. In experiment 1, 24 mice were injected with approx. 1×10^5 *cph1/cph1 efg1/efg1* mutant cells in 0.5 ml saline (groups A and B), whereas the mice in the control (groups a and b) were injected with 0.5 ml saline alone. The observation that mice injected with 0.5 ml saline alone appeared to be healthy at least for two weeks before exposed to the wild-type cells (group b) negates any effects of the injection method *per se* influencing the results obtained. Then, after one week (groups A and a) or two weeks (group B and b), mice were injected with approx. 1×10^6 wild-type cells and were monitored daily for survival. The date of injecting the wild-type cells was recorded as day 1. All mice, except group B, were dead within 6 d after the injections. Approximately one quarter of group B mice survived 10 d after the injection. This result suggests that injections of the *cph1/cph1 efg1/efg1* mutant cells two weeks prior to that of

Table 1 – Daily survival of treated mice

Group (no. of mice)	Treatment[a]	Days after injection of the wild-type cells														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
		Daily survival rate (%)														
Experiment 1																
A (12)	Mutant → Wild-type	100	100	100	75	0										
a (12)	Saline → Wild-type	100	100	100	100	67	0									
B (12)	Mutant → → Wild-type	100	100	92	58	42	42	25	25	25	25	17	17	17	17	17
b (10)	Saline → → Wild-type	100	100	100	0											
Experiment 2																
C (5)	Mutant → Wild-type	100	100	100	100	0										
D (12)	Mutant → → Wild-type	100	100	100	50	17	17	8	8	8	8	8	8	8	8	8
E (12)	Mutant → → → Wild-type	100	100	100	50	33	25	25	25	25	25	25	8	8	0	
F (9)	Mutant → →Mutant → → → Wild-type	100	100	100	44	11	11	11	11	11	11	11	0			
G (10)	Mutant → →Saline → → → Wild-type	100	100	100	60	40	30	30	20	20	20	20	20	20	20	20
H (11)	Mutant → →Mutant → → → → → → → Wild-type	100	100	100	27	18	9	9	9	9	9	9	9	0		

a Stands for one week.

the wild-type cells were able to partially protect the mice from death as a result of exposure to the wild-type cells ($P = 0.02$).

To further investigate the possibility that the *cph1/cph1 efg1/efg1* mutant cells may function as an effective protection agent, we conducted experiment 2 in which the mice were subjected to various additional treatments (Table 1). Injection of the *cph1/cph1 efg1/efg1* mutant cells one week prior to that of the wild-type cells (group C) did not improve the survival of the mice, consistent with the result of experiment 1 (group A). Conversely, the survival periods of the mice were prolonged in groups D to H when the mutant cells were injected at least two weeks before the injection of the wild-type cells. The effects of injecting the *cph1/cph1 efg1/efg1* mutant cells in different groups were similar despite the variation in treatments. The results of experiments 1 and 2 suggest that injections of the *cph1/cph1 efg1/efg1* mutant cells two weeks or earlier prior to that of the lethal wild-type cells are able to partially protect mice from exposure to *C. albicans*. Nevertheless, the protection is not complete with the dosage used. In addition, an additional injection of the *cph1/cph1 efg1/efg1* cells did not boost the protective effects (groups F and H).

Discussion

The induction of the expression of cytokines, including granulocyte/monocyte colony-stimulating factor (GM-CSF), interferon-gamma (IFN-gamma), interleukin 1 (IL-1) and tumour necrosis factor (TNF-a) in mice treated with an attenuated *Candida albicans* strain (PCA02) was observed 6 h after infection and then persisted for many days. Furthermore, the ability to enhance cytokine production by the attenuated cells was correlated with the induction of antimicrobial protection (Vecchiarelli *et al.* 1989). Consistent with previous studies (Bistoni *et al.* 1986; Fernandez-Arenas *et al.* 2004), mice infected with the *cph1/cph1 efg1/efg1* mutant cells two or more weeks before challenge with a virulent strain, showed at least some degree of protection. In earlier reports, the injection of viable attenuated cells conferred considerable protection against the challenge of highly virulent strains of *C. albicans* only when the inoculum was as large as 10^6 cells (Bistoni *et al.* 1986; Fernandez-Arenas *et al.* 2004).

In this study, we have shown that injection of a lower dosage (1×10^5 cells) of the *cph1/cph1 efg1/efg1* mutant cells was sufficient to partially protect mice from the wild-type cells. Accordingly, even though the *cph1/cph1 efg1/efg1* mutant cells still proliferated in the kidneys of the mice, the amount of cells was ten-fold less than that of the wild-type cells (Chen *et al.* 2006). Therefore, we tested the idea that immunization with an additional dose of the *cph1/cph1 efg1/efg1* mutant cells might enhance the protective effect. Our data suggests that the protective effect was not enhanced by a second injection (Table 1). The observation that the *cph1/cph1 efg1/efg1* mutant cells are still able to persist asymptomatically in mice may prolong the cytokine response of mice in conferring protection. The mechanism of protection by injecting the *cph1/cph1 efg1/efg1*mutant cells needs further investigation by monitoring the immune response of mice. Hence, our findings further emphasize the importance of using viable, attenuated cells and appropriate inoculum dosage to achieve the best possible protective effects against systemic candidiasis and candidaemia in murine models of infection.

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