SHORT COMMUNICATION

Virulence of a wild-type parent and a petite mutant of *Candida albicans* toward rats

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The dimorphic yeast *Candida albicans* is an opportunistic pathogen and is isolated most frequently from patients with candidosis. Much attention has been focused on factors determining the degree of virulence of *C. albicans*. The ability of the yeast to produce extracellular enzymes, such as proteinase and phospholipase, has been suggested to be one of the factors¹⁾. The cellular form, yeast or hypha, has been also proposed to be related to the virulence in studies using morphological mutants although a sound conclusion has not been as yet formed on the correlation between the cellular form and the virulence¹⁾.

Another important problem in the evaluation of pathogenicity of *C. albicans* is a possible influence of the growth capacity of each fungal strain on pathogenicity. Auxotrophic mutants whose growth *in vivo* is limited by their strict nutritional requirements have provided evidence to support the view that the capacity of growth of each strain correlates closely with pathogenicity²⁻⁵⁾.

We have succeeded in isolating petite (respiration-deficient) mutants of *C. albicans*⁶⁾, and some of their morphological and physiological characteristics have been reported elsewhere⁶⁻⁸⁾. The petite mutants have been also shown to be avirulent toward mice, and the avirulence of the petite mutants has been discussed in relation to their capacity of growth⁹⁾. In the present study, we compare the

virulence of a petite mutant for rats with that of its wild-type parent.

C. albicans K was isolated from a patient with oral candidosis in this laboratory¹⁰⁾ and used as the wild-type parent strain. The petite mutant KRD-19 was derived from the wild-type strain by treatment with acriflavine at an elevated temperature as reported previously⁶⁾. The wild-type parent and the petite KRD-19 were grown in liquid PYG medium⁶⁾ at 37 °C with shaking for 16 h and 45 h, respectively. Cells were harvested by centrifugation and washed three times in sterile physiological saline (SPS). The cells were then diluted with SPS to give concentrations used as inocula. Doses of the wild-type strain ranged from 2×10^7 to 7×10^7 cells/rat, and doses of KRD-19 were 5×10^7 and 1×10^8 . Male and female Sprague-Dawley rats, 4-week old, were separately grouped to give 10 or 12 rats per group. These rats were inoculated with 0.4 ml of a suspension of cells through a lateral tail vein. Rats in control groups received 0.4 ml of SPS.

The rats were maintained for 3 weeks in animal chambers controlled at 24 °C. Food (MF type, Oriental Yeast Co., Tokyo) and water were provided *ad libitum*. The number of survivors, the body weight and any unusual behaviour or appearance were daily recorded. Rats, that succumbed to candidal infection within 3 weeks or were sacrificed at the end

Table 1 Mortality of rats after intravenous injection of the wild-type parent strain K or the petite mutant KRD-19 of *C. albicans*

Strain	Dose (cells/rat)	Male			Female		
		Mortality (dead/ injected)	Mean survival time (days) ^a	LD ₅₀ ^b	Mortality (dead/ injected)	Mean survival time (days) ^a	LD ₅₀ ^b
K	2 × 10 ⁷	0/12	—		0/12	—	
	2.4 × 10 ⁷	0/12	—		1/12	7.0	
	3 × 10 ⁷	0/12	—	4.9 × 10 ⁷	0/12	—	4.8 × 10 ⁷
	4 × 10 ⁷	2/12	7.0	(4.5 × 10 ⁷ —	2/12	6.0	(4.3 × 10 ⁷ —
	5 × 10 ⁷	8/12	5.3	5.4 × 10 ⁷) ^c	7/12	5.6	5.2 × 10 ⁷) ^c
	6 × 10 ⁷	11/12	4.3		12/12	3.2	
	7 × 10 ⁷	11/12	3.5		11/12	4.1	
KRD-19	5 × 10 ⁷	0/10	—	> 1 × 10 ⁸	0/12	—	> 1 × 10 ⁸
	1 × 10 ⁸	0/10	—		0/12	—	

^a The mean survival time of rats that died within the experimental period of 21 days.

^b The LD₅₀ was calculated by probit analysis.

^c The range for 95% confidence.

of the experimental period, were subjected to autopsy. Their kidneys were fixed, dehydrated and then embedded in paraffin by conventional procedures. Thin sections of the kidneys were stained with periodic-Schiff reagent (PAS).

Loss of the appetite, a decline of active movement, hemorrhage from the eyes or nose, and diarrhoea were observed at an early stage of the infection in rats that had received relatively high doses of the wild-type strain. Furthermore, it is of interest that wryneck and arthritis were induced in a dose-dependent manner in rats infected with the wild-type cells. Results of these subjects will be reported elsewhere. In contrast, no such changes were observed in rats inoculated with the petite mutant KRD-19.

Mortality rates of male and female rats infected with the wild-type strain or the petite mutant are shown in Table 1. In infections with the wild-type strain, the number of survivors and the mean survival time were gradually decreased by increasing the inoculum size. The 21-day LD₅₀-values for the wild-type strain were 4.9 × 10⁷ cells/rat for male and 4.8 × 10⁷ for female, indicating there is no significant difference in the susceptibility to the wild-type strain between male and female rats. The petite mutant KRD-19 was unable to cause fatal infection even at a dose of 1 × 10⁸

cells.

Changes in the body weight of male rats infected with the wild-type strain or KRD-19 are shown in Fig. 1. Immediately after inoculation, a repression or a decrease of the body weight was induced dependently on the inoculum size in rats in groups infected with the wild-type strain. However, the body weight of rats in groups inoculated with the petite mutant increased in parallel with that of rats in the control group. Changes in the body weight in female rats infected with the wild-type strain or KRD-19 showed a tendency almost identical to that observed with male rats (data not shown).

The kidney has been known to be the primary infectious site by intravenously introduced *C. albicans*¹⁾. Edema with enlargement and discolouration of the kidney were most commonly observed in rats which succumbed to infection with the wild-type cells within the 3-week experimental period. Similarly, kidneys from rats which received the wild-type cells but survived for the experimental period showed edema, discolouration, scarring and focal lesions. However, these pathological changes were less extensive than those observed in the kidneys of mice⁹⁾. Kidneys from rats infected with KRD-19 showed the same gross appearance as those from uninfected rats. Growth of *C. albicans* in

Fig. 1 Changes in the mean body weight of male rats in groups infected with the wild-type strain K (A) and the petite mutant KRD-19 (B). Doses (cells/rat) were: (A) $\circ$, 0; $\bullet$, 2×10^7 ; Δ , 3×10^7 ; $\blacktriangle$, 4×10^7 , and (B) $\circ$, 0; $\blacksquare$, 5×10^7 ; $\square$, 1×10^8 . Each asterisk indicates the death of one rat.

hyphal and pseudohyphal forms was observed in thin sections of the kidneys from rats infected with the wild-type cells. In contrast, fungal elements and pathological changes were not found in thin sections of kidneys from rats infected with the petite mutant KRD-19. These results indicate that KRD-19 cells cannot survive and multiply in the kidney.

In rats, most isolates of *C. albicans* has been reported to cause lethal infection at doses of 5×10^2 – 5×10^4 cells/g body weight¹¹. The LD₅₀-value, 4.8×10^7 cells/rat, for the wild-type strain is equivalent to 5.6×10^5 cells/g body weight, showing that the wild-type strain K is an isolate with relatively low virulence as reported previously in the case of experimental infections in mice⁹.

In the previous study using mice, we reported that the petite mutant KRD-19 was unable to colonize the kidney and consequently failed to cause fatal infection⁹. Similarly, the petite mutant was shown to be avirulent toward rats in the present study. Although the inability to produce extracellular proteinase⁹ and the poor capacity of yeast hypha transition of the petite mutant^{8,9} may be related to some extent to its avirulence, the

major cause for the petite mutant being avirulent is assumed to result from its poor capacity of growth: the doubling time *in vitro* of the petite mutant is about 4.2 h, three-times longer than that of the wild-type parent⁶, and the poor capacity of growth of the petite mutant favours the host with an attempt to reject fungal cells before invasion of the fungal cells into the tissues of the host. A similar correlation between the capacity of growth and the degree of virulence has been proposed in studies using auxotrophic mutants²⁻⁵. Our results presented in this paper, together with those obtained with auxotrophic mutants, indicate that full understanding of the growth capacity of each strain is required for the assessment of respective pathogenicity in experimental infections.

Summary: The virulence of a wild-type parent and of a petite mutant of *C. albicans* was examined in experimental infections in rats. The petite mutant was unable to cause fatal infection whereas the wild-type strain gave the LD₅₀-value of 4.8×10^7 cells/rat. The avirulence of the petite mutant is discussed on the basis of its biological properties and concluded to result most probably from the poor capacity for growth.

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