INFECTION AND IMMUNITY, Sept. 2002, p. 5256–5258 Vol. 70, No. 9
0019-9567/02/$04.00+0 DOI: 10.1128/IAI.70.9.5256–5258.2002

NOTES

Contribution of *Candida albicans ALS1* to the Pathogenesis of Experimental Oropharyngeal Candidiasis

Yasuki Kamai,[1]* Mikie Kubota,[1] Yoko Kamai,[2] Tsunemichi Hosokawa,[2] Takashi Fukuoka,[1] and Scott G. Filler[3]

Biological Research Laboratories[1] and Laboratory Animal Science and Toxicology Research Laboratories,[2] Sankyo Co., Ltd., Shinagawa-ku, Tokyo 140-8710, Japan, and Harbor-UCLA Research and Education Institute, Torrance, California 90502[3]

Received 2 January 2002/Returned for modification 17 February 2002/Accepted 8 May 2002

We investigated the contribution of *Candida albicans ALS1*, which encodes a candidal adhesin, to the pathogenesis of experimental murine oropharyngeal candidiasis. Our results indicate that the *ALS1* gene product is important for the adherence of the organism to the oral mucosa during the early stage of the infection.

Candida albicans causes both hematogenously disseminated and mucosal infections. The ability of *C. albicans* to adhere to various host constituents plays an important role in colonizing the host and then initiating and maintaining an infection. Several adherence genes have been characterized (6, 7, 12). The *C. albicans ALS1* (agglutinin-like sequence) gene encodes an adhesin that mediates attachment to endothelial cells (4, 9). In this study, we used *C. albicans* mutants in which one or both alleles of *ALS1* were disrupted to investigate the role of Als1p in adherence to epithelial cells during experimental murine oropharyngeal candidiasis (OPC).

We used strains constructed from *C. albicans* CAI4 (3), as described previously (5), and SC5314, the wild-type parent strain of CAI4 (Table 1). The growth rates of all strains in vitro are similar (5).

Experimental OPC was induced as described previously (10). All animal experiments were carried out according to the guidelines of the Institutional Animal Care and Use Committee of Sankyo Co., Ltd. Specific pathogen-free male ddY mice (5 weeks old; Japan SLC, Inc., Shizuoka, Japan) were immunosuppressed with 4 mg of cortisone acetate administered subcutaneously on the day before and 1 day after inoculation. Mice received tetracycline hydrochloride (Achromycin V; Lederle Japan Ltd., Tokyo, Japan) in their drinking water (0.5 mg/ml), starting the day before inoculation. Before inoculation, mice were anesthetized by intraperitoneal injection with 26 μg of dimorpholamine (Theraptique; Eisai Co., Ltd., Tokyo, Japan), 207 μg of xylazine (Bayer Yakuhin Ltd., Osaka, Japan), and 1.04 mg of pentobarbital sodium (Nembutal; Dainippon Pharmaceutical Co., Ltd., Osaka, Japan), and cotton wool balls (3-mm diameter) containing 10^4 blastospores were placed sublingually in the oral cavity for 2 h.

To determine the viable counts in the oral tissue, each mouse was sacrificed and the mandibular soft tissue, including the tongue, was dissected free of the teeth and bone. The excised tissue was homogenized in saline, after which serial dilutions were plated onto Sabouraud dextrose agar containing 10 μg of chloramphenicol/ml for colony counting. For histopathological study, the excised tissue was fixed with formalin and embedded in paraffin, after which thin sections were prepared and stained with periodic acid-Schiff (PAS) stain.

The adherence of *C. albicans* to the tongue ex vivo was determined as follows. Tongues were excised from sacrificed mice, added to each well of a 24-well culture plate containing 10^4 *C. albicans* cells in phosphate-buffered saline, and incubated at 35°C for 30 min with gentle rotation. Next, the tongues were washed three times with phosphate-buffered saline and homogenized in saline, and serial dilutions were plated onto Sabouraud dextrose agar containing chloramphenicol. The viable counts adhering to each tongue were determined by colony counting.

We first investigated the contribution of *ALS1* to the virulence of *C. albicans* in experimental OPC. Figure 1 shows the viable counts in the oral tissue on days 1, 2, and 3 postinoculation. On day 1, the *als1*/*als1* mutant was the least virulent

TABLE 1. Strains

Strain	Designation	Genotype
SC5314	Wild-type	
I–13	*ALS1*/*als1*	Δ*als1*::*hisG-URA3-hisG*/*ALS1* Δ*ura3*::*imm434*/Δ*ura3*::*imm434*
3–13–22	*als1*/*als1*	Δ*als1*::*hisG*/Δ*als1*::*hisG-URA3-hisG* Δ*ura3*::*imm434*/Δ*ura3*::*imm434*
13R11	*als1*/*als1*+ *ALS1*	Δ*als1*::*hisG*/Δ*als1*::*hisG-ALS1-URA3-hisG* Δ*ura3*::*imm434*/Δ*ura3*::*imm434*

* Corresponding author. Mailing address: Biological Research Laboratories, Sankyo Co., Ltd., 2-58 Hiromachi 1-chome, Shinagawa-ku, Tokyo 140-8710, Japan. Phone: 81-3-3492-3131. Fax: 81-3-5436-8565. E-mail: ykamai@shina.sankyo.co.jp.

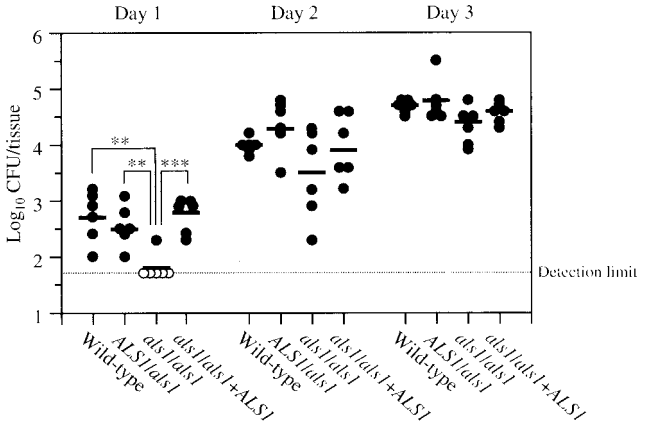

FIG. 1. Viable *C. albicans* counts in oral tissue of mice inoculated with *C. albicans* strains on days 1, 2, and 3. Each circle represents the result for an individual mouse. The closed circles indicate results that were above the limit of detection (1.7 $\log_{10}$ CFU/tissue), and the open circles represent results that were below the limit of detection. When the culture of the oropharynx was sterile, the value of the detection limit was used for the statistical analysis. The bars indicate the mean value ($n = 6$). **, $P < 0.01$; ***, $P < 0.001$(Tukey's test).

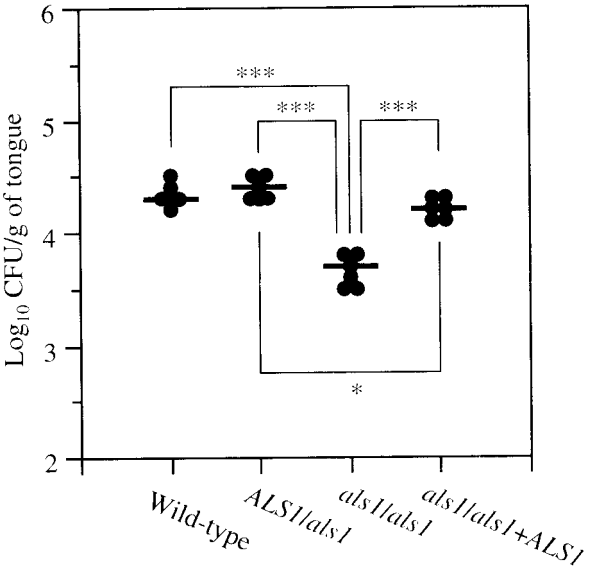

FIG. 3. Adherence of *C. albicans* strains to tongues of mice ex vivo. The closed circles indicate data from individual mice and each bar indicates the mean value ($n = 6$). *, $P < 0.05$; ***, $P < 0.001$ (Tukey's test).

among the strains used. In five out of six mice inoculated with the *als1*/*als1* mutant, the viable counts were below the limit of detection (<1.7 $\log_{10}$ CFU/tissue). The levels of viable counts were similar for the wild-type, *ALS1*/*als1*, and *als1*/*als1*+*ALS1* strains. On days 2 and 3, the viable counts increased progressively, and there was a trend towards a reduced viable count in mice inoculated with the *als1*/*als1* mutant compared to mice inoculated with the other strains, but these differences were not significant.

ALS1 is important for hyphal formation as well as adherence (5, 8). Therefore, we examined the oral tissues microscopically to determine the morphology of the different strains. Figure 2 shows representative micrographs of PAS-stained sections of the oral tissues of mice inoculated with the wild-type strain (Fig. 2A) and the *als1*/*als1* mutant (Fig. 2B) on day 1. The infectious foci in mice inoculated with the wild-type strain were larger than those in mice inoculated with the *als1*/*als1* mutant. However, both strains formed filaments of similar length. The histopathology results with the *ALS1*/*als1* and the *als1*/*als1*+*ALS1* mutants were similar to those of mice inoculated with the wild-type strain (data not shown).

Next, we investigated the adherence of the various strains to the tongues of mice after a 30-min incubation ex vivo (Fig. 3). The *als1*/*als1* mutant was significantly less adherent to the

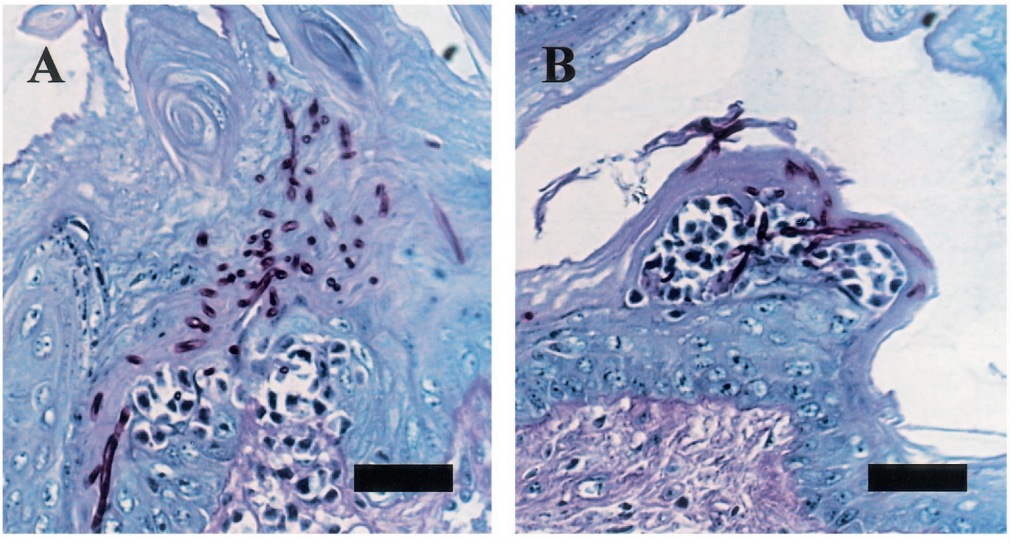

FIG. 2. Histopathological analysis of oral tissues of mice inoculated with the wild-type strain (A) or *als1*/*als1* strain (B) on day 1 (PAS stain). Bar, 30 μm.

tongue than the other strains. Although the *als1*/*als1*+*ALS1* mutant was significantly less adherent than the *ALS1*/*als1* mutant, the absolute difference in adherence was small compared with the differences between the *als1*/*als1* mutant and the other strains and it was probably not biologically significant. From these experiments, there does not appear to be a gene dosage effect. In general, the relative difference in adherence among the various strains in the ex vivo assay parallels the oral fungal burden of the mice infected with these strains on day 1 in the in vivo experiment. Therefore, the reduced virulence of the *als1*/*als1* mutant early in the infection may have been due to reduced adherence to the oropharyngeal mucosa.

Our results demonstrate that *ALS1* is important during the early stages of OPC in the mouse model. Previously, we found that *ALS1* also contributes to virulence in the mouse model of hematogenously disseminated candidiasis (5). In both models, the tissue fungal burden of mice infected with the *als1*/*als1* mutant was significantly less than that of mice infected with the wild-type strain on day 1, but the number of organisms at the infection site subsequently returned to wild-type levels afterward (5). These results suggest that there may be compensatory upregulation of a gene(s) that can eventually substitute for the lack of the *ALS1* gene product. It is possible that these genes are other members of the *ALS* gene family (6, 9, 12).

In this OPC model, the homozygous *ALS1* null mutant filamented normally in the oropharynx. On the other hand, in the disseminated infection model, this mutant produced shorter filaments than wild-type or *als1*/*als1*+*ALS1* strains in the kidney after a similar duration of infection, but the lengths of filaments were similar for all strains by 40 h of infection (5). Multiple pathways have been identified that regulate filamentation in *C. albicans* (1, 2, 11). These results suggest that a filamentous pathway other than the *ALS1* pathway may operate in the oropharynx earlier than in the kidney, or a filamentous pathway that operates in the oropharynx may be different from that in the kidney. The lack of an observed filamentation defect of the *als1*/*als1* mutant in the oropharynx combined with its impaired ability to adhere to the murine tongue ex vivo suggest that the reduced early virulence of this mutant is predominantly the result of reduced adherence. Collectively, these results support the concept that adherence to the oropharynx is important for induction of OPC and that methods to block this adherence are likely to be efficacious in preventing and/or treating this infection.

S.G.F. was supported in part by U.S. Public Health Service grants R01 DE13974, R01 AI19990, and P01 AI37194.

REFERENCES

1. **Braun, B. R., and A. D. Johnson.** 2000. *TUP1*, *CPH1* and *EFG1* make independent contributions to filamentation in *Candida albicans*. Genetics **155:**57–67.
2. **Brown, A. J., and N. A. Gow.** 1999. Regulatory networks controlling *Candida albicans* morphogenesis. Trends Microbiol. **7:**333–338.
3. **Fonzi, W. A., and M. Y. Irwin.** 1993. Isogenic strain construction and gene mapping in *Candida albicans*. Genetics **134:**717–728.
4. **Fu, Y., G. Rieg, W. A. Fonzi, P. H. Belanger, J. E. Edwards, Jr., and S. G. Filler.** 1998. Expression of the *Candida albicans* gene *ALS1* in *Saccharomyces cerevisiae* induces adherence to endothelial and epithelial cells. Infect. Immun. **66:**1783–1786.
5. **Fu, Y., A. S. Ibrahim, D. C. Sheppard, Y.-C. Chen, S. W. French, J. Cutler, S. G. Filler, and J. E. Edwards, Jr.** 2002. *Candida albicans* Als1p: an adhesin that is a downstream effector of the *EFG1* filamentation pathway. Mol. Microbiol. **44:**61–72.
6. **Gale, C. A., C. M. Bendel, M. McClellan, M. Hauser, J. M. Becker, J. Berman, and M. K. Hostetter.** 1998. Linkage of adhesion, filamentous growth, and virulence in *Candida albicans* to a single gene *INT1*. Science **279:**1355–1358.
7. **Gaur, N. K., and S. A. Klotz.** 1997. Expression, cloning, and characterization of a *Candida albicans* gene, *ALA1,* that confers adherence properties upon *Saccharomyces cerevisiae* for extracellular matrix proteins. Infect. Immun. **65:**5289–5294.
8. **Hoyer, L. L., S. Scherer, A. R. Shatzman, and G. P. Livi.** 1995. *Candida albicans* ALS1: domains related to *Saccharomyces cerevisiae* sexual agglutinin separated by a repeated motif. Mol. Microbiol. **15:**39–54.
9. **Hoyer, L. L.** 2001. The *ALS* gene family of *Candida albicans*. Trends Microbiol. **9:**176–180.
10. **Kamai, Y., M. Kubota, Y. Kamai, T. Hosokawa, T. Fukuoka, and S. G. Filler.** 2001. New model of oropharyngeal candidiasis in mice. Antimicrob. Agents Chemother. **45:**3195–3197.
11. **Lengeler, K. B., R. C. Davidson, C. D'souza, T. Harashima, W. C. Shen, P. Wang, X. Pan, M. Waugh, and J. Heitman.** 2000. Signal transduction cascades regulating fungal development and virulence. Microbiol. Mol. Biol. Rev. **64:**746–785.
12. **Staab, J. F., S. D. Bradway, P. L. Fidel, and P. Sundstorm.** 1999. Adhesive and mammalian transglutaminase substrate properties of *Candida albicans* Hwp1. Science **283:**1535–1538.

Editor: T. R. Kozel