MYCOLOGY

The relative pathogenicity of *Candida krusei* and *C. albicans* in the rat oral mucosa

Y. H. SAMARANAYAKE, P. C. WU, L. P. SAMARANAYAKE* and P. L. HO†

Departments of Pathology and †Microbiology, Faculty of Medicine and *Faculty of Dentistry, University of Hong Kong, Hong Kong

The relative pathogenicity of *Candida krusei* and *C. albicans* was investigated by assessing their colonisation and infectivity of the Sprague-Dawley rat oral mucosa. During an initial 21-week period with intermittent oral inoculation, both *Candida* spp. demonstrated variable surface colonisation of the oral mucosa. After 3 days of oral inoculation, both yeast species were recovered from all animals. During the 21-week period the mean oral load of *C. albicans* in the control group of rats varied between $(26-274) \times 10^1$ cfu/ml whereas the two test groups of rats carrying *C. krusei* CK9 and CK13 had a mean load of $(2-10) \times 10^1$ cfu/ml. Although oral colonisation by *C. albicans* was greater than that of *C. krusei*, neither species induced candidal infection during this period. Subsequent immunosuppression of the rats by intramuscular cyclophosphamide (40 mg/kg body weight) initiated *C. albicans* infection of the dorsal tongue (around the conical papillae area) after 4 weeks, in all of three animals while similar lesions due to *C. krusei* were seen – albeit after 5–7 weeks – in three of eight animals. Characteristic histological changes of mucosal candidosis were discernible on the lingual mucosa of rats infected with both *Candida* spp. including parakeratosis, absence of a stratum granulosum, thickened rete ridges, micro-abscess formation and polymorph infiltration of the lingual epithelium. Although both species produced fungal hyphae that penetrated the epithelium up to the prickle cell layer, *C. albicans* hyphae tended to be relatively more profuse. Taken together these results substantiate, for the first time in an animal model, the clinical evidence that *C. krusei*, once considered an innocuous commensal, is capable of transforming into an invasive pathogen under conditions of immunosuppression.

Introduction

The role played by various *Candida* spp. in initiating oral epithelial lesions is ill understood. Cawson and Lehner [1] first suggested a possible aetiological relationship between leukoplakia and candidal infection on finding mycelial elements of *C. albicans* infiltrating the cornified layer of the epithelium in some cases of human oral leukoplakia [2]. Since then several workers have attempted to produce lesions resembling human oral leukoplakia in cultured tissue [3], chick chorio-allantoic membrane [4] and animal models [5, 6]. Jones and Russell [7, 8] pioneered these animal studies and demonstrated histological changes similar to the human disease in the posterior dorsum of the tongue of

Sprague-Dawley rats chronically infected with *Candida* spp. Since then, the Sprague-Dawley rat has served as one of the more useful animal models for investigating the epithelial changes caused by *Candida* spp. in the oral cavity [9–11]. However, the data from the latter studies almost exclusively pertain to *C. albicans*, the most virulent *Candida* spp., and indicate that the host–fungal interactions depend to a large extent on the nature of the yeast strain used, as well as the carbohydrate and antibiotic content of the rat diet [9, 12].

Despite the relative abundance of data on oral lesions induced by *C. albicans* in animal models, there is sparse information on the less pathogenic species of *Candida* such as *C. krusei*. As it is well established that patients with leukaemia, bone marrow transplants and human immunodeficiency virus infection may be readily infected by these lesser known emerging pathogens [13–15], it is important to establish their

Received 28 Nov. 1997; revised version accepted 4 March 1998.

Corresponding author: Professor L. P. Samaranayake.

pathogenic attributes *in vivo* with an animal model. Therefore, the aims of the present investigation were to compare the potential of *C. krusei* and *C. albicans* to colonise and infect the oral mucosa in a conventional Sprague-Dawley rat model, and to assess the susceptibility to mucosal infection with these yeasts following immunosuppression by intravenous cyclophosphamide.

Materials and methods

Candida isolates and growth conditions

A strain of *C. albicans* (OSU #3) that previously had produced lesions in a Sprague-Dawley rat model [9, 10] was kindly supplied by Dr C. M. Allen, of the Ohio State University College of Dentistry, Columbus, OH, USA. Two different isolates of *C. krusei* (CK9 and CK13) were tested; both were isolated from oral lesions of denture stomatitis patients attending the Oral Medicine Clinic of the Glasgow Dental Hospital and School, Glasgow. These two isolates were specifically selected from a battery of 20 *C. krusei* isolates because of their greater virulence attributes of adherence and hydrophobicity as described in previous studies [16, 17]. All three isolates were speciated by the germ-tube test and API20C identification test kits (API Laboratory Products, Basingstoke, Hants). They were stored in distilled water at -70°C [18], subcultured monthly on Sabouraud's Dextrose Agar (SDA; Gibco Ltd, Paisley) and maintained at 4°C during the experimental period. Purity of cultures was ensured by regular identification.

Sprague-Dawley rats

A total of 15 locally bred, 4-week-old male rats (200 g, average weight) was used for the study. For the experiment, the animals were randomly assigned into three groups of five rats each and each group was housed in a separate metal cage in a conventional animal house. The three groups were coded as the control group – for inoculation with *C. albicans* – and test groups 1 and 2 for inoculation with *C. krusei* CK9 and CK13, respectively. The rats were fed a carbohydrate-rich diet (CRD) for 22 weeks (PMI Feeds Incorporation, USA). Thereafter they were given standard rat pellets for a further 7 weeks. All rats were given tetracycline hydrochloride 0.1% in their drinking water throughout the experimental period as described by Russell and Jones [19].

Yeast inoculum

The *C. albicans* and *C. krusei* isolates were cultured on SDA for 24 h at 37°C . The harvested organisms were resuspended in sterile distilled water to give a yeast concentration of $c.10^8$ cells/ml, as assessed by haemocytometric counting (Hawksley Limited, London). Microscopic examination of gram-stained colo-

nies of the two *Candida* spp. revealed no hyphal formation.

Oral inoculation of Sprague-Dawley rats with Candida spp.

Initially, the 15 rats were given tetracycline 0.1% in their drinking water *ad libitum* for a period of 4 weeks. Their oral cavities were then swabbed and examined to rule out indigenous candidal growth, before experimental inoculation with the respective *Candida* isolate. For the latter purpose, all rats were inoculated orally with 0.1 ml of the yeast inoculum (10^7 yeasts/ml), with the aid of a sterile pipette, three times per week over the 29-week experimental period.

Quantification of oral carriage of Candida spp.

Following inoculation, a semi-quantitative estimation of the *Candida* spp. on oral tissues was performed once every week by a single operator. For this purpose, an experienced technician restrained each rat on a smooth surface, by holding the body with one hand and opening the mouth by pressing firmly on either side of the temporomandibular joint area with the thumb and fingers of the other hand. This method of opening the mouth facilitated both the inoculation of yeasts and subsequent swab sampling. In order to avoid cross-contamination of yeast strains the animals were handled with sterile cotton gloves designated for each group.

The oral cavity of each rat was sampled thoroughly with a dry sterile cotton wool swab. This was accomplished by inserting the swab into the mouth up to the oropharynx and firmly sampling the tongue and the mucosal surfaces by rotating the swab for *c.* 5 s. The swab contents were then dispersed by mild agitation in 5 ml of sterile distilled water; 50 μl of the latter suspension were then spiral plated (Spiral Systems, OH, USA) on to SDA and incubated at 37°C for 48 h to estimate the number of cfu of candida (gram-stained smears of the suspensions examined microscopically revealed no hyphal formation). The identity of *Candida* isolates was confirmed once every 2 weeks to rule out exogenous oral yeast infection, if any.

Initial gross observations of the oral tissues and subsequent experimental protocol

At the end of 21 weeks of oral inoculation with the *Candida* strains, the oral cavities of all rats were examined under short ether anaesthesia. Each rat was held by a technician as described previously, the mouth was opened and the tongue and oral mucosa including the palate were thoroughly examined by a single examiner with a magnifying glass, overhead lamp and a bright light. No grossly visible candidal infection or 'white patches' were noted in the Sprague-Dawley rat oral mucosa during this period of inoculation with

either *C. albicans* or *C. krusei*. To substantiate these gross findings, at the end of 21 weeks, a histopathological study of the tongues of two animals from the *C. albicans* group, and one each from the two *C. krusei* groups was also performed. The criterion used to select these four rats was their consistently high oral yeast load compared with the values for the other animals in their respective groups.

The tongue was examined visually, dissected and immersed in buffered formalin 10%, for 48 h. Three areas of the tongue were sampled for histopathological studies. The length of the samples, measured sagittally along the mid-dorsum comprised: (i) a 2-mm cross-sectional length anterior to the conical papillae area; (ii) the length of the whole conical papillae area (c. 4 mm); and (iii) a 4-mm cross-sectional length posterior to the conical papillae area. These three regions were separated by horizontal sectioning of the tongue. Any lesions observed on the separated pieces were identified and blocked; the dimensions of these blocks depended on the size of the lesion encountered. The blocks were embedded in paraffin and serial 5- μ m thick sections were cut from each specimen. Randomly selected tissue sections (one from every 10 serial sections) were stained with haematoxylin and eosin, mounted in permount and examined for histological changes, in a conventional manner. In addition, periodic acid-Schiff stained sections were used to visualise mycelial penetration of the lingual epithelium.

Immunosuppression by cyclophosphamide

The remaining 11 animals (three from the *C. albicans* group and four each from the *C. krusei* groups) were immunosuppressed with cyclophosphamide (Endoxan-ASTA; ASTA Medica AG, Germany). The rats were anaesthetised with ether, and venous blood samples were collected from the rat tail end before the initial administration of cyclophosphamide. After the blood was collected, cyclophosphamide (200 mg dissolved in 10 ml of sterile distilled water) was injected intramuscularly at a dose of 40 mg/kg body weight. Repeated doses of cyclophosphamide were administered at 1-week intervals to all the remaining rats for a period of 8 weeks. On these occasions, venous blood samples were collected before cyclophosphamide administration and all blood samples were subjected to leucocyte counts to assess the degree of leucopenia. For this purpose, a 1 in 20 dilution of blood was made in acetic acid 2% to lyse red cells and the leucocytes were counted with a Neubauer counting chamber [20].

Naked eye examinations of the tongue and mucosal surfaces of the 11 remaining rats were made once weekly after blood was collected from the animals. All the animals were restrained and examined by a single examiner in a standard manner, with a magnifying glass, overhead lamp and a bright light. Whenever a

'white patch' with an associated lesion was seen on gross examination, the animal was killed and samples were subjected to histopathological examination as described above.

Results

The experiment comprised two parts. In the first part the rats were maintained in their normal healthy state albeit with a carbohydrate-rich diet (CRD) and with tetracycline in their water, while in the second part they were, in addition, immunocompromised by the administration of cyclophosphamide.

Leucocyte counts

The leucocyte counts of all the animals were depressed consistently immediately after administration of cyclophosphamide. The total mean (SD) leucocyte count at the beginning of the experiment (i.e., week 21, before cyclophosphamide administration) for the control group inoculated with *C. albicans* was $1.40 (0.38)/\mu\text{l} \times 10^4$ and then fell to $0.5 (0.20)/\mu\text{l} \times 10^4$, 1 week after cyclophosphamide administration (end of week 22). During the following 4 weeks all three rats demonstrated a consistent depression in their leucocyte counts, at the end of which the mean (SD) leucocyte count was $0.31 (0.23)/\mu\text{l} \times 10^4$. At this stage all three rats had white patches on their lingual surfaces.

In test group 1 (inoculated with *C. krusei* CK9), the mean (SD) leucocyte count was $1.60 (0.36)/\mu\text{l} \times 10^4$ before cyclophosphamide administration and, 1 week after drug administration (at the beginning of week 22), the count decreased to $0.61 (0.16)/\mu\text{l} \times 10^4$. Thereafter during the weeks before they were killed, all four rats in this group showed considerable fluctuation in counts, but in general a gradual overall reduction. Two animals (T1.1 and T1.3) whose leucocyte counts gradually declined during the experimental period (seventh and fifth week after cyclophosphamide administration) had white patches on their tongue surfaces. However, lesions were not observed in a third animal (T1.2) whose leucocyte counts declined dramatically ($0.30/\mu\text{l} \times 10^4$) at the end of the eighth week, and in another animal (T1.5) with a high leucocyte count ($3.10/\mu\text{l} \times 10^4$). These results demonstrate that the leucocyte counts of the rats did not stabilise, but showed considerable fluctuation during the 8 weeks of drug administration. The second test group of rats which harboured *C. krusei* CK13 on the lingual surface showed similar variations in leucocyte counts, and a relatively comparable association between the numbers of leucocytes and the development of white patches.

Thus, rats inoculated with *C. albicans* showed an invariable association between decreased leucocyte counts and the development of white patches, whereas

this relationship was less clear in the two groups harbouring *C. krusei*.

Oral carriage of Candida spp. in Sprague-Dawley rats

Before cyclophosphamide administration. None of the Sprague-Dawley rats in the three designated groups was colonised by *Candida* spp. at the beginning of the experiment. At the end of week 1, after three inoculations, *Candida* spp. were recovered from the mouths of all animals. A marked variation in the mean oral candidal load among the three groups was observed during the experimental period (Fig. 1).

The weekly variation in the load of *C. albicans* in the five animals in the control group was quite similar. Thus during the first 7 weeks of the experiment, the mean candidal load varied between $(26-32) \times 10^1$ cfu/ml, but thereafter between weeks 8 and 14 the group as a whole demonstrated a gradual increase in yeast load varying between $(37-274) \times 10^1$ cfu/ml. Oral colonisation began to decline during weeks 20-21 in all animals (Fig. 1a).

The *C. krusei* load also varied considerably during weeks 1-21 (Fig. 1b and c) although the relative candidal load was consistently (3-4-fold) lower than with *C. albicans* ($p < 0.001$). The *C. krusei* group as a whole demonstrated an increased mean yeast load during weeks 12-16, after which the candidal load varied widely (Fig. 1).

A variable load of *C. krusei* was also seen within each group of rats. For instance, in the group inoculated with strain CK13, two rats demonstrated a consistently high oral load when compared with the other three counterparts throughout the experimental period (data not shown).

After cyclophosphamide administration. Generally, the mean candidal load in all three groups of rats was consistently higher before cyclophosphamide administration than in the post-administration period. Furthermore, the load of *C. albicans* was relatively (12-fold) higher than that of either *C. krusei* isolate. The results of oral carriage of yeasts in the three groups of rats after cyclophosphamide administration are summarised in Fig. 1. It should be noted that none of the weekly oral samples revealed hyphal elements of either candidal species, throughout the study period. The above data demonstrated clearly the superior ability of *C. albicans* to colonise the Sprague-Dawley rat oral mucosa when compared with *C. krusei*, both in immunocompetent and immunosuppressed animals.

Pathological findings before cyclophosphamide administration. At the end of 21 weeks of oral inoculation with *C. albicans*, a histopathological study of the rat tongue was conducted on two randomly selected

animals. These two rats showed no evidence of candidal infection in either the haematoxylin and eosin or periodic acid-Schiff stained sections, although the candidal load in the rats (C3 and C4) at the time of death varied from 22.3×10^1 to 30.48×10^1 cfu/ml.

No grossly visible lesions were noted in the Sprague-Dawley rat oral mucosa during the first 21 weeks of oral inoculation with either *C. krusei* CK9 or CK13. Histopathological examination of a randomly selected rat from each group provided no histological evidence of candidal infection, although both rats (T1.4 and T2.2) harboured *C. krusei* during the 21-week period. When they were killed, the oral candidal load in the two rats was 2.03×10^1 cfu/ml.

Pathological findings after cyclophosphamide administration. On naked eye examination of the control group inoculated with *C. albicans*, white patches on the dorsal tongue mucosa were evident, with depapillation of the conical papillae region and smooth erythematous areas anterior and posterior to this region. An increasing incidence of such lingual lesions was observed in all animals after administration of cyclophosphamide, from the second week onwards (Table 1, Fig. 2). Thus all three rats in the control group demonstrated white patches on the tongue surface during week 25. The oral candidal load for two animals, when they were killed, were similar (6.09×10^1 cfu/ml); the third showed a higher candidal load (30.48×10^1 cfu/ml). The diameters of the lingual lesional areas of these rats were: 2-3 mm, 1.8-2.4 mm and 2.8 mm respectively, indicating a lack of a relationship between the oral candidal load and the size of the lesion.

Histopathological changes of tissue sections incorporating the white patches demonstrated loss of normal lingual papillae and replacement by a smooth layer of parakeratotic and acanthotic epithelium (Fig. 3a). The rete ridges appeared thickened with blunt tips. The superficial layers of epithelium showed marked intercellular and intracellular oedema with polymorph infiltration. Intra-epithelial vesicles were noted in the prickle cell layer, many containing polymorphs. The basal and parabasal cells were hyperplastic with increased mitotic figures and loss of nuclear palisading. The corium showed slightly increased vascularity and mild oedema with scanty infiltrate of mononuclear cells. Fungal hyphae were observed penetrating mainly the parakeratin layer of the superficial epithelium (Fig. 3b). The slender hyphae invaded the cornified epithelium at right angles to the epithelial surface, but terminated short of the prickle cell layer. The length of *C. albicans* hyphae varied between 5 and 17 μ m. No *Candida* elements were identified in the intra-epithelial vesicles. No microscopic abnormality was identified in sections taken from the grossly normal parts of the tongue.

The animals had to be immunocompromised for a longer period with cyclophosphamide for lingual

Fig. 1. The mean oral load of: **a**, *C. albicans*; **b**, *C. krusei* CK9; **c**, CK13 before and after (→) administration of cyclophosphamide in SD rats. (Note the scale difference in the Y axis.)

Table 1. Results of the visual examination of the oral cavities of Sprague-Dawley rats in the control group (C) and the test groups (CK9 and CK13) inoculated orally with *C. albicans* and *C. krusei*, respectively, with weekly administration of cyclophosphamide (40 mg/kg)

Candida group	Cyclophosphamide administration period									
	Week 22	Week 23	Week 24	Week 25	Week 26	Week 27	Week 28	Week 29		
<i>C. albicans</i> OSU#3										
C1	...	...	Red depapillated area posterior to CP	*White patches anterior and posterior to CP area (K)						
C2	...	Depapillated erythematous depression on CP	White patches in and around CP	*Two smooth erythematous areas on the posterior tongue (K)						
C5	...	Slight erythema in and around CP	Small areas of depapillation on and behind CP	*White patches posterior to CP (K)						
<i>C. krusei</i> CK9										
T1.1	...	...	Slight erythema behind CP	...	Red depapillated depression posterior to CP	Increased size of depapillated lesion	*Depapillated area further increased in size; another area of erythema on the right side (K)			
T1.2	...	...	Slight erythema on CP	...	...	...	Erythematous lesion in front of CP	Red lesion in front of CP persisted (K)		
T1.3	...	...	...	...	*Red depapillated area behind CP (K)					
T1.5	...	...	...	...	...	Erythema on and behind CP (K)				
<i>C. krusei</i> CK13										
T2.1	...	...	...	...	...	...	Small erythematous area on CP	No visual abnormality (K)		
T2.3	...	...	Slight erythema on CP	...	White patches on CP (K)					
T2.4	...	...	Slight erythema on posterior tongue	...	...	Three small depressed areas of depapillation on CP	*A large depapillated, depressed area behind CP; separate smaller lesion posterior to this (K)			
T2.5	...	...	...	...	Loss of papillae behind CP	The lesion appeared to be improving	Further healing of lesion (K)			

K, killed; CP, conical papillae.

*Histopathological findings consistent with candidal infection.

Fig. 2. Macroscopic appearance of Sprague-Dawley rat tongue C1, infected with *C. albicans* OSU#3 after 25 weeks of sequential oral inoculation with the yeast and 4 weeks of cyclophosphamide administration. Note the areas of homogeneous leucoplakia (→) and speckled leucoplakia (→).

lesions to be detected visually with *C. krusei* than with *C. albicans*. The time required for discernible changes on the tongue mucosa varied between 3 and 8 weeks and 3 and 7 weeks with *C. krusei* isolates CK9 and CK13, respectively, compared with 2–4 weeks for *C. albicans* (Table 1). A total of three of eight rats infected with *C. krusei* demonstrated candidal infection after cyclophosphamide treatment, compared with all three rats in the *C. albicans* group. The lingual mucosal lesions and their histopathological changes in both groups of rats infected with *C. krusei* CK9 and CK13 were essentially very similar to the lesions produced by *C. albicans* OSU#3. PAS-stained sections showed hyphal infiltration of the cornified layers by *C. krusei* (Fig. 4b) which were generally shorter than those of *C. albicans*, and penetrated a relatively short depth of the keratinised epithelium varying between 3.73 and 5.07 μm and 5.01–8.34 μm for strains CK9 and CK13, respectively compared with 5.00–17.00 μm for *C. albicans*. However, the number of hyphae penetrating the oral epithelium was relatively fewer and more sparsely distributed for *C. krusei* than with *C. albicans*. *C. albicans* hyphae penetrating the oral epithelium appeared in clusters on the lingual

epithelium, compared with isolated, individual hyphae in the case of *C. krusei*. Oral lesions were recorded for the other five rats, but no yeasts were demonstrated histologically. Any lesions observed appeared to recover during a short period (Table 1). For instance, after 3 weeks of inoculation with *C. krusei* CK9, rat T1.2 in test group 1 showed mild erythema on the conical papillae area, but during the following 3 weeks of the test period the mucosal surface returned to normal, although an ill-defined slightly erythematous area appeared in front of the conical papillae area during the eighth week.

Discussion

A world-wide increase in *C. krusei* infection in immunocompromised patient groups has been described recently [15]. In these individuals the clinical presentation of the disease is influenced not only by the compromised immune status, but also by other factors such as the presence of catheters due to intravenous hyperalimentation, oral prostheses, endocrine disturbances and use of antibiotics or steroids [21]. Despite the increased morbidity caused by *C. krusei*, the approaches used in the investigation of mucosal candidosis have focused mainly on the most virulent species, *C. albicans*, and to our knowledge there are no animal studies pertaining to oral *C. krusei* infection.

Several animal models such as monkeys, rabbits, rats and mice have been developed to study the pathogenesis of oral candidosis. The disadvantages of the monkey and the rabbit model are their cost, and inconsistent oral infection in different hands [22–25]. Hence a smaller, less expensive rat model developed by Jones and Adams [5] has proved to be the most popular for the study of mucosal candidosis [6, 9–12, 19, 26–29]. Such animal studies have contributed significantly towards the understanding of the relationship between *C. albicans* and its host. Hence the Sprague-Dawley rat model was chosen for the current study, and has proved to be reliable for experimental oral infection with *C. krusei*, albeit under immunosuppression.

Tetracycline was used throughout the current study as it is known that a tetracycline-laced diet and repeated yeast inoculation are necessary to maintain prolonged oral colonisation and infection in Sprague-Dawley rats [19, 28–33]. Although there is a single report indicating that tetracycline exposure is not an essential prerequisite for initiating rat oral lesions, the authors acknowledged that the drug increases the overall size of the lesions [10]. In addition to tetracycline, a diet high in carbohydrate further promotes the oral carriage of yeasts and their infectivity [19]. For these reasons, and also because of the well-known low pathogenicity of *C. krusei*, it was decided to administer a carbohydrate-rich diet plus tetracycline. Nonetheless, these measures together with repeated yeast inoculation

Fig. 3. (a) Histopathological section of leucoplakia on dorsal tongue of rat C1 infected with *C. albicans* OSU#3. Note the loss of filiform papillae and vesicle formation and polymorph infiltration in the parakeratotic, oedematous epithelium (H & E, $\times 200$). (b) Section illustrating *C. albicans* hyphae infiltrating the cornified layers of the inflamed lingual epithelium (PAS, $\times 400$).

failed to initiate oral lesions in immunocompetent rats, with either *Candida* species. This may be due to inbreeding of rats as *C. albicans* OSU#3 is known to initiate infection under these experimental conditions.

Most studies that have employed animal models to

mimic oral candidosis have recorded the carriage of candida as positive or negative by the culture of swabs. In this investigation, a semi-quantitative technique was used to assess oral yeast carriage, as described previously [9,28]. The consistent oral carriage of *C. albicans* OSU#3 observed in this study

Fig. 4. (a) Histopathological section of a depapillated area on dorsal tongue of rat T1.1 infected with *C. krusei* CK9. Note the loss of papillae and orthokeratinisation. The infected superficial layers of the epithelium are oedematous with polymorph infiltration and vesicle formation (PAS, $\times 200$). (b) Periodic acid-Schiff stain to confirm epithelial invasion by *C. krusei* (CK9) hyphae (PAS, $\times 400$).

over 21 weeks was different to the sporadic yeast isolation reported by Allen *et al.* [28] who employed the identical yeast strain. This discrepancy may be due to the tetracycline-free, low carbohydrate diet employed by Allen *et al.* [28]. Dietary carbohydrates

potentiate oral candidal carriage in a number of ways, including enhancement of yeast adhesion to mucosae [34], and production of metabolic carboxylic acids leading to a reduction in pH [35] with consequent activation of acidic proteases [36] and phospholipases

[37]. Tetracycline, on the other hand, helps to suppress the commensal bacteria, thereby promoting yeast growth [38, 39].

It would appear that this is the first documented study on the oral carriage of *C. krusei* in an animal model. The results indicated that *C. krusei* isolates were able to colonise the rat tongue mucosa, but in smaller numbers than *C. albicans*, despite the use of equal inocula at an identical frequency and the same conditions. Thus, on occasions, some animals failed to yield *C. krusei* in swab cultures, whereas *C. albicans* was grown from all swab cultures throughout the experimental period of 29 weeks (Fig. 1). Furthermore, in terms of the numbers of organisms colonising the oral mucosa, the prevalence of *C. albicans* was much higher (c. 12-fold) than that of *C. krusei*, implying clearly that the colonisation potential is an intrinsic ability of each *Candida* spp., and may vary between isolates within a given species. Nonetheless, further studies with large numbers of isolates of each species need to be conducted to verify such inter-species attributes.

The lower oral carriage of *C. krusei*, in general, may reflect its reduced ability to adhere to buccal epithelial cells when compared with *C. albicans*. In-vitro studies have shown reduced adherence of *C. krusei* to buccal and vaginal epithelial cells in comparison with *C. albicans*, *C. tropicalis*, *C. stellatoidea* and *C. parapsilosis* [40]. In one study, an 11-fold increase in adherence of *C. albicans* to buccal epithelial cells compared with *C. krusei* was observed [41]. These observations broadly concur with our previous studies in which adhesion of *C. krusei* to buccal epithelial cells from healthy individuals was 10 times less than that of *C. albicans* [16].

Although *C. albicans* was isolated consistently from all five immunocompetent rats, no candidal infection was observed either on gross or histopathological examination of tongue tissues. In previous similar studies conducted by Russell and Jones [19] and Hassan *et al.* [32], candidal infection, indicated by mycelial penetration of the superficial lingual epithelium, was noted in 63% and 60% of rats, respectively. It is difficult to explain why the animals in the present study did not develop infection despite the fact that the *C. albicans* OSU#3 strain used was capable of initiating oral lesions previously [9, 10]. Perhaps the attenuated virulence of a subcultured isolate or the higher natural immunity of rats derived from a local stock may account for this observation.

Under cyclophosphamide-mediated immunosuppressed conditions, *C. albicans* initiated oral lesions in all three rats. Lingual infection confirmed by histopathological observation resembled features described by previous investigators [19, 32]. However, large vesicles with polymorph infiltration were also observed in the stratum spinosum (Fig. 4a), a feature not previously

reported. This may be due to candidal infection superimposed on cyclophosphamide-mediated changes in a rapidly proliferating epithelial cell compartment [42].

The inability of *C. krusei* to infect healthy animals was not surprising, as this opportunist pathogen is isolated mainly from patients with reduced defences [43–45]. However, both *C. krusei* isolates were able to induce lingual infection to some extent in animals that were rendered immunocompromised. Hyphal penetration of the stratum corneum of the lingual mucosa was evident, resembling the histopathological changes induced by *C. albicans*. However, the intra-epithelial vesicles and hyphal penetration appeared to a lesser extent in *C. krusei* lesions. Examination of the erythematous lingual areas of the five rats in test groups 1 and 2 that did not show evidence of candidal infection revealed only mild cellular disorganisation in the basal and parabasal cell layers with occasional lymphocytic infiltration. These observations have shown that oral candidosis can be induced, albeit with some difficulty, in the rat model by the repeated oral inoculation of immunosuppressed rats with *C. krusei*. The fact that only c. 33% of compromised animals developed the infection substantiates the lesser virulence potential of *C. krusei* compared with *C. albicans*.

To conclude, these results confirm that *C. krusei* is an opportunist pathogen that may cause disease when host defences are impaired, and concur with clinical epidemiological data which designate the organism as an emerging pathogen, especially in compromised hosts [15]. Furthermore, the Sprague-Dawley rat model appears to be useful for future workers to simulate *in vivo* the pathogenicity of *Candida* spp. with low virulence, such as *C. parapsilosis*, *C. glabrata*, etc., under immunocompromised situations.

This research was supported by the Committee for Research and Conference Grants of the University of Hong Kong (grant nos. 335/268/0004 and 335/268/0006).

References

1. Cawson RA, Lehner T. Chronic hyperplastic candidiasis – candidal leukoplakia. *Br J Dermatol* 1968; **80**: 9–16.
2. Cawson RA. Chronic oral candidosis, denture stomatitis and chronic hyperplastic candidosis. In: Winner HI, Hurley R (eds) Symposium on *Candida* infections. Edinburgh, Livingstone. 1966: 138–153.
3. Howlett JA. The infection of rat tongue mucosa *in vitro* with five species of *Candida*. *J Med Microbiol* 1976; **9**: 309–316.
4. Partridge BM. The use of the chorioallantoic membrane of the developing chick for culture of dermatophytes: a modified technique; a preliminary report upon its use for serial passage. *J Invest Dermatol* 1959; **32**: 605–619.
5. Jones JH, Adams D. Experimentally induced acute oral candidosis in the rat. *Br J Dermatol* 1970; **83**: 670–673.
6. Adams D, Jones JH. Life history of experimentally induced acute oral candidiasis in the rat. *J Dent Res* 1971; **50**: 643–644.
7. Jones JH, Russell C. The histology of chronic candidal

- infection of the rat's tongue and its relevance to human oral leukoplakia. *J Clin Pathol* 1973; **26**: 390.
8. Jones JH, Russell C. The time scale of penetration of the rat's oral epithelium by *C. albicans*. *International Research Communications System* 1973; **73-12**, 6-3-5.
 9. Allen CM, Beck FM. Strain-related differences in pathogenicity of *Candida albicans* for oral mucosa. *J Infect Dis* 1983; **147**: 1036-1040.
 10. Allen CM, Beck FM, Lurie FA, Pinsky HM. Role of tetracycline in pathogenesis of chronic candidiasis of rat tongues. *Infect Immun* 1985; **47**: 480-483.
 11. Allen CM, Beck FM. Differences in mucosal reaction related to *Candida albicans* isolates. *J Oral Pathol* 1987; **16**: 89-93.
 12. Allen CM. Animal models of oral candidiasis: a review. *Oral Surg Oral Med Oral Pathol* 1994; **78**: 216-221.
 13. Horn R, Wong B, Kiehn TE, Armstrong D. Fungemia in a cancer hospital: changing frequency, earlier onset, and results of therapy. *Rev Infect Dis* 1985; **7**: 646-655.
 14. Wingard JR, Merz WG, Rinaldi MG, Johnson TR, Karp JE, Saral R. Increase in *Candida krusei* infection among patients with bone marrow transplantation and neutropenia treated prophylactically with fluconazole. *N Engl J Med* 1991; **325**: 1274-1277.
 15. Samaranayake YH, Samaranayake LP. *Candida krusei*: biology, epidemiology, pathogenicity and clinical manifestations of an emerging pathogen. *J Med Microbiol* 1994; **41**: 295-310.
 16. Samaranayake YH, Wu PC, Samaranayake LP, So M, Yuen KY. Adhesion and colonisation of *Candida krusei* on host surfaces. *J Med Microbiol* 1994; **41**: 250-258.
 17. Samaranayake YH, Wu PC, Samaranayake LP, So M. Relationship between the cell surface hydrophobicity and adherence of *Candida krusei* and *Candida albicans* to epithelial and denture acrylic surfaces. *APMIS* 1995; **103**: 707-713.
 18. Silverman S, Migliorati CA, Epstein JB, Samaranayake LP. Laboratory diagnosis of oral candidosis. In: Samaranayake LP, MacFarlane TW (eds) *Oral candidosis*. London, Wright. 1990: 213-237.
 19. Russell C, Jones JH. Effects of oral inoculation of *Candida albicans* in tetracycline-treated rats. *J Med Microbiol* 1973; **6**: 275-279.
 20. Darcie JV, Lewis SM. Basic haematological techniques, In: *Practical haematology*, 7th edn. London, Churchill Livingstone. 1991.
 21. Odds FC. *Candida* and candidosis, 2nd edn. London, Bailliere Tindall. 1988.
 22. Budtz-Jorgensen E. Denture stomatitis IV. An experimental model in monkeys. *Acta Odontol Scand* 1971; **29**: 513-526.
 23. Budtz-Jorgensen E. Cellular immunity in acquired candidiasis of the palate. *Scand J Dent Res* 1973; **81**: 372-382.
 24. Olsen I, Haanaes HR. Experimental palatal candidosis and saliva-flow in monkeys. *Scand J Dent Res* 1977; **85**: 135-141.
 25. Mackinnon JE. Nuevo sentido de variacion en *Mycotorula (monilia) albicans*. *Archivos Sociedad de Biologia de Montevideo* 1936; **7**: 162-163.
 26. Russell C, Jones JH. The effects of oral inoculation of the yeast and mycelial phases of *Candida albicans* in rats fed on normal and carbohydrate rich diets. *Arch Oral Biol* 1973; **18**: 409-412.
 27. Russell C, Jones JH, Gibbs ACC. The carriage of *Candida albicans* in the mouths of rats treated with tetracycline briefly or for a prolonged period. *Mycopathologia* 1976; **58**: 125-129.
 28. Allen CM, Blozis GG, Rosen S, Bright JS. Chronic candidiasis of the rat tongue: a possible model for human median rhomboid glossitis. *J Dent Res* 1982; **61**: 1287-1291.
 29. Fisker AV, Rindom Schiott C, Philipsen HP. Long-term oral candidosis in rats. *APMIS* 1982; **90**: 221-227.
 30. Jones JH, Russell C, Young C, Owen D. Tetracycline and the colonization and infection of the mouths of germ-free and conventionalized rats with *Candida albicans*. *J Antimicrob Chemother* 1976; **2**: 247-253.
 31. Russell JH, Jones JH. The histology of prolonged candidal infection of the rat's tongue. *J Oral Pathol* 1975; **4**: 330-339.
 32. Hassan OE, Jones JH, Russell C. Experimental oral candidal infection and carriage of oral bacteria in rats subjected to a carbohydrate-rich diet and tetracycline treatment. *J Med Microbiol* 1985; **20**: 291-298.
 33. Fisker AV, Rindom Schiott C, Philipsen HP. Short-term oral candidosis in rats, with special reference to the site of infection. *APMIS* 1982; **90**: 49-57.
 34. Samaranayake LP, MacFarlane TW. On the role of dietary carbohydrates in the pathogenesis of oral candidosis. *FEMS Microbiol Lett* 1985; **27**: 1-5.
 35. Samaranayake LP, Hughes A, Weetman DA, MacFarlane TW. Growth and acid production of *Candida* species in human saliva supplemented with glucose. *J Oral Pathol* 1986; **15**: 251-254.
 36. Samaranayake LP, Hughes A, MacFarlane TW. The proteolytic potential of *Candida albicans* in human saliva supplemented with glucose. *J Med Microbiol* 1984; **17**: 13-22.
 37. Samaranayake LP, Raeside J, MacFarlane TW. Factors affecting the phospholipase activity of *Candida* species *in vitro*. *Sabouraudia* 1984; **22**: 201-207.
 38. Seelig MS. Mechanisms by which antibiotics increase the incidence and severity of candidiasis and alter the immunological defenses. *Bacteriol Rev* 1966; **30**: 442-459.
 39. Solomon P. Oral moniliasis complicating combined broad-spectrum antibiotic and antifungal therapy. *N Engl J Med* 1961; **265**: 847-848.
 40. King RD, Lee JC, Morris AL. Adherence of *Candida albicans* and other *Candida* species to mucosal epithelial cells. *Infect Immun* 1980; **27**: 667-674.
 41. Tobgi RS. Differences in the pathogenic potential of *Candida* species especially *C. albicans*. PhD thesis, University of Glasgow, 1989.
 42. Ferguson MM. Oral mucous membrane markers of internal disease; part II: disorders of the endocrine system, haemopoietic system and nutrition. In: Dolby AD (ed) *Oral mucosa in health and disease*, Oxford; Blackwell. 1975: 249-250.
 43. Merz WG, Karp JE, Schron D, Saral R. Increased incidence of fungemia caused by *Candida krusei*. *J Clin Microbiol* 1986; **24**: 581-584.
 44. Nguyen VQ, Penn RL. *Candida krusei* infectious arthritis. A rare complication of neutropenia. *Am J Med* 1987; **83**: 963-965.
 45. McQuillen DP, Zingman BS, Meunier F, Levitz SM. Invasive infections due to *Candida krusei*: report of ten cases of fungemia that include three cases of endophthalmitis. *Clin Infect Dis* 1992; **14**: 472-478.