Avirulence of Candida albicans auxotrophic mutants in a rat model of oropharyngeal candidiasis

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Abstract

The virulence of Candida albicans strain SC5413 and two isogenic derivatives have been investigated in a rat model of oropharyngeal candidiasis. The results demonstrate that both mutant strains are avirulent in this animal model while the parental strain readily initiates infection. Avirulence is not related to altered growth characteristics or the inability of the strains to undergo yeast-to-hyphal morphogenesis. The potential importance of nutritional sufficiency as a virulence factor as well as the possibility of utilizing such strains in the development of an in vitro expression technology system for Candida albicans is discussed.

Keywords: Candida albicans; Hyposalivation; Pathogenesis; Virulence factor

1. Introduction

Candida albicans is a member of the indigenous human flora and is an important opportunistic fungal pathogen. The organism is primarily responsible for minor oral and vaginal infections in the immunocompetent host, and for infections ranging from superficial to systemic in individuals immunocompromised by disease or therapies [1]. In this regard, C. albicans promotes the largest percentage of oropharyngeal infections of fungal origin in patients with AIDS [1–5]. In recognition of the increased prominence of C. albicans as a pathogen, an expansion of research aimed at defining factors integral to virulence, as well as towards understanding the basic biology of the organism, has occurred [6,7].

We have begun experiments to identify factors required in the establishment and progression of oropharyngeal candidiasis. As a first step the pathogenicity of certain C. albicans auxotrophic mutants have been investigated in a rat model of oropharyngeal candidiasis.

2. Materials and methods

2.1. Organisms and culture conditions

Experiments were performed utilizing auxotrophic strains derived using molecular genetic techniques by Fonzi and Irwin [8] such that the strains differed from the parent by only defined mutations. A parental
prototrophic strain, SC5314, and the derivatives, CA14 ($\Delta$ura3::imm434/$\Delta$ura3::imm434) and CA18 ($\Delta$ade2::hisG/$\Delta$ade2::hisG $\Delta$ura3::imm434/$\Delta$ura3::imm434), were used in all experiments [8]. Stock cultures were maintained on YPD agar, and liquid cultures were grown in YNB medium supplemented to 25 µg ml$^{-1}$ with both adenine and uridine [8]. Samples taken from the oral cavity of animals inoculated with the appropriate C. albicans strain were assayed for the organism on Sabouraud agar.

2.2. Growth rate determination

Overnight cultures of strains SC5314, CA14 and CA18 were grown in YNB medium containing adenine and uridine to 25 µg ml$^{-1}$. Aliquots were removed and diluted in fresh medium to an OD$_{600}$ of 0.1. At appropriate time intervals the samples were taken and the OD$_{600}$ of the respective culture was determined.

2.3. Rat model of oropharyngeal candidiasis

The rat model of oropharyngeal candidiasis has been described [9]. Briefly, weanling, specific pathogen-free Sprague-Dawley rats, 19–21 days of age, identified by litter, were obtained commercially (Charles River Labs, Inc., Kingston, NY). To insure that animals were free of endogenous C. albicans, oral swabs were taken and plated onto YEPD and/or Sabouraud agar. Subsequently, animals were subjected to surgery where hyposalivation was achieved by ligating the parotid ducts and removing the submandibular and sublingual salivary glands.

Oral infection was initiated using a cotton-tipped applicator saturated with an actively growing culture of C. albicans to approximately 10$^6$ cfu. During the course of the experiment rats were fed diet NIH 2000 containing 56% sucrose and sucrose (10% w/v) was added to the drinking water.

Seven and 14 days after inoculation, the level of colonization of the rats with C. albicans was determined by plating oral swabs onto Sabouraud agar. These time points were chosen as it was previously shown [9,10] that a stable level of colonization of C. albicans is attained over this time period.

3. Results and discussion

3.1. Avirulence of CA14 and CA18 in the rat model of oral candidiasis

After surgery to promote hyposalivation, rats were inoculated with either strain SC5314, CA14 ($\Delta$ura3::imm434/$\Delta$ura3::imm434) or strain CA18 ($\Delta$ade2::hisG/$\Delta$ade2::hisG $\Delta$ura3::imm434/$\Delta$ura3::imm434). The results shown in Table 1 demonstrate that neither auxotrophic strain was capable of initiating infection at an inoculum to 10$^6$ cfu. In contrast, infection was easily established by the parental strain (Table 1). Furthermore, strain SC5314 was transmissible to uninfected cagemates while the auxotrophic strains were not demonstrating that: (i) the model system closely mimics the natural course

### Table 1

**Comparison of infection and transmission of C. albicans strain SC5314 and isogenic mutants CA14 and CA18 in desalivated rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Infecting strain</th>
<th>Number of rats infected</th>
<th>Number of recipients infected/total</th>
<th>Number of C. albicans (cfu/jaw X 10$^4$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desal. donor</td>
<td>SC5314</td>
<td>5/5</td>
<td>-</td>
<td>9.54</td>
</tr>
<tr>
<td>Desal. donor</td>
<td>CA14</td>
<td>0/5</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Desal. donor</td>
<td>CA18</td>
<td>0/5</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Transmission</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Desal. donor to desal. recip.</td>
<td>SC5314</td>
<td>-</td>
<td>5/5</td>
<td>2.21</td>
</tr>
<tr>
<td>Desal. donor to intact recip.</td>
<td>SC5314</td>
<td>-</td>
<td>5/5</td>
<td>2.00</td>
</tr>
<tr>
<td>Desal. donor to desal. recip.</td>
<td>CA14</td>
<td>-</td>
<td>0/5</td>
<td>0</td>
</tr>
<tr>
<td>Desal. donor to intact recip.</td>
<td>CA14</td>
<td>-</td>
<td>0/5</td>
<td>0</td>
</tr>
<tr>
<td>Desal. donor to desal. recip.</td>
<td>CA18</td>
<td>-</td>
<td>0/5</td>
<td>0</td>
</tr>
<tr>
<td>Desal. donor to intact recip.</td>
<td>CA18</td>
<td>-</td>
<td>0/5</td>
<td>0</td>
</tr>
</tbody>
</table>
of infection; and (ii) the number of cfu required for transmission is significantly less than that (10^6 cfu) required to establish infection in primary animals.

Since no significant differences in the in vitro growth rates of the three strains were observed (Fig. 1) it is unlikely that the failure of strains CAI4 and CAI8 to establish infection is solely dependent on this factor. Likewise, the yeast-to-hyphal conversion, a potential virulence factor of *C. albicans* [6], occurs normally in the mutant strains (data not shown), thereby suggesting the inability to undergo morphogenesis is not responsible for avirulence.

The results demonstrate that *C. albicans* strains deficient in uracil and adenine or uracil biosynthesis are avirulent in the rat model system of oropharyngeal candidiasis. It has been shown previously that *C. albicans* strains carrying mutations in either *ADE2* or *URA3* exhibit a low level of pathogenicity in a mouse systemic model of candidiasis [11,12]. Other studies have demonstrated that putative virulence factors that participate in the establishment of infection may vary depending on the target site [6]. Thus, the data reported suggest that specific nutrient auxotrophies may be of general significance in influencing the virulence of individual strains. This conclusion also lends support to the idea that careful choice of target biosynthetic enzymes may prove useful in the development of new antifungal agents [12].

Perhaps more importantly, the results suggest a pathway that may lead to development of an in vivo expression technology system (IVET) [13] for the study of oral candidiasis. A model IVET system has been described for use with Salmonella typhimurium [13], and was designed to detect genes expressed exclusively in vivo under the premise that genes that contribute to virulence will be identified. The requisites for an IVET for study of oropharyngeal candidiasis include: (i) an appropriate animal model; (ii) a strain that shows reduced virulence from an isogenic parent by virtue of mutation to a defined biosynthetic gene; and (iii) the ability to select clones of interest by design and use of suitable cloning vehicles. The use of the rat model and companion strains as presented herein satisfy the first two requirements; in regard to the latter issue, an appropriate cloning vehicle to complement these strains might be obtained by inclusion of *C. albicans* *URA3* and *ADE2* in plasmid construction. Both genes have been partially characterized [14,15] and use of *ADE2* to initially select a library of *C. albicans* transformants would be straightforward. Inclusion of a promoterless *URA3* in the vector would make available both positive and negative selection techniques for tracking *Ura^+* and *Ura^-* phenotypes [16] (analogous to the promoterless *purA-lacZ* fusion that provides in vivo and in vitro selection, respectively, in the *S. typhimurium* IVET [13]). For example, cloning of a fragment upstream from a truncated *URA3* gene might restore in vivo growth if the fragment contained a functional promoter (analogous to restoration of *purA* function [13]). In order to detect sequences that are expressed only in vivo (analogous to lac^-* selection [13]) cells recovered from an infected host could be grown on non-selective medium and then replica-plated to medium containing 5-fluoro-orotic acid [8,16]. Only cells that are *Ura^-* should grow on the latter medium, and such survivors would putatively contain sequences of interest. Experiments are in progress to test these possibilities.
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References