View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by University of Queensland eSpace

Available online at www.sciencedirect.com
SCIENCE DIRECT
Microbes and Infection xx (2005) 1–9
Microbes and Infection
www.elsevier.com/locate/micinf

Original article

Isolates of *Candida albicans* that differ in virulence for mice elicit strain-specific antibody-mediated protective responses

Y. Hu [1], C.S. Farah, R.B. Ashman*

Department of Oral Biology and Pathology, School of Dentistry, University of Queensland, Brisbane, Qld 4072, Australia

Received 27 April 2005; accepted 26 August 2005

Abstract

Three distinct isolates of *Candida albicans* were used to establish systemic and oral infections in inbred mice that are genetically resistant or susceptible to tissue damage. Patterns of infection differed significantly between both yeasts and mouse strains. Systemic infection conferred significant protection against re-challenge with the homologous, but not the heterologous yeast; however, the protective effect was more evident in the tissue-susceptible CBA/CaH mice than in the resistant BALB/c strain. In contrast, oral infection induced protection against both homologous and heterologous oral challenge, although this was significant only in the CBA/CaH mice. CBA/CaH mice produced antibodies of both IgG1 and IgG2a subclasses, whereas BALB/c mice produced predominantly IgG1. Western blotting demonstrated considerable differences between epitopes recognised by serum antibodies from mice of both strains after immunisation with each of the three yeasts. Thus, different strains of yeast show considerable specificity in antibody responses elicited by either systemic or oral infection.
© 2005 Elsevier SAS. All rights reserved.

Keywords: Virulence; Antibody; Protection

1. Introduction

Candida albicans is a common commensal that causes both mucocutaneous and disseminated infections, particularly in those who are debilitated or immunocompromised. Inbred mice differ in their susceptibility to systemic infection with the yeast [1–3], and some progress has been made in identifying the genetic basis of these differences [4–6]. In oral candidiasis, the increased tissue susceptibility of CBA/CaH mice relative to BALB/c has been confirmed [7], and DBA/2 mice show slightly different patterns of oral colonisation compared to BALB/c [8,9]. However, the extent to which host responses are related to global virulence properties of different *Candida* strains has not yet been evaluated.

Clinical isolates of the yeast differ in virulence for mice, as demonstrated by variations in patterns of mortality and fungal burden after systemic challenge. A virulent isolate from a patient with vaginal candidiasis showed higher colonisation of the oral mucosa, and a stronger ability to cause disruption of keratin than did a strain from transient candidaemia [10], and wild-type strains have been shown to differ in their ability to cause tissue damage in the absence of quantitative differences in infection [11]. Active regulation of enzyme secretion may result in increased virulence, and high-proteinase isolates were significantly more pathogenic for mice [12], but the increased virulence was not associated with a single molecular type or category identifiable through DNA fingerprinting or pulsed-field electrophoretic karyotype. Secretory aspartyl proteinases (SAPs) have been implicated in infection of the oral mucosa [13], but no differences were detected in the ability of SAP knockout strains to invade the stomach, or to disseminate to internal organs such as the brain [14], indicating that individual SAPs participated in, but were not essential for virulence. Some of these differences may be attributable to the

* Corresponding author. Tel.: +61 7 3365 3039; fax: +61 7 3365 1109.
E-mail address: r.ashman@uq.edu.au (R.B. Ashman).
[1] Present address: Guanghuan College of Stomatology, Sun Yat-Sen University, Guangzhou, P.R. China.

1286-4579/$ - see front matter © 2005 Elsevier SAS. All rights reserved.
doi:10.1016/j.micinf.2005.08.011

properties of the tissues targeted in the various experimental models, whereas others may be related to the specific virulence factors that are expressed under various conditions, either in vivo or in vitro.

Recovery from primary *Candida* infection is attributed to the actions of both T cells and phagocytes [15], but antibodies play a dominant role in protection against secondary challenge. Monoclonal and polyclonal antibodies to *C. albicans* can protect against both systemic and oral candidiasis [16,17], although antibodies with defined specificities show different degrees of protection [18−20]. B-cell epitope recognition and antibody isotype also differ markedly between inbred strains of mice, in that BALB/c mice produced high levels of IgG1, whereas CBA/H produced predominantly IgG2a [21].

The aims of this study were to compare the virulence of three clinical isolates of *C. albicans* in mouse models of systemic and oral candidiasis, to examine the role of antibody in the protection of susceptible or resistant inbred mice against systemic and oral challenge, and to identify patterns of epitope recognition by serum antibody from these mice.

2. Materials and methods

2.1. Mice

Inbred female mice were purchased from the Animal Resource Centre, Western Australia. Mice were kept in filter top cages in a PC2 facility, and given food and water ad libitum. Animal experiments were approved by the Animal Experimentation Ethics Committee of the University of Queensland, and carried out in accordance with the National Health and Medical Research Council's Australian Code of Practice for Care and Use of Animals for Scientific Purposes (1997).

2.2. Yeasts

Candida albicans isolates 3630 and 3683 were obtained from the Australian Medical Mycology Reference Laboratory (AMMRL) at the Royal North Shore Hospital, Sydney. Strain 3630 was from the nail of a patient with cutaneous candidiasis, and 3683 was an oral isolate. *C. albicans* SC5314, isolated from a patient with disseminated candidiasis [22], was a gift from Dr P. Sundstrom, Ohio State University. Yeasts were stored at −70 °C in 15% (v/v) glycerol in Sabouraud's broth and grown in Sabouraud's broth for 48 hours at room temperature with continuous agitation.

2.3. Oral infection

Oral inoculation of yeast, and the swabbing procedures were performed under halothane anaesthesia (Veterinary Companies of Australia, Artarmon, NSW, Australia) using an inhalation apparatus (Fluortec, Mediquip, Brisbane, Qld, Australia) and a scavenging system (Omnicon Fresh Air Cannister, Bickford Inc, NY, USA). Mice were inoculated with 1×10^8 yeasts in 20 µl sterile PBS, and infection monitored by swabbing the oral cavity using sterile cotton ear, nose and throat (ENT) swabs (Sarstedt, Adelaide, SA, Australia) and plating on Sabouraud's agar plates (MicroDiagnostics, Qld, Australia). The plates were incubated at 37 °C for 48 h and colony-forming units (CFU) counted. The results were expressed according the following scoring system [23]: Score 0 = no detectable yeasts; Score 1 = 1−10 CFU; Score 2 = 11−100 CFU; Score 3 = 110−1000 CFU; Score 4 = 1000+ CFU.

2.4. Systemic infection

Mice were injected with 3×10^5 *C. albicans* 3630 and 3683 but 4×10^4 SC5314, in 200 µl PBS via the tail vein. A reduced concentration was used for SC5314, because preliminary experiments showed that mice inoculated with 3×10^5 cells died within 1 week of injection (data not shown). Where necessary for comparisons between yeasts, the dose for 3630 and 3683 was also reduced to 4×10^4. Mice were killed on day 5 after either primary infection or secondary challenge. The brains and kidneys were harvested, weighed, suspended in 1 ml sterile PBS, and homogenised. One hundred microlitres of suspension was titrated in duplicate on Sabouraud's agar plates, incubated at 37 °C for 48 h, and colonies counted. The results were expressed as $\log_{10}$ colony-forming units per gram of tissue. For immunisation, mice were inoculated as above. At week 6, they were challenged with 3×10^5 yeasts and killed on day 5 for assessment of CFU in the brain and kidneys as described above.

2.5. Extraction of Candida antigen

Yeasts were grown for 48 h at room temperature. 1×10^9 cells were harvested and washed twice with 10 ml sterilised distilled water. The yeast pellet was resuspended in an equal volume of Protein Extraction Buffer and mixed with an equal volume of 0.4 mm glass beads. The yeasts were vortexed for 15 min at 4 °C, after which 1 ml of 0.1% SDS was added while vortexing for 1 min to ensure complete cell lysis. The protein extract was collected by centrifuging at 2000 rpm for 20 min, and stored at −70 °C until used. The concentration of antigen was estimated using BCA Protein Assay Kit (Pierce Chemical, Rockford, IL, USA) according to the manufacturer's instructions.

2.6. SDS−PAGE and Western blotting

SDS−PAGE and immunoblotting were performed according to manufacturer's instructions (Bio-Rad Laboratories, Hercules, CA, USA). Briefly, the proteins were prepared in a sample buffer and electrophoresed on a 7.5% SDS−PAGE gel after heating for 5 min at 95 °C, and then transferred onto a 0.2 µm nitrocellulose membrane (Bio-Rad) for antibody binding. The concentration of antigens for SDS−PAGE was 20 µg in 20 µl sample buffer/well. A pre-stained precision

ARTICLE IN PRESS

protein standard and a biotinylated standard (Bio-Rad) were electrophoresed in parallel with the antigens.

2.7. Collection of immune serum

CBA/CaH and BALB/c mice were immunised either systemically or orally with the three strains of yeast as described above. Blood was obtained by direct heart puncture on weeks 2, 5 and 8 after systemic infection, and on weeks 1, 3, 5 and 8 after oral infection. Sera were collected by centrifugation of blood at 14,000 rpm for 15 min at 4 °C. Sera from mice infected with the same yeast strain were pooled and stored at −20 °C until used.

2.8. Antibody detection

The ECL antibody detection kit (Amersham, USA) was used. Briefly, the membranes were treated with blocking buffer, and washed three times with PBS-T. The membranes were incubated with the appropriate mouse serum, and detected by incubation with horseradish peroxidase (HRPO)-labelled IgG1, IgG2a and IgM goat anti-mouse conjugates (CALTAG, Burlingame, CA, USA) for 1 h at room temperature. The membranes were washed three times, and bands detected with ECL reagents and ECL Hyperfilm. The films were converted to digital images with a Nikon Coolpix 995 digital camera (Nikon Co, Japan) and analysed using Quantity One Software (Bio-Rad, USA).

2.9. Statistics

All quantitative data were analysed using one-way analysis of variance (ANOVA) and Student's *t*-test, as implemented in GraphPad Prism Version 2.01 (GraphPad Inc., San Diego, CA, USA).

3. Results

We have previously noted that mice infected systemically with yeast strain SC5314 showed a dramatically different pattern of mortality when compared with strain 3630, routinely used in our laboratory. Deaths resulting from systemic infection with 3×10^5 yeasts of strain 3630 typically occurred within 10 days, after which there was no further mortality [24]. After infection with the same dose of SC5314, there was no mortality during this early period, but the long-term survival rate was very low. Mice began to die at about 20 days, and deaths continued for several months.

3.1. Systemic infection

The virulence of three different isolates of *C. albicans*, 3630, 3683, and SC5314, was compared by determining the levels of infection in brain and kidney of 'tissue susceptible' (CBA/CaH) and 'tissue resistant' (BALB/c) mice after intravenous challenge. In BALB/c mice, infection with 3630 produced the highest fungal burden in the brain (Fig. 1), whereas infection by SC5314 was significantly less severe. Using this latter strain of yeast, a substantial proportion (5/9) of the animals failed to demonstrate infection of the brain. It should be emphasised that this was not an artefact of inoculation, as the kidneys of these mice were colonised at levels not significantly different to other mice in the group, and doubling the challenge dose (to 6×10^5) did not increase the proportion of animals whose brains became infected (data not shown). SC5314 caused the most severe infection in the kidney, whereas infections caused by the other two *Candida* strains were comparable in severity.

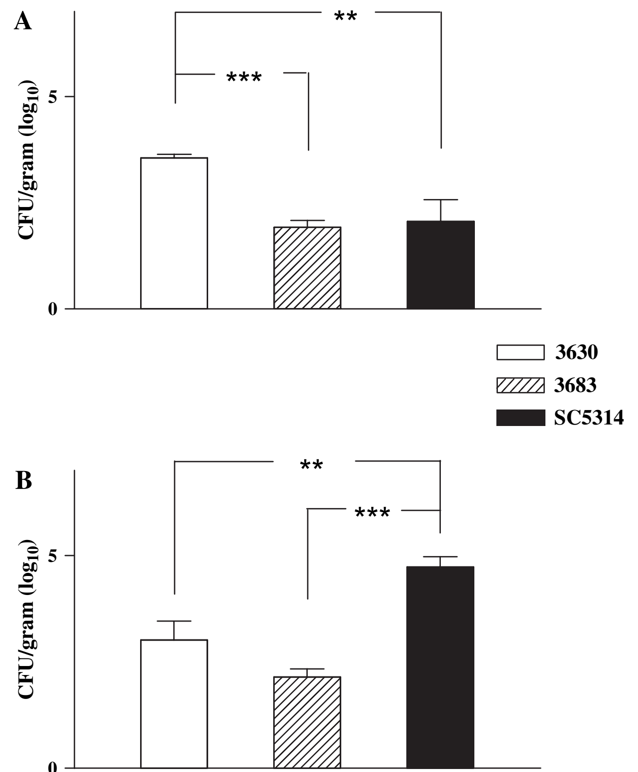

Fig. 1. Fungal burden in brain (A) and kidney (B) of BALB/c mice 4 days after systemic infection with 4×10^4 *Candida albicans* 3630, 3683 or SC5413. Bars represent mean ± SEM log_{10} CFU/g of tissue for a minimum of 7 mice/group. $^{**}P < 0.01$, $^{***}P < 0.001$.

In CBA/CaH mice, the SC5314 strain was most virulent, showing the highest fungal burden in the kidney (Fig. 2). In only one case (1/9) did this yeast fail to infect the brain, and when this animal was excluded, the severity of infection was equivalent to that induced by 3630. Yeast 3683 was significantly less virulent for both organs. When infected with 3630, the fungal burden in the kidney of CBA/CaH mice was significantly higher compared to that in BALB/c (4.16 ± 0.17 vs. 3.13 ± 0.51, $P < 0.05$). However, both the brain and kidney of CBA/CaH mice were more susceptible to infection with SC5314 compared to BALB/c mice (brain: 4.15 ± 0.05 vs. 2.06 ± 0.52, $P < 0.001$ and kidney: 5.93 ± 0.27 vs. 4.73 ± 0.24, $P < 0.01$).

Tissue lesions that developed following systemic challenge with SC5314 were comparable to those previously described after infection with 3630, and were more severe in CBA/CaH

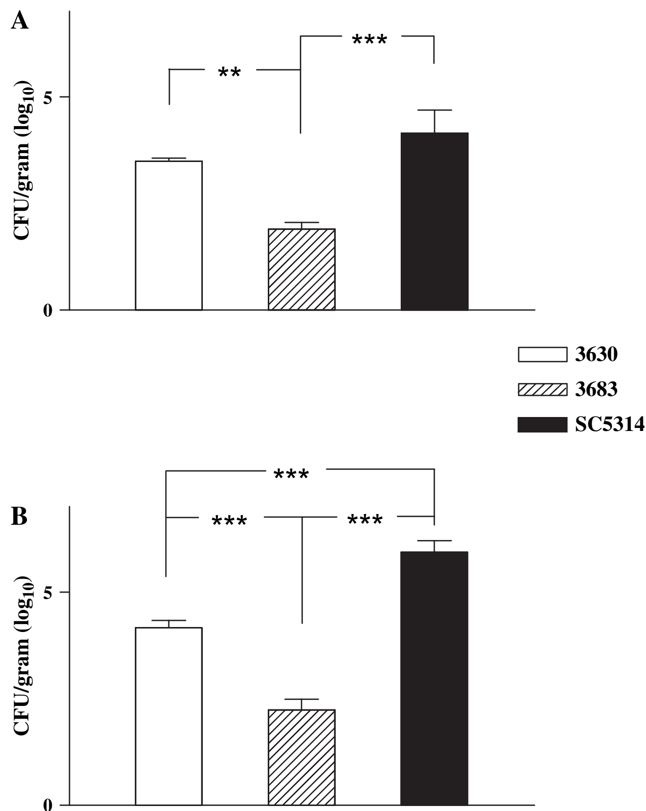

Fig. 2. Fungal burden in brain (A) and kidney (B) of CBA/CaH mice 4 days after systemic infection with 4×10^4 *Candida albicans* 3630, 3683 or SC5413. Bars represent mean ± SEM $\log_{10}$ CFU/g of tissue for a minimum of 7 mice/group. $^{**}P < 0.01$, $^{***}P < 0.001$.

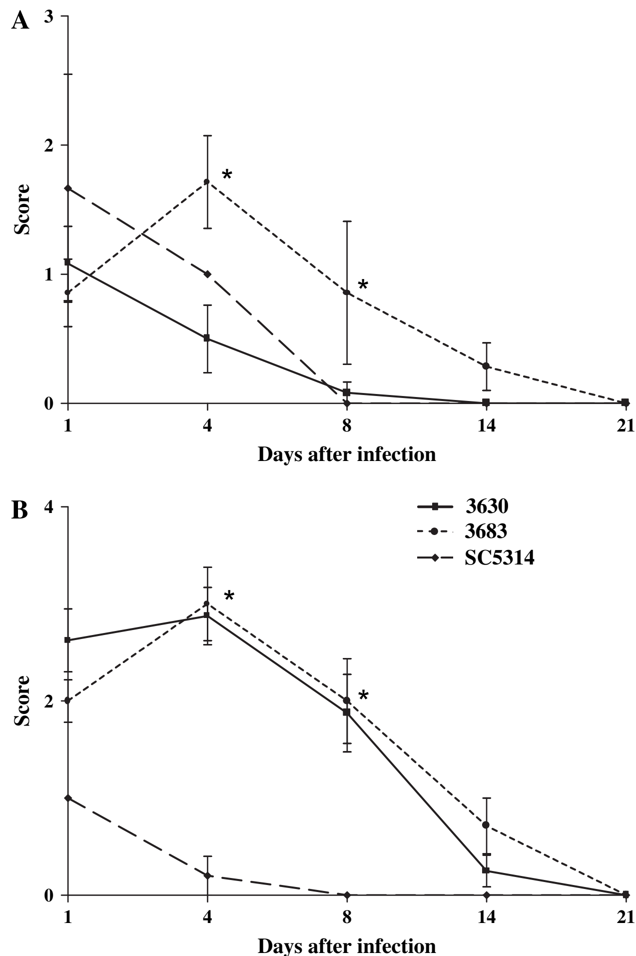

Fig. 3. Oral infections with three strains of yeasts in BALB/c (A) and CBA/CaH (B) mice. Data points represent the score (mean ± SEM) for a minimum of 7 mice/time point in each group. There was a significant difference between 3683 and 3630 (A), and between SC5314 and 3683 or 3630 (B) on days 4 and 8. $^{*}P < 0.05$.

than in BALB/c mice (data not shown), confirming that tissue susceptibility in the host was independent of *Candida* virulence.

3.2. Oral infection

In oral infection, there was a strong interaction between the yeast and host, that was not seen in systemic infection. In BALB/c mice, yeast 3683 caused a more severe infection ($P < 0.05$) of slightly longer duration than either 3630 or SC5314 (Fig. 3A). The latter failed to infect the oral cavity in 4/7 of the mice. In the CBA/CaH mouse strain, the level and duration of infection by 3683 and 3630 were equivalent. As in BALB/c mice, SC5314 produced detectable infections in only 5/10 mice (Fig. 3B). Nevertheless, the relative susceptibility of CBA/CaH and BALB/c mice was similar to that after systemic infection, in that CBA/CaH mice developed significantly more severe infections than did BALB/c mice after oral inoculation with either strain 3630 or 3683 but not SC5314. The apparent lack of virulence expressed by SC5314 after oral challenge was further evaluated by inoculation into BALB/c nude mice, which are acutely susceptible to infection with strain 3630 [23]. In 2/5 mice, the infection failed to take, and although oral colonisation persisted significantly longer in athymic mice than in euthymic controls, it was mild in nature (maximum mean score, 2.3 ± 0.3 SEM at day 4), and decreased during the 21-day period of observation.

3.3. Protection against systemic infection

In the following experiments, BALB/c and CBA/CaH mice were immunised with the three strains of *C. albicans*, and challenged with the homologous and heterologous strains 5 weeks later. The protective effect of immunisation against challenge with the homologous yeasts is shown in Table 1.

Immunisation of CBA/CaH mice induced strong protective responses in both brain and kidneys, whereas the response induced in BALB/c mice varied with the yeast strain used for immunisation. Isolate 3630 protected the brain but not the kidneys, whereas in mice immunised with SC5314, the pattern was reversed. After infection with 3683, protective responses were only demonstrated in the brain of CBA/CaH mice, but the kidneys in the CBA/CaH showed no protective effect, whereas infection in the kidney of BALB/c mice was significantly enhanced.

ARTICLE IN PRESS

Table 1
Protective effect of systemic immunisation against challenge with the homologous yeasts

Mouse strain	Treatment	Yeast strain					
		3630		3683		SC5314	
		Brain	Kidney	Brain	Kidney	Brain	Kidney
BALB/c	Naïve	3.7 ± 0.1	5.0 ± 0.2	1.8 ± 0.1	2.6 ± 0.1	2.1 ± 0.5	4.2 ± 0.6
	Immunised	2.3 ± 0.3***	4.4 ± 0.3	1.6 ± 0.2	4.0 ± 0.7*	1.7 ± 0.2	1.8 ± 0.3*
CBA/CaH	Naïve	4.5 ± 0.2	5.8 ± 0.2	2.7 ± 0.1	2.9 ± 0.2	4.1 ± 0.5	5.9 ± 0.3
	Immunised	3.0 ± 0.2**	4.0 ± 0.4***	1.9 ± 0.2**	3.3 ± 0.5	1.5 ± 0.1**	2.8 ± 0.4***

The data represent the mean ± SEM of the $\log_{10}$ CFU/g in tissue, calculated from at least 7 mice for each group. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

In general, infection with any one yeast strain failed to protect against heterologous challenge (Table 2), although some protection was noted in the kidneys but not the brain of CBA/CaH mice after immunisation with isolate 3630 and challenge with SC5314.

3.4. Protection against oral infection

BALB/c and CBA/CaH mice were immunised by oral infection with the three strains of yeasts, and the level of protection induced was evaluated by oral challenge with yeast strain 3630 five weeks later (Fig. 4). In contrast to systemic challenge, similar levels of protection were induced in CBA/CaH mice, regardless of the strain of yeasts used for immunisation, whereas BALB/c mice were not protected. However, when the mice were challenged with 3683, only immunisation with the homologous yeast induced significant protection (Fig. 5).

3.5. B-cell epitope recognition

BALB/c and CBA/CaH mice were immunised with each of the three strains of *C. albicans*, and the serum tested for reactivity against antigenic preparations from the same three strains. Following systemic immunisation, specific antibody production was detectable at 2 weeks after immunisation (data not shown), and the strength of the antibody response appeared to reach a plateau within 5–8 weeks after infection. CBA/CaH mice produced antibody of both IgG1 and IgG2a subclasses, to a wider range of antigenic determinants than did BALB/c mice, which produced predominantly IgG1 (Fig. 6). No IgM reactivity was detected in serum from either CBA/CaH or BALB/c mice immunised with any of the three isolates of yeasts (data not shown). BALB/c mice only recognised antigens in the 45–48 kDa group, regardless of the strain of yeast used for immunisation. Antibodies produced

Table 2
Protective effect of systemic immunisation against challenge with the heterologous yeasts

Yeast used for:		Mouse strain			
		BALB/c		CBA/CaH	
Primary infection	Challenge	Brain	Kidney	Brain	Kidney
3630	SC5314	1.9 ± 0.7	3.9 ± 1.0	2.7 ± 0.1	4.3 ± 0.4*
None	SC5314	2.1 ± 0.5	4.2 ± 0.6	4.1 ± 0.5	5.9 ± 0.3
3630	3683	1.4 ± 0.1	2.5 ± 0.2	1.9 ± 0.2	4.1 ± 0.5
None	3683	1.8 ± 0.1	2.6 ± 0.1	2.7 ± 0.1	2.9 ± 0.2
SC5314	3630	2.9 ± 0.2	4.6 ± 0.2	3.6 ± 0.3	5.9 ± 0.2
3683	3630	2.9 ± 0.2	4.5 ± 0.3	3.5 ± 0.5	5.4 ± 0.4
None	3630	3.7 ± 0.1	5.0 ± 0.2	4.5 ± 0.2	5.8 ± 0.2

The data represent the mean ± SEM of the $\log_{10}$ CFU/g in tissue, calculated from at least 7 mice for each group. *$P < 0.05$.

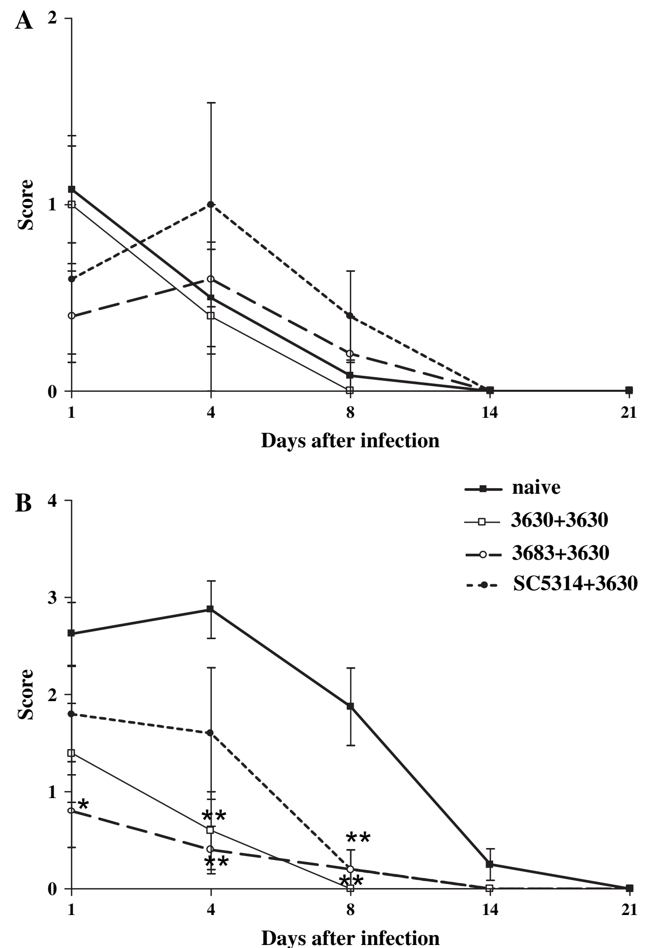

Fig. 4. Oral protection in BALB/c (A) and CBA/CaH (B) mice after oral immunisation with 1×10^8 yeasts of strains 3630, 3683, or SC5314 *C. albicans*, and challenge with the same dose of 3630. Data points represent the score (mean ± SEM) for a minimum of 7 mice/time point in each group. *Significant difference ($P < 0.05$) between (3683 + 3630) and the naïve group on day 1; **Significant difference ($P < 0.01$) between both (3630 + 3630) and (3683 + 3630) compared to the naïve group on day 4, and all three groups compared to the naïve group on day 8.

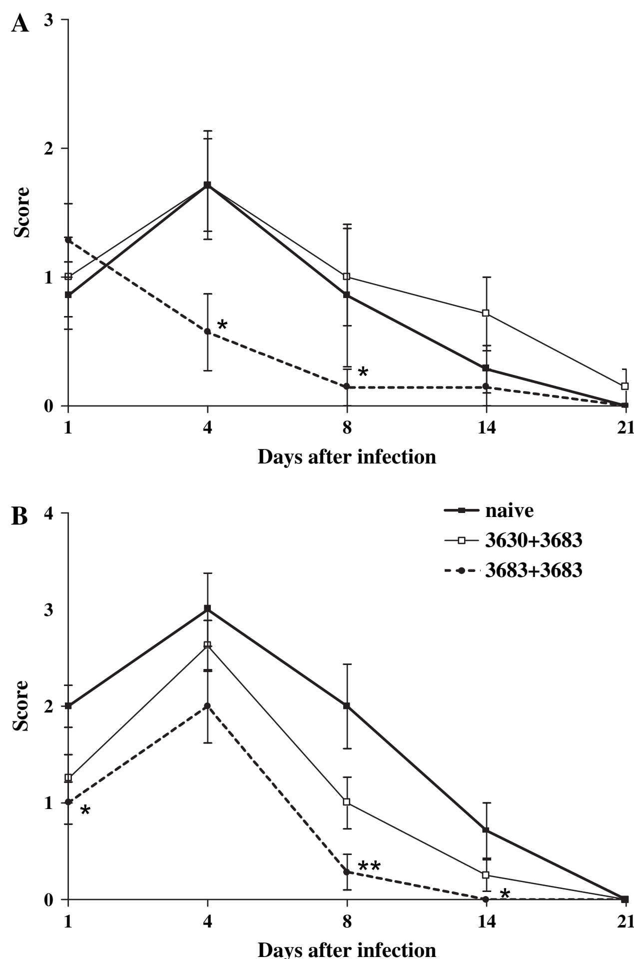

Fig. 5. Oral protection in BALB/c (A) and CBA/CaH (B) mice after oral immunisation with 1×10^8 yeasts of strain 3630 or 3683, and challenge with the same dose of strain 3683. Data points represent the score (mean ± SEM) for a minimum of 7 mice/time point in each group. Significant difference between the immunised group and the controls at $*P < 0.05$ or $**P < 0.01$, respectively.

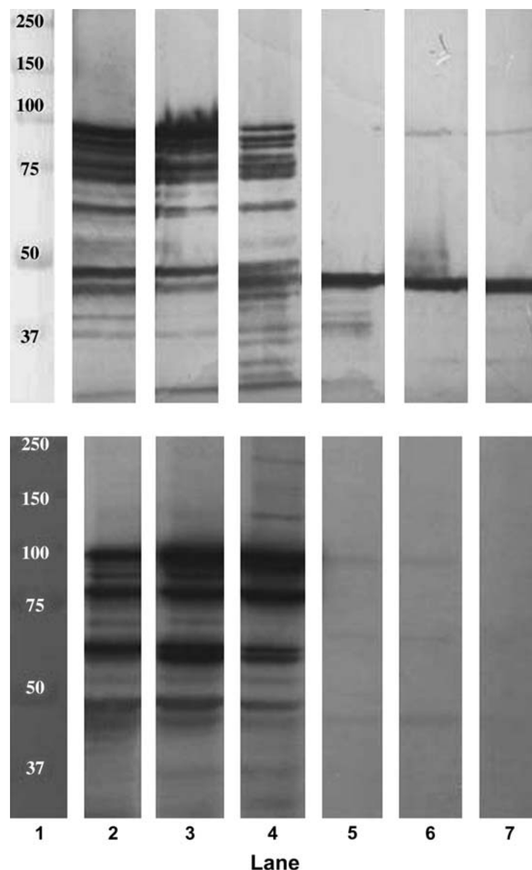

Fig. 6. Western blot showing reactivity of IgG1 (upper panel) and IgG2a (lower panel) in sera from CBA/CaH and BALB/c mice 5 weeks after systemic infection with *C. albicans* 3630. Lane 1, molecular weight standard; lanes 2–4, antigen preparations of *C. albicans* 3630, 3683 and SC5314 reacted with sera from CBA/CaH mice; lanes 5–7, the same preparations reacted with sera from BALB/c mice.

after oral immunisation tended to show both a more restricted range, and a lower intensity of recognition than those elicited by systemic immunisation (data not shown).

4. Discussion

Establishing satisfactory criteria to define the virulence of *C. albicans* has been the subject of considerable debate, but the gold standard must be colonisation and infection in vivo. Yeast strain 3630, routinely used in our laboratory, has been shown to establish reproducible infections in the brain, kidney and other tissues of inbred mice [25], and in the oral cavity [7]. The present experiments have confirmed these results, and further shown that yeast 3683, derived from an oral infection, was more efficient in infecting the oral mucosa, while retaining an ability to establish consistent systemic infections. In contrast, yeast SC5314, from a systemic infection, induced a very severe infection in the kidney, but was quite inefficient in infecting either the brain or the oral cavity.

Virulence in vivo has been associated with differences in proteinase production. Type I strains of *C. albicans*, that could invade the chorioallantoic membrane (CAM), were able to produce proteinase, whereas type II strains that were not able to penetrate the CAM did not secrete the enzyme [26]. Kidney tissues of mice infected with the type I strains demonstrated extensive invasion by fungal cells, whereas fungal cells were rarely found in those infected with the type II strains [27]. Thus, the inefficiency with which yeast SC5314 infects the brain and oral cavity suggests that this strain may show reduced expression of an enzyme essential for attachment to either epithelial or endothelial cells. Nevertheless, in a model of contact lens-facilitated keratitis in rabbits, *C. albicans* SC5314 penetrated deeper into the cornea than did another clinical isolate [11], indicating that mechanisms of adherence and/or invasion by the yeast may be highly specific for different tissues.

Establishment of an oral infection, in particular, has been associated with expression of SAP1, SAP3, SAP4, SAP7, and SAP8 [28], whereas SAP2 and SAP5 were present in both patients with asymptomatic *Candida* carriage and in those with oral disease. Thus, comparison of SC5314 and 3683 may be useful in correlating proteinase production with the efficiency of yeast infectivity at various body sites.

However, in addition to the role of proteinases, adhesion of the yeast to epithelial cell walls is promoted by fungal wall components such as mannose, C3d receptors, mannoprotein, and saccharins [29–31], as well as hydrophobicity [32] and the ability to bind to host fibronectin [33]. Therefore, virulence may be a global property of the yeast, representing contributions from numerous different factors [34]. The yeast may also demonstrate considerable genetic plasticity, compensating for reduced function of one gene by the up-regulation of others not normally expressed [35].

A significant finding from this series of experiments was the specificity of protection elicited after immunisation with any particular isolate of *C. albicans*. Mouse strain specificity in the expression of antibody-mediated protection has previously been reported [36], in that immunisation with *C. albicans* 3630 induced a strong protective response against systemic challenge in CBA/CaH mice, but not in BALB/c mice, but isolate-specific protection was unexpected. This result suggests that the dominant B-cell epitopes expressed by the various yeast isolates are significantly different. In only one case was cross-protection observed, and this was restricted to the kidney.

Inbred mice differ not only in their susceptibility or resistance to *C. albicans* infection, but also show a specificity of organ responsiveness, in that different organs appear to be protected by different components of the immune system. For example, neutrophil depletion causes an acute increase in the susceptibility of the heart and kidney, whereas the brain is only slightly affected [37]. Thus, antibody may, depending on its specificity and isotype, act to protect different organs—in BALB/c mice, the brain but not the kidney, and the kidney but not the brain after immunisation with SC5314. After immunisation and re-challenge with 3683, both strains of mice demonstrated enhanced infection in the kidney, while simultaneously exhibiting protective responses in the brain. Macrophages from both BALB/c and CBA/CaH mice demonstrated a depressed killing of 3683 opsonised with homologous immune serum (see accompanying paper), so the different responses of brain and kidney may reflect a predominance of these cell types in protection of the kidney, whereas microglia may be the major effector cell in defence of the brain.

After oral infection, the situation appears even more complicated. Immunisation with any of the three isolates protected CBA/CaH, but not BALB/c mice, against challenge with 3630, whereas protection against challenge with 3683 could only be induced by immunisation with the homologous yeast. One possible explanation may be the persistence of mannans or mannoproteins of 3630 in the oral mucosa, that act to induce a continuing low level of activation in the resident macrophages. This activation may then manifest as cross-protection. An alternative is that there are functional differences between dendritic cells of the oral cavity and the spleen in processing of the yeast antigens. In vitro, dendritic cells have been reported to discriminate between yeast and hyphal forms of *C. albicans*, producing IL-12 and priming for Th1 cell responses after contact with the yeast form, whereas contact with hyphae induced IL-4 production and inhibited IL-12 and Th1 priming [38]. This discrimination could extend to B-cell epitopes of different yeasts, resulting in different antibody profiles after oral or systemic priming.

BALB/c mice respond to infection with *C. albicans* by producing antibodies, predominantly of the IgG1 isotype, against a restricted range of epitopes, whereas CBA/CaH mice make antibodies of both IgG1 and IgG2a isotypes, against a much more diverse spectrum of antigens. In the present experiments, the general patterns in BALB/c and CBA/CaH mice were consistent with those reported [21,39], and were reproduced after infection with each of the three yeast strains used in this study. There were, however, marked differences both between isotypes, between yeast strains, and between routes of immunisation, in the antibody profiles elicited. After infection with 3630, IgG2a of CBA/CaH mice recognised the largest number of different specificities, many not present in the other two yeast strains, but the IgG1 isotype also detected numerous separate and non-overlapping specificities. A similar pattern was seen after infection with 3683, whereas the profiles elicited by infection with SC5314 were similar for both IgG1 and IgG2a isotypes. In contrast, only one major reactivity, most probably against the enzyme enolase [21,39], was detected in the responses of BALB/c mice to the three strains of *Candida*.

After immunisation with live *C. albicans* 3630, both CBA/H and BALB/c mice show increased resistance to infection; however, the tissue-susceptible CBA/H mice develop much stronger protective responses than do tissue-resistant BALB/c mice [40]. This protection was also evident after infection with strains 3683 and SC5314, and correlated with the diverse antibody reactivity generated by this mouse strain. Surprisingly, many of the major antigenic reactivities reported in clinical studies, such as those against proteins of molecular mass 29 kDa, 36 kDa [41], 58 kDa [41,42], 70 kDa [43], and the 90 kDa heat shock protein [44], were not identified in these mice, although the range of antigens detected was not as extensive as that reported by Pitarch et al. [39], possibly because of the sensitivity of the two-dimensional gel electrophoresis used in their study.

In contrast, BALB/c mice showed a restricted range of antibody responses, predominantly to antigens of 45–48 kDa. Although immunisation with purified proteins, such as enolase, that migrate with this group, stimulates humoral and cell-mediated immune responses [45], either the limited antibody diversity in BALB/c mice is inadequate to confer significant protection, or BALB/c mice have a high level of innate resistance against *C. albicans* infection, and the protective effect of the antibodies are only evident in specific yeast/organ combinations. Nevertheless, it seems clear that each of the yeast strains possesses a relatively distinct antigenic profile, and that protective responses may involve contributions from numerous distinct antigenic entities.

It was noted that the pattern of epitopes recognised after infection of the oral cavity differed from those elicited by systemic immunisation, although again, the patterns for each of the yeasts are almost non-overlapping. Two explanations seem tenable. First, the virulence factors expressed by the yeast in colonisation and invasion of the oral cavity may be substantially different from those expressed in systemic infection,

and second, processing and presentations of proteins of the yeast by antigen-presenting cells (APC) in the lymph nodes draining the oral mucosa may be different to that of splenic APC. The use of techniques for the generation of antibody responses in vitro may be necessary to resolve these points.

Although the tissue susceptibility of inbred mouse strains is comparable in oral and systemic infection, patterns of oral colonisation by the three yeast isolates differed from tissue infection in systemic disease, a result consistent with the hypothesis [15] that the mechanisms of host recovery from oral and systemic candidiasis may be separate and distinct.

Acknowledgements

The research was supported by grants from the National Health and Medical Research Council of Australia and the Australian Dental Research Foundation.

References

[1] G. Marquis, S. Montplaisir, M. Pelletier, P. Auger, W.S. Lapp, Genetics of resistance to infection with *Candida albicans* in mice, Br. J. Exp. Pathol. 69 (1988) 651–660.

[2] R.B. Ashman, J.M. Papadimitriou, Production and function of cytokines in natural and acquired immunity to *Candida albicans* infection, Microbiol. Rev. 59 (1995) 646–672.

[3] R.B. Ashman, *Candida albicans*: pathogenesis, immunity and host defence, Res. Immunol. 149 (1998) 281–288.

[4] R.B. Ashman, A. Fulurija, J.M. Papadimitriou, Evidence that two independent host genes influence the severity of tissue damage and susceptibility to acute pyelonephritis in murine systemic candidiasis, Microb. Pathog. 22 (1997) 187–192.

[5] R.B. Ashman, A gene (*Carg1*) that regulates tissue resistance to *Candida albicans* maps to chromosome 14 of the mouse, Microb. Pathog. 25 (1998) 333–335.

[6] R.B. Ashman, A. Fulurija, J.M. Papadimitriou, A second *Candida albicans* resistance gene (*Carg2*) regulates tissue damage, but not fungal clearance, in sub-lethal murine systemic infection, Microb. Pathog. 25 (1998) 349–352.

[7] C.S. Farah, S. Hong, S. Wanasaengsakul, S. Elahi, G. Pang, T. Gotjamanos, G.J. Seymour, R.L. Clancy, R.B. Ashman, Irradiation-induced oral candidiasis in an experimental murine model, Oral. Microbiol. Immunol. 16 (2001) 358–363.

[8] J. Chakir, L. Cote, C. Coulombe, N. Deslauriers, Differential pattern of infection and immune response during experimental oral candidiasis in BALB/c and DBA/2 (H-2d) mice, Oral. Microbiol. Immunol. 9 (1994) 88–94.

[9] S. Elahi, G. Pang, R. Clancy, R.B. Ashman, Cellular and cytokine correlates of mucosal protection in murine model of oral candidiasis, Infect. Immun. 68 (2000) 5771–5777.

[10] W.P. Holbrook, J.A. Sofaer, J.C. Southam, Experimental oral infection of mice with a pathogenic and a non-pathogenic strain of the yeast *Candida albicans*, Arch. Oral. Biol. 28 (1983) 1089–1091.

[11] D.M. O'Day, W.S. Head, C. Csank, D.J. Shetlar, R.D. Robinson, G.W. McCollum, R. Yang, T.L. Zhu, M.X. Wang, Differences in virulence between two *Candida albicans* strains in experimental keratitis, Invest. Ophthalmol. Vis. Sci. 41 (2000) 1116–1121.

[12] F. De Bernardis, P. Chiani, M. Ciccozzi, G. Pellegrini, T. Ceddia, G. D'Offizzi, I. Quinti, P.A. Sullivan, A. Cassone, Elevated aspartic proteinase secretion and experimental pathogenicity of *Candida albicans* isolates from oral cavities of subjects infected with human immunodeficiency virus, Infect. Immun. 64 (1996) 466–471.

[13] J.R. Naglik, S.J. Challacombe, B. Hube, *Candida albicans* secreted aspartyl proteinases in virulence and pathogenesis, Microbiol. Mol. Biol. Rev. 67 (2003) 400–428.

[14] M. Kretschmar, A. Felk, P. Staib, M. Schaller, D. Hess, M. Callapina, J. Morschhauser, W. Schafer, H.C. Korting, H. Hof, B. Hube, T. Nichterlein, Individual acid aspartic proteinases (Saps) 1–6 of *Candida albicans* are not essential for invasion and colonization of the gastrointestinal tract in mice, Microb. Pathog. 32 (2002) 61–70.

[15] R.B. Ashman, C.S. Farah, S. Wanasaengsakul, Y. Hu, G. Pang, R.L. Clancy, Innate versus adaptive immunity in *Candida albicans* infection, Immunol. Cell Biol. 82 (2004) 196–204.

[16] S.J. Challacombe, Immunologic aspects of oral candidiasis, Oral Surg. Oral Med, Oral Pathol. 78 (1994) 202–210.

[17] P.L. Fidel Jr., Immunity to Candida, Oral Dis, 8 (Suppl. 2) (2002) 69–75.

[18] F. De Bernardis, M. Boccanera, D. Adriani, E. Spreghini, G. Santoni, A. Cassone, Protective role of antimannan and anti-aspartyl proteinase antibodies in an experimental model of *Candida albicans* vaginitis in rats, Infect. Immun. 65 (1997) 3399–3405.

[19] Y.M. Han, R.P. Morrison, J.E. Cutler, A vaccine and monoclonal antibodies that enhance mouse resistance to *Candida albicans* vaginal infection, Infect. Immun. 66 (1998) 5771–5776.

[20] R. Matthews, J. Burnie, Antifungal antibodies: a new approach to the treatment of systemic candidiasis, Curr. Opin. Invest. Drugs. 2 (2001) 472–476.

[21] P.J. Costantino, N.F. Gare, J.R. Warmington, Humoral immune responses to systemic *Candida albicans* infection in inbred mouse strains, Immunol. Cell Biol. 73 (1995) 125–133.

[22] A.M. Gillum, E.Y. Tsay, D.R. Kirsch, Isolation of the *Candida albicans* gene for orotidine-5′-phosphate decarboxylase by complementation of *S. cerevisiae* ura3 and *E. coli* pyrF mutations, Mol. Gen. Genet. 198 (1984) 179–182.

[23] C.S. Farah, S. Elahi, K. Drysdale, G. Pang, T. Gotjamanos, G.J. Seymour, R.L. Clancy, R.B. Ashman, Primary role for $CD4^+$ T lymphocytes in recovery from oropharyngeal candidiasis, Infect. Immun. 70 (2002) 724–731.

[24] R.B. Ashman, J.M. Papadimitriou, in: E. Kurstak, G. Marquis (Eds.), Immunology of Fungal Infection, vol. 47, Marcel Dekker, New York, 1989, pp. 347–371.

[25] J.M. Papadimitriou, R.B. Ashman, The pathogenesis of acute systemic candidiasis in a susceptible inbred mouse strain, J. Pathol. 150 (1986) 257–265.

[26] K. Shimizu, Y. Kondoh, K. Tanaka, Proteinase production and pathogenicity of *Candida albicans*. I. Invasion into chorioallantoic membrane by *C. albicans* strains of different proteinase activity, Microbiol. Immunol. 31 (1987) 1045–1060.

[27] Y. Kondoh, K. Shimizu, K. Tanaka, Proteinase production and pathogenicity of *Candida albicans*. II. Virulence for mice of *C. albicans* strains of different proteinase activity, Microbiol. Immunol. 31 (1987) 1061–1069.

[28] J.R. Naglik, C.A. Rodgers, P.J. Shirlaw, J.L. Dobbie, L.L. Fernandes-Naglik, D. Greenspan, N. Agabian, S.J. Challacombe, Differential expression of *Candida albicans* secreted aspartyl proteinase and phospholipase B genes in humans correlates with active oral and vaginal infections, J. Infect. Dis. 188 (2003) 469–479.

[29] T. Kanbe, R.K. Li, E. Wadsworth, R.A. Calderone, J.E. Cutler, Evidence for expression of the C3d receptor of *Candida albicans* in vitro and in vivo obtained by immunofluorescence and immunoelectron microscopy, Infect. Immun. 59 (1991) 1832–1838.

[30] D. Brassart, A. Woltz, M. Golliard, J.R. Neeser, In vitro inhibition of adhesion of *Candida albicans* clinical isolates to human buccal epithelial cells by Fuc alpha1–2Gal beta-bearing complex carbohydrates, Infect. Immun. 59 (1991) 1605–1613.

[31] M. Ghannoum, K. Abu Elteen, Correlative relationship between proteinase production, adherence and pathogenicity of various strains of *Candida albicans*, J. Med. Vet. Mycol. 24 (1986) 407–413.

[32] K.C. Hazen, D.L. Brawner, M.H. Riesselman, M.A. Jutila, J.E. Cutler, Differential adherence of hydrophobic and hydrophilic *Candida albicans* yeast cells to mouse tissues, Infect. Immun. 59 (1991) 907–912.

[33] S.A. Klotz, R.L. Smith, A fibronectin receptor on *Candida albicans* mediates adherence of the fungus to extracellular matrix, J. Infect. Dis. 163 (1991) 604–610.

[34] N.A. Gow, A.J. Brown, F.C. Odds, Fungal morphogenesis and host invasion, Curr. Opin. Microbiol. 5 (2002) 366–371.

[35] M. Schaller, H.C. Korting, W. Schafer, J. Bastert, W. Chen, B. Hube, Secreted aspartic proteinase (Sap) activity contributes to tissue damage in a model of human oral candidosis, Mol. Microbiol. 34 (1999) 169–180.

[36] R.B. Ashman, J.M. Papadimitriou, Strain dependence of antibody-mediated protection in murine systemic candidiasis, J. Infect. Dis. 168 (1993) 511–513.

[37] A. Fulurija, R.B. Ashman, J.M. Papadimitriou, Neutrophil depletion increases susceptibility to systemic and vaginal candidiasis in mice, and reveals differences between brain and kidney in mechanisms of host resistance, Microbiology 142 (1996) 3487–3496.

[38] C.F. d'Ostiani, G. Del Sero, A. Bacci, C. Montagnoli, A. Spreca, A. Mencacci, P. Ricciardi-Castagnoli, L. Romani, Dendritic cells discriminate between yeasts and hyphae of the fungus *Candida albicans*. Implications for initiation of T helper cell immunity in vitro and in vivo, J. Exp. Med. 191 (2000) 1661–1674.

[39] A. Pitarch, R. Diez-Orejas, G. Molero, M. Pardo, M. Sanchez, C. Gil, C. Nombela, Analysis of the serologic response to systemic *Candida albicans* infection in a murine model, Proteomics 1 (2001) 550–559.

[40] R.B. Ashman, J.M. Papadimitriou, Murine candidiasis: strain dependence of host responses after immunization, Immunol. Cell Biol. 66 (1988) 231–237.

[41] C. Weis, R. Kappe, H.G. Sonntag, Western blot analysis of the immune response to *Candida albicans* antigens in 391 long-term intensive care patients, Mycoses 40 (1997) 153–157.

[42] J.P. Martinez, M.L. Gil, J.L. Lopez Ribot, W.L. Chaffin, Serologic response to cell wall mannoproteins and proteins of *Candida albicans*, Clin. Microbiol. Rev. 11 (1998) 121–141.

[43] P.M. Sundstrom, G.E. Kenny, Enzymatic release of germ tube-specific antigens from cell walls of *Candida albicans*, Infect. Immun. 49 (1985) 609–614.

[44] R.C. Matthews, J.P. Burnie, D. Howat, T. Rowland, F. Walton, Autoantibody to heat-shock protein 90 can mediate protection against systemic candidosis, Immunology 74 (1991) 20–24.

[45] P. Sundstrom, J. Jensen, E. Balish, Humoral and cellular immune responses to enolase after alimentary tract colonization or intravenous immunization with *Candida albicans*, J. Infect. Dis. 170 (1994) 390–395.