Evaluation of the intranasal challenge route in mice as a mucosal model for *Candida albicans* infection

Patricia Londoño,^{1†} Xiao M. Gao,¹ Frances Bowe,¹ William L. McPheat,² George Booth² and Gordon Dougan¹

Author for correspondence: Gordon Dougan. Tel: +44 171 5945256. Fax: +44 171 594 5255.
e-mail: g.dougan@ic.ac.uk

¹ Biochemistry Department, Imperial College of Science, Technology and Medicine, Exhibition Road, London SW7 2AY, UK

² ZENECA Pharmaceuticals, Alderley Park, Macclesfield, UK

The intranasal route was used to study *Candida albicans* infections in mice. Mice from two different inbred strains were challenged intranasally with *C. albicans* and the level of local and systemic colonization was monitored. DBA/2 mice were highly susceptible to challenge and viable *C. albicans* disseminated from the lungs to deeper tissues, including kidneys, liver and spleen within 48 h. In contrast, in BALB/c mice challenged in the same manner, *C. albicans* were retained within the lungs and cleared. Local and systemic anti-*C. albicans* immune responses were investigated. BALB/c mice exhibited higher titres of serum and mucosal anti-*C. albicans* IgA than DBA/2 mice. Splenocytes from BALB/c mice, but not from DBA/2 mice, produced detectable levels of interleukin-4 and -5 following stimulation with *C. albicans* antigens. Both DBA/2- and BALB/c-derived splenocytes produced interferon- γ and interleukin-10 in response to similar stimulation. In conclusion, the intranasal route provided a simple, non-invasive murine model for investigating *C. albicans* infection through mucosal surfaces.

Keywords: *Candida albicans*, *in vivo* animal models, immunity, fungal infection, cytokines

INTRODUCTION

Candida albicans is frequently isolated from the commensal flora of healthy humans. However, *C. albicans* is also a significant cause of opportunistic infections and has emerged in recent years as a major cause of nosocomial infections (Pfaller, 1996). *C. albicans* infection in the immunocompromised host often involves colonization of vital organs such as the brain, liver and kidneys (Dupont, 1995; Klingspor *et al.*, 1996). More common is the development of chronic mucosal candidiasis in the oropharyngeal or urogenital tracts, particularly in HIV-infected patients (Klein *et al.*, 1984; Samaranayake & Holmstrom, 1989). Experimental approaches to investigating the immunology and pathogenicity of *C. albicans* have been substantially limited by the range of small animal models available for *in vivo*

studies. The most frequently reported infection model utilizes intravenous (i.v.) challenge of inbred mice which can lead to a reproducible systemic infection with serious renal and cerebral compromise (Papadimitriou & Ashman, 1986; Romani *et al.*, 1993). Yet, clinical *C. albicans* infections most frequently develop from a mucosal site, with the gastrointestinal and urogenital tracts serving as important *C. albicans* reservoirs. In rodents *C. albicans* has a limited ability to colonize the intestinal tract and to cause systemic disease after oral challenge. Significant colonization of the gastro-intestinal mucosa only occurs in adult immunocompetent mice if prolonged antibiotic treatment is given before and after *C. albicans* oral or intragastric challenge (Cenci *et al.*, 1995; Greenfield & Joyce, 1993; Helstrom & Balish, 1979). Germ-free mice orally infected with *C. albicans* may develop localized mucosal infections in the oro-intestinal tract (Jensen *et al.*, 1996; Lacasse *et al.*, 1993). However, intestinal infections resulting from oral challenge are usually self-limiting and do not spread to other organs, except if the mice are artificially immunosuppressed or exhibit severe genetic immunodeficiencies (Jensen *et al.*, 1993, 1994; Samonis *et al.*, 1994).

[†] Present address: Peptide Therapeutics, 321 Cambridge Science Park, Cambridge, UK.

Abbreviations: ConA, Concanavalin A; HIY, heat-inactivated yeast cells; IFN, interferon; IL, interleukin; i.n., intranasal; i.v., intravenous.

Recently, the respiratory tract of mice has been explored as a mucosal challenge route using various enteric bacterial pathogens. For example, the human pathogens *Shigella flexneri* and *Shigella sonnei* are unable to infect mice when inoculated orally, but when administered to the respiratory tract they invade the murine pulmonary epithelia, evoking a pathology which shows some resemblance to the colitis which characterizes intestinal shigellosis in humans (Mallett *et al.*, 1995, 1993; van de Verg *et al.*, 1995; Voino-Yasenetsky & Voino-Yasenetsky, 1962). This model has been exploited to study the immunobiology of shigellosis and to evaluate potential vaccines (Barzu *et al.*, 1996; Noriega *et al.*, 1996; Phalipon *et al.*, 1995; Sizemore *et al.*, 1997). There is evidence from calves and pigs that the upper respiratory tract is also a susceptible site for *Salmonella* invasion. *Salmonella dublin* and *Salmonella typhimurium* can spread systemically to organs such as the liver, spleen and intestines upon challenge through the upper respiratory tract (Fedorka-Cray *et al.*, 1995; Gray *et al.*, 1995; Roof & Doitchinoff, 1995; Stabel *et al.*, 1995). *Salmonella typhi*, a highly host-restricted human intestinal pathogen, is incapable of inducing a progressive systemic disease in mice following oral inoculation. However, when delivered intranasally, *S. typhi* can elicit strong systemic and mucosal immune responses which can be used to predict the behaviour of the strain in human oral inoculation trials (Barry *et al.*, 1996; Galen *et al.*, 1997).

Challenge through the respiratory tract and intranasal (i.n.) challenge in particular, has not been explored in experimental models of *C. albicans* infection as extensively as oro-gastric challenge. Intratracheal deposition has been used to infect mice (Hurley, 1966; Nugent & Onofrio, 1983; Sawyer, 1990) and concomitant systemic spread was only observed if mice were treated with steroids after challenge (Hurley, 1966; Nugent & Onofrio, 1983). Similarly, i.n. challenge has been reported to result in systemic dissemination in mice rendered neutropenic by cyclophosphamide treatment (Fallon *et al.*, 1997). In this study we have shown that, following i.n. infection of immunocompetent adult mice, *C. albicans* can elicit an inflammatory response in the lungs and subsequently disseminate to kidneys, liver and spleen in a susceptible (DBA/2) but not in a resistant (BALB/c) mouse strain. We have analysed the anti-*C. albicans* immune responses elicited by i.n. challenge in these mice and have described the differences found in mucosal and systemic antibody and cellular responses. We propose that the model described here could be used to investigate the process of systemic *C. albicans* spread from mucosal surfaces and the role of mucosal immune responses in candidiasis.

METHODS

Growth and characterization of *C. albicans*. A well characterized, clinical serotype A isolate of *C. albicans*, named MG3, was used throughout this study (characterization carried out in Dr David Coleman's laboratory, Department of Microbiology, Trinity College, Dublin). This strain is germ-tube-

positive and chlamydospore-positive and yields the chlamydospore presentation profile typical of *C. albicans*. It yields blue-green colonies on Chromagar *Candida* medium, grows well at 30, 37 and 42 °C and its carbohydrate assimilation profile is characteristic of *C. albicans*. MG3 has DNA fingerprint characteristics typical of *C. albicans* serotype A, has the ability to germinate in the presence of bovine serum and is resistant to cycloheximide. The strain was routinely maintained in Potato Dextrose agar (PDA; Difco). Stocks of live yeast to be used for animal infections were prepared from 18-h-old cultures of *C. albicans* MG3 in Sabouraud broth (Difco) at 37 °C (cell density of at least 1×10^8 c.f.u. ml⁻¹). Yeast cells were harvested by centrifugation at 800 g for 10 min. After three washes with endotoxin-free PBS (Sigma), yeast cells were resuspended in approximately 0.2 vols endotoxin-free PBS, aliquoted, snap-frozen and stored at -70 °C. Aliquots were thawed immediately before inoculation and their concentration adjusted to 3×10^8 – 4×10^8 c.f.u. ml⁻¹. To confirm stock viability and enumerate viable yeast cells, aliquots of different dilutions of thawed stocks were plated on PDA. Colonies were enumerated (c.f.u.) after 24–36 h incubation at 37 °C. No significant decrease in the viability of stocks was observed over storage periods longer than 6 months.

i.n. and i.v. infection of mice and enumeration of viable *C. albicans* in mouse organs. BALB/c and DBA/2 mice were purchased from Harlan. Mice of the same sex and age (8–12 weeks old) were used for all the experiments. For i.n. infections, mice were anaesthetized with halothane and, unless otherwise stated, 30 µl PBS containing 1×10^7 live yeast cells was very slowly dropped over their nares using a Gilson Pipetman. For i.v. infections, 100 µl PBS containing 10^8 – 10^9 yeast cells were injected into a tail vein. The animals were maintained under observation and those showing symptoms of distress were sacrificed by cervical dislocation, a standard method for humane culling of small rodents listed in the UK Animals (Scientific Procedures) Act 1986. To follow the course of infection, groups of four challenged mice were sacrificed on different days after infection. For enumeration of *C. albicans* in mouse organs, lungs, liver, spleen and kidneys were removed and homogenized using a glass homogenizer (lungs) or a Stomacher (other organs). Viable *C. albicans* were counted by plating different dilutions of the homogenates on PDA, as described above. For assessment of LD₅₀, groups of 6 mice were infected either intranasally or intravenously with maximum doses of 3×10^7 or 1×10^6 live yeast cells, respectively. Mice were kept under observation for 45 (i.n. challenge) or 52 (i.v. challenge) d and deaths were recorded on a daily basis. The Reed–Muench method was used to calculate LD₅₀ (Welkos & O'Brien, 1994).

Histology. Lungs from mice infected 4 h previously with 1×10^7 yeast cells were fixed in 10% (v/v) neutral buffered formalin, embedded in paraffin wax and sectioned. Sections were either stained with hematoxylin and eosin or immunostained using the Vectastain ABC Reagent (Anti-Rabbit IgG Kit; Vector Laboratories). The primary antiserum used was a commercial rabbit polyclonal antiserum against *C. albicans* diluted 1/400 (Dako). Endogenous peroxidase was blocked for 3 min with 0.3% H₂O₂ in methanol. Counter staining was performed with Mayer's Haemalum.

Cell proliferation and cytokine assays. Naive or *C. albicans*-infected mice were splenectomized on different days after challenge. Single cell suspensions from pooled spleens of 3–4 mice per group were prepared in DMEM supplemented with 5% (v/v) heat-inactivated foetal bovine serum, 2 mM L-

glutamine, 50 mM 2-mercaptoethanol and 10 mM HEPES (all from Sigma). Aliquots (200 µl) of 4×10^5 splenocytes were stimulated with different numbers of heat-inactivated yeast cells (HIY), killed by incubation at 60 °C for 2 h. Controls included cells stimulated with either medium or Concanavalin A (ConA; Sigma). Cellular proliferation was measured by incorporation of [³H]thymidine (Amersham) during the last 18–20 h of 96-h-old cultures. For cytokine analysis, 1 ml aliquots of 2×10^6 splenocytes were cultured with HIY (1.2×10^6 yeast cells ml⁻¹), medium only or ConA (2.5 µg ml⁻¹). Supernatants from quadruplicate samples were collected at various times after stimulation and stored at -70 °C. For quantification of interferon-γ (IFNγ), interleukin-10 (IL10), IL4 and IL5 in culture supernatants, commercial ELISA systems were used (Pharmingen). Limits of detection for cytokine ELISA were: 62.5 pg IL4 or IL5 ml⁻¹, 125 pg IL10 ml⁻¹ and 312 pg IFNγ ml⁻¹.

Antibody responses. Sera from groups of 3–4 mice, obtained on different days of *C. albicans* infection, were pooled for titration. Levels of specific anti-*C. albicans* antibodies were measured by ELISA. Nunc maxisorb plates were coated with a sonicate extract of live *C. albicans* MG3 yeast cells (30 µg ml⁻¹ in sodium carbonate buffer, pH 9.6). Serum samples were used in duplicates from a 1:50 dilution. Biotinylated anti-mouse IgG, IgA (Sigma) or IgG1, IgG2a, IgG2b or IgG3 (Pharmingen) were used as secondary antibodies. These were detected with a streptavidin-horseradish peroxidase conjugate (Dako). Titres were defined as the lowest dilution of sera to give an A_{492} above the mean A_{492} of naive sera + 2 × SEM.

Specific anti-*C. albicans* IgA was measured in lung lavages from groups of 4–6 mice obtained as described previously (Douce *et al.*, 1995). To compare the levels of specific anti-*C. albicans* IgA in the lavages, titres were expressed as ELISA units (EU) (µg total IgA)⁻¹, thus preventing errors due to differences in total content of IgA. The total content of IgA present on pooled lavage samples was assessed in a sandwich ELISA using plates coated with anti-mouse IgA antibody as described by Elson *et al.* (1984). The content of specific anti-*C. albicans* IgA on lavages was measured using the same ELISA as for serum antibodies. The number of EU present on the lavages was established by comparison to a reference mouse serum that contained a high titre of anti-*C. albicans* IgA antibodies, to which an arbitrary concentration (EU ml⁻¹) was assigned.

RESULTS

Susceptibility of BALB/c and DBA/2 mice to *C. albicans* MG3 i.n. challenge

C. albicans MG3 challenge through either the i.v. or i.n. routes showed that BALB/c mice were more resistant to *C. albicans* MG3 infection than DBA/2 mice (Table 1). The i.v. data, included as control, agree with previously published observations (Ashman *et al.*, 1993; Hector *et al.*, 1982; Romani *et al.*, 1993). All DBA/2 mice intravenously challenged with a dose of 10^5 yeast cells succumbed to the infection, whereas more than 50% of the BALB/c mice survived a similar challenge. The i.v. LD₅₀ for BALB/c mice was at least four times greater than for DBA/2 mice (Table 1). Intranasally challenged BALB/c mice could survive a dose of 3×10^7 yeast cells, whereas more than half of the DBA/2 mice succumbed to the same challenge. The i.n. LD₅₀ for BALB/c mice was at least two times higher than for DBA/2 mice

Table 1. Survival of BALB/c and DBA/2 mice upon *C. albicans* challenge

The table shows the number of surviving mice at the end of the observation period/total number of mice challenged. Mice were kept under observation for 45 (i.n. challenge) or 52 (i.v. challenge) d.

Dose*	BALB/c	DBA/2
i.v. challenge		
1×10^5	4/6	0/6
1×10^4	5/6	5/6
1×10^3	6/6	6/6
LD ₅₀	$> 1.3 \times 10^5$	2.5×10^4
i.n. challenge		
3×10^7	6/6	2/6
1×10^7	6/6	6/6
3×10^6	6/6	6/6
LD ₅₀	$> 5.1 \times 10^7$	2.3×10^7

* *C. albicans* MG3 c.f.u. per mouse.

(Table 1). Examination of dead DBA/2 mice after i.n. challenge showed heavy *C. albicans* MG3 loads in the kidneys, comparable to those observed after i.v. infection (data not shown). BALB/c mice that died following i.v. challenge also showed heavy *C. albicans* loads on their kidneys ($> 1 \times 10^4$ c.f.u. per organ).

Kinetics of infection after i.n. challenge

Following i.n. challenge with 1×10^7 live yeast cells, viable *C. albicans* MG3 were recovered from the lungs of BALB/c and DBA/2 mice at different time points (Fig. 1). The course of lung infection was very similar in both mouse strains during the first 48 h after challenge. However, by day 5 the *C. albicans* load in the lungs of DBA/2 mice was significantly higher (approx. 50-fold higher) than that of BALB/c mice ($P < 0.05$, as calculated by Student's *t* test). By day 7, no significant difference was observed in the number of *C. albicans* recovered from the lungs of both strains of mice.

BALB/c mice were not only able to clear *C. albicans* from their lungs more rapidly, but they could also avoid significant *C. albicans* spread to other organs such as kidneys, liver (Table 2, Fig. 1) and spleen (Table 2). In contrast, the majority of DBA/2 mice exhibited significant colonization of one or more of these organs (Table 2). *C. albicans* colonization of the kidneys and liver peaked between 2 and 5 d after challenge (Fig. 1). A positive correlation was found between the *C. albicans* load in the lungs and that of kidneys or liver (data not shown).

Histopathology of *C. albicans* infection in the lung

The lungs of mice were examined after i.n. challenge to determine the early fate of *C. albicans* MG3 cells within host tissues. At 4 h after challenge there were detectable

Fig. 1. Course of *C. albicans* infection in the lungs (a), kidneys (b) and liver (c) of intranasally challenged BALB/c (□) and DBA/2 (○) mice. Mice were challenged with 1×10^7 live yeast cells. The results shown correspond to the mean $\pm$ SEM of two experiments. For each experiment, 4 animals were sacrificed per time point. Counts shown for day 0 were obtained 4 h after challenge. Limits of detection were 20 c.f.u. per organ in lungs and 50 c.f.u. per organ in liver and kidneys.

Table 2. Systemic dissemination of *C. albicans* after i.n. challenge

The table shows the number of organs from which viable *C. albicans* were recovered/total number of organs examined. The limit of detection was 50 c.f.u. per organ. Results shown were obtained with groups of 8 or 9 mice per strain sacrificed 2 or 5 d after challenge.

Mouse strain	Kidneys	Liver	Spleen	Total*
BALB/c	1/16	3/16	1/16	3/16
DBA/2	7/17	9/17	7/17	10/17

* Number of mice from which viable yeast cells were recovered from one or more of the organs shown per total number of mice examined.

C. albicans MG3 adjacent to the respiratory bronchioles in the lungs of both BALB/c and DBA/2 mice. No discernible differences in the histology of lungs prepared from *C. albicans* MG3-infected DBA/2 or BALB/c mice were found at this stage of the infection. *C. albicans* MG3 in hyphal and yeast forms were visible in airways and also in surrounding tissue by immunostaining with a *C. albicans*-specific rabbit serum and hematoxylin and eosin. An inflammatory infiltrate, composed of polymorphonuclear lymphocytes and mononuclear cells, was also detectable in sections from both mouse strains (data not shown).

Cellular response against *C. albicans* MG3 upon i.n. challenge

Anti-*C. albicans* cellular responses, as determined in splenocyte proliferation assays, were detected in both DBA/2 and BALB/c mice from 7 d after i.n. challenge. The response peaked on day 7 in BALB/c mice and on

day 14 in DBA/2 mice and remained detectable for at least 50 d after challenge in both strains of mice (data not shown). The profiles of cytokine secretion by the proliferating splenocytes showed differences (Fig. 2). While both splenocyte populations produced IFN γ and IL10, production of IL4 and IL5 was only detected in BALB/c, but not DBA/2, splenocytes. Similar results were obtained with splenocytes harvested 7, 14 (Fig. 2) or 21 d (data not shown) after challenge. As expected, all the cytokines measured were secreted by DBA/2 and BALB/c splenocyte populations in response to ConA stimulation (data not shown).

Antibody response to *C. albicans* MG3

Humoral responses against *C. albicans* MG3 were detected in both BALB/c and DBA/2 mice following *C. albicans* MG3 i.n. challenge. A significant anti-*C. albicans* IgG response appeared earlier in DBA/2 mice (day 7) than in BALB/c mice (day 14) (Fig. 3). The antibody response did not significantly change over a period of 90 d (data not shown). IgG1, IgG2a, IgG2b and IgG3 antibody subclasses contributed to the anti-*C. albicans* response in BALB/c mice, although IgG2a appeared to be slightly dominant. In contrast, in DBA/2 mice, IgG2b was clearly the predominant IgG subclass and anti-*C. albicans* IgG1 was not detected.

Following i.n. challenge, BALB/c, but not DBA/2 mice exhibited a systemic IgA response against *C. albicans* MG3 (Fig. 3). The titre of the response in BALB/c mice was higher on day 14 than on day 7. The local production of IgA paralleled the systemic response (Table 3). Specific anti-*C. albicans* IgA was detected in lung lavages of BALB/c, but not DBA/2 mice after primary i.n. infection. However, this pattern of local IgA production changed after a secondary i.n. infection with *C. albicans* MG3 as anti-*C. albicans* IgA was found in lung lavages of both strains of mice (Table 3).

Fig. 2. Cytokine production by splenocytes from *C. albicans*-infected mice. Splenocytes were obtained from mice before and 7 or 14 d after i.n. challenge with 1×10^7 live yeast cells. Concentrations of IL4, IL5, IL10 and IFN γ shown were measured in supernatants of splenocytes stimulated with 1.2×10^6 HIY for 72 (IL10 and IFN γ) or 96 (IL4 and IL5) h. Experiments were performed two (BALB/c mice) or three (DBA/2 mice) times with similar results. Splenocytes cultured in medium only did not produce detectable amounts of any of the cytokines measured.

DISCUSSION

In this study we have shown that i.n. administration of *C. albicans* to adult immunocompetent mice may be used as a model to study the pathogenesis and immunobiology of *C. albicans* infection. Both local and systemic immune responses can be evaluated after i.n. challenge, thus potentially allowing an analysis of the contribution of both of these arms of the immune response to protection against *C. albicans* infection originated from mucosal surfaces. DBA/2 mice (H-2^d) have been shown previously to be more susceptible to i.v. *C. albicans* challenge than BALB/c mice (also H-2^d) (Ashman *et al.*, 1993; Hector *et al.*, 1982; Romani *et al.*, 1993). This pattern of susceptibility was also apparent following i.n. challenge. BALB/c mice survived i.n. challenge with 3×10^6 – 3×10^7 live yeast cells, while DBA/2 mice succumbed to a similar challenge in a dose-dependent manner. Interestingly, the *C. albicans* loads observed in the kidneys of dead DBA/2 mice after i.n. challenge resembled those detected in mice that succumbed to lethal i.v. challenge ($>1 \times 10^4$ c.f.u. per organ). In addition, DBA/2, but not BALB/c mice, also succumbed to i.n. challenge with four other *C. albicans* isolates, although *C. albicans* MG3, the isolate used in this study, exhibited the highest relative virulence (data not shown).

The course of *C. albicans* infection in intranasally challenged BALB/c and DBA/2 mice was also considerably different. In BALB/c mice *C. albicans* were cleared steadily from the lungs with no significant systemic spreading of the infection. In DBA/2 mice there was a delay in the initiation of *C. albicans* clearance from the lung and systemic *C. albicans* colonization was

evident (Fig. 1 and Table 2). Significant numbers of live *C. albicans* were recovered from the kidneys, spleen and liver of DBA/2 mice (Table 2). In particular, after i.n. challenge, only DBA/2 mice exhibited *C. albicans* loads in their kidneys at levels comparable to those observed after i.v. challenge (data not shown).

In this study a significant infiltrate of both mononuclear cells and neutrophils was observed in the lungs of both BALB/c and DBA/2 mice after *C. albicans* i.n. challenge (data not shown). Nugent & Onofrio (1983) have reported that in Swiss Webster mice, pulmonary macrophages, together with infiltrating neutrophils, provide the first line of protection against intratracheal challenge with live *C. albicans* yeast cells. More recently, Fallon *et al.* (1997) found that neutropenic Swiss Webster mice are highly susceptible to *C. albicans* i.n. challenge. Infiltration and accumulation of inflammatory cells, especially mononuclear cells and neutrophils, have also been observed in the gastro-intestinal mucosa of gastrically challenged BALB/c and DBA/2 mice rendered susceptible to transient *C. albicans* colonization by prolonged antibiotic treatment. However, in this case both BALB/c and DBA/2 mice eventually cleared the local gastro-intestinal infection in the absence of systemic colonization (Bistoni *et al.*, 1993; Cenci *et al.*, 1995). Although comparable early inflammatory responses were elicited by i.n. *C. albicans* challenge in the lungs of BALB/c and DBA/2 mice, differences were observed between these murine strains in the rate of *C. albicans* clearance from the lungs after 48 h of challenge. In DBA/2 mice the delay in *C. albicans* clearance could have resulted from either the temporary establishment of a balance between *C. albicans* killing and *C. albicans*

Fig. 3. Serum antibody levels against *C. albicans* in BALB/c (a) and DBA/2 (b) mice challenged intranasally with live yeast cells. Antibody titres were determined in sera obtained 7 (■) and 14 (□) d after challenge, as described in Methods. Values shown represent the titre of pooled sera from 3–4 mice per group. Titres beneath 1/50 were below detection limits of the assay. SEM was below 5%.

growth or from the discrete activation of a candidastatic rather than candidacidal immune mechanism. This mechanism was probably systemic rather than local to the lungs since colonization occurred in various organs simultaneously. DBA/2 mice are known to lack the complement molecule C5. Although this deficiency has been eliminated as a factor associated with their increased susceptibility to acute i.v. challenge (Sawyer, 1990; Ashman *et al.*, 1991), it may still play a role during mucosal infections.

Resistance to i.v. *C. albicans* challenge in BALB/c mice is accompanied by a Th1 cellular response, while susceptibility of DBA/2 mice is associated with development of a Th2 response (Romani *et al.*, 1995).

Table 3. Specific anti-*C. albicans* IgA response in lung lavages of intranasally challenged mice

Lung lavages were carried out in groups of 4–6 mice sacrificed 15 d after primary or 21 d after secondary i.n. *C. albicans* challenge. Values shown were calculated as described in Methods and represent the titre of pooled lavages obtained from each particular group of mice. SEM was below 5%. ND, Not detected.

Infection	Titre [EU ($\mu\text{g total IgA}$) ⁻¹]	
	BALB/c	DBA/2
Primary	1.67	ND
Secondary	9.35	18.91

Intragastrically challenged DBA/2 and BALB/c mice develop Th1 and a combination of Th1 and Th2 anti-*C. albicans* responses, respectively (Bistoni *et al.*, 1993; Cenci *et al.*, 1995). Challenge of mice by topical application of *C. albicans* in the oral mucosa generates detectable T-cell responses in DBA/2 but not BALB/c mice (Chakir *et al.*, 1994). In this study significant differences were found in the pattern of cytokine expression by *C. albicans*-activated splenocytes from intranasally infected BALB/c and DBA/2 mice. The cellular responses observed resemble those reported in the same mice upon intragastric *C. albicans* challenge (Bistoni *et al.*, 1993; Cenci *et al.*, 1995). As a result of i.n. challenge, *C. albicans*-dependent IL4 and IL5 secretion was only detected in BALB/c splenocyte cultures, while *C. albicans*-dependent IL10 and IFN γ secretion was detected in both BALB/c and DBA/2 splenocyte populations. The differences in cytokine response correlated with the anti-*C. albicans* antibody response of these mice. The presence of specific mucosal and serum IgA as well as IgG1 responses in BALB/c, but not in DBA/2 mice could be attributed to the synthesis of IL5 and IL4 by BALB/c-, but not DBA/2-activated lymphocytes. Furthermore, the production of IgG3 and IgG2a in both mouse strains may be linked to the presence of comparable IFN γ responses (Abbas *et al.*, 1996). The animal model of i.n. *C. albicans* infection described here could be explored further to determine the effect of mucosal antibody responses during *C. albicans* infection. Significantly, in this study resistant BALB/c, but not susceptible DBA/2 mice exhibited detectable levels of local mucosal and also humoral IgA against *C. albicans* after primary i.n. challenge. Interestingly, DBA/2 mice also exhibit defective production of IgA after intragastric, but not i.v. *C. albicans* challenge (Bistoni *et al.*, 1993; Cenci *et al.*, 1995).

In summary, the data presented here indicate the following. (i) Lethal systemic candidiasis may be induced in healthy adult mice by i.n. *C. albicans* challenge with no requirement for artificial immunosuppression or antibiotic treatment. (ii) i.n. *C. albicans* challenge results in transient lung colonization of both DBA/2 and

BALB/c mice; however, only in DBA/2 mice is this accompanied by systemic spreading of the organism. (iii) i.n. *C. albicans* challenge elicits cellular immune responses in DBA/2 and BALB/c mice which resemble their respective responses to intragastric rather than i.v. challenge. (iv) After primary i.n. *C. albicans* infection, systemic and local anti-*C. albicans* IgA responses are detected in mice that rapidly clear the organism from their lungs (BALB/c), but not in those in which systemic spreading occurs (DBA/2).

The i.n. model of *C. albicans* infection is not based on clinical observations. Pulmonary candidiasis is a very rare occurrence in clinical practice. In humans systemic *C. albicans* infections are believed to originate in the intestinal rather than the respiratory tract. Nevertheless, the i.n. challenge model mirrors the early stage of this process, i.e. dissemination of the yeast from a mucosal surface. This process is by-passed by the most commonly used i.v. challenge model and cannot be reproduced in normal adult mice by oral challenge. The advantages of using an i.n. model of *C. albicans* infection have been highlighted recently (Fallon *et al.*, 1997). This model will be very valuable in studying the generation of systemic, and especially mucosal, immune responses during *C. albicans* colonization of mucosal surfaces as well as their contribution to prevention of systemic dissemination of infection.

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