Increased tissue resistance in the nude mouse against *Candida albicans* without altering strain-dependent differences in susceptibility

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Strain differences in tissue responses to infection with *Candida albicans* were examined in nude mice having susceptible (CBA/CaH) and resistant (BALB/c) parentage. Homozygous (nu/nu) mice of both strains were more resistant to systemic infection with *C. albicans* than heterozygous (nu/+ ) littermates as indicated by a reduction in both the severity of tissue damage and colony counts in the brain and kidney. However, the tissue lesions in nu/nu CBA/CaH mice were markedly more severe than those in nu/nu mice with the BALB/c background. This pattern was reflected in the greater fungal burden in the CBA/CaH strain. Analysis of cDNA from infected tissues using a competitive polymerase chain reaction excluded interferon-γ (IFN-γ), tumour necrosis factor-a (TNF-a), and interleukin 6 (IL-6) as mediators of the enhanced resistance of the nude mice. The results confirm that the different patterns of lesion severity in BALB/c and CBA/CaH mice do not involve T lymphocyte-mediated pathology, and are consistent with the hypothesis that strain-dependent tissue damage is not dependent on the effector function of macrophages or their precursors.

**Keywords** *Candida albicans*, nude mice, resistance, susceptibility

**Introduction**

The yeast *Candida albicans* is an important opportunistic pathogen. Systemic candidiasis in inbred mice closely resembles human disease in that the lesions are similar in nature and distribution [1], and the brain and kidney are major targets for infection. However, there are significant differences among inbred strains in mortality [2,3], colony counts in infected organs [4,5], and the severity of tissue lesions [6]. These last two variables, which reflect different aspects of the host response, are subject to regulation by at least two different genes [7].

In experimental systemic infection, the severity of tissue damage is regulated by a single gene that segregates in Mendelian fashion [8], and is linked to the function of bone marrow-derived cells. These observations suggested that strain-dependent differences in the severity of tissue damage might be related to differences in production or function of inflammatory cells in the different inbred strains, although an immunopathological effect of *Candida*-specific T lymphocytes was not definitively excluded. The phagocytic and candidacidal activities of macrophages and monocytes are important mechanisms of host resistance to *C. albicans*, and there is evidence that both their functional responses to the yeast [9] and their protective effects [10] vary between inbred strains. The candidacidal activity of phagocytic cells can be regulated by T cell-derived cytokines [11], and a dominance of either the Th1 or Th2 subset has been linked to the resistance or susceptibility of different mouse strains [12].

Nude mice have minimal T lymphocyte function, but display enhanced early resistance to infection with *C. albicans* [13–15] and other bacterial pathogens [16]. The mechanisms mediating this increased resistance have not been defined, but the monocyte/macrophage system in these mice is activated [17,18], and phagocytosis by blood leucocytes (both monocytes and neutrophils) is increased [19]. The hypothesis that strain-specific differences in lesion severity were related to variations in the non-specific inflammatory response was tested by comparing...
the severity of infection in nude mice having CBA/CaH and BALB/c backgrounds. Euthymic mice of these two strains develop severe and mild lesions, respectively [6].

Materials and methods

Mice

BALB/c and CBA/CaH mice homozygous for the mu/mu mutation and heterozygous controls were purchased from the Animal Resources Centre, Perth, and the Walter and Eliza Hall Institute, Melbourne, respectively. Only female mice, 6–8 weeks of age, were used in experiments. All animals were housed and used in accordance with the NH&MRC/CSIRO/Australian Agricultural Council's Code of Practice for the Care and Use of Animals for Experimental Purposes in Australia, 1985. The experimental protocols were approved by the University of Western Australia Animal Ethics Committee, and in accordance with its guidelines, the minimum number of mice necessary to obtain statistically valid results have been used.

Yeast

Candida albicans isolate 3630 was obtained from the Mycology Reference Laboratory at the Royal North Shore Hospital, Sydney. The strain is kept in small aliquots at −70 °C in 15% glycerol in Sabouraud glucose broth. Prior to use, a loop of the frozen stock was inoculated into Sabouraud glucose broth, and grown for 2 days at 25 °C with constant agitation. Blastocoenidia were washed in phosphate buffered saline, and adjusted to the appropriate concentration for use.

Histology

Mice were sacrificed at 1 and 4 days after intravenous infection with $3 \times 10^5$ C. albicans blastocoenidia, and brains and kidneys removed for histological examination and quantitative culture. A portion of each organ was fixed in formalin, sectioned and stained with either haematoxylin and eosin (H&E), or periodic acid-Schiff (PAS). Random sections were coded and examined blind, and re-evaluated when the code had been broken.

Quantitation

Tissue samples were weighed and homogenized in PBS using an Ultra Turrax T-25 homogenizer (IKA Labortechnik, Staufen, Germany) at 13 500 rev min⁻¹ at 25 °C. The samples were diluted appropriately, and 100 μl aliquots plated on Sabouraud glucose agar containing chloramphenicol. The plates were incubated at 37 °C for 2 days, and the colonies counted. Each assay was performed in duplicate, and the results were expressed as colony-forming units per gram of tissue. The data from two experiments were pooled, giving a minimum of seven mice for each timepoint. The experiments were analysed using Student’s t-test. Differences were considered significant at a level of $P < 0.05$.

RNA preparation and reverse transcription

Total cellular RNA was prepared from infected brains and kidneys using Ultraspec (Biotec Laboratories, Houston, TX) according to the manufacturer’s protocol. The concentration and purity of the RNA samples were determined by spectrophotometry at 260 and 280 nm. Two micrograms of each sample was then reverse transcribed using an oligo d(T)₁₅ primer and AMV reverse transcriptase according to the manufacturer’s instructions (Promega Corporation, Madison, WI). Briefly, 5 mM MgCl₂, 1 × reverse transcription buffer, 1 mM each dNTP, 0.5 units RNasin, 15 units AMV reverse transcriptase and 0.5 μg oligo (dT)₁₅ primer were incubated in a 20 μl reaction mix at 42 °C for 1 h, heated to 99 °C for 5 min, then cooled on ice. The cDNA was stored at −20 °C until used.

Primer selection and synthesis

Oligonucleotide primer sequences were obtained from published data [20], and synthesized at the Australian Neuromuscular Research Institute, Perth, Western Australia. For each primer pair, optimum conditions for amplification were established in preliminary experiments. Plasmid MCQ (pMCQ) was generously donated by Dr Cornelia Platzer, Humboldt University, Berlin, Germany. This plasmid (control fragment) contains the primer sequences for interleukins 1-6, interferon-γ (IFN-γ), tumour necrosis factor (TNF), lymphotoxin, and β-actin, arranged so that the products amplified by cytokine-specific primers differ in size from those amplified from the target DNA, and thus can be distinguished by electrophoresis through an agarose gel [21].

Polymerase chain reaction (PCR)

The amplification mix consisted of 50 ng cDNA, 200 μM dNTPs, 0.5 unit Taq polymerase (Biotech International, Perth, Western Australia), 1 × reaction buffer, either 1 mM or 2 mM MgCl₂ and either 1 μl or 2 μl primers in a total volume of 25 μl. The mixture was overlaid with paraffin oil, then amplified using a PTC-100 thermal cycler (MJ Research, Inc.). For each sample, controls included both undigested and digested RNA, as well as the appropriate cDNA. Each amplification included
pMCQ as a positive control, and a negative control containing all the reagents except the sample. The PCR amplification protocol was identical to that of Platzer et al. [21] (94 °C, 1 min; 60 °C, 2 min; 72 °C, 2 min; for 35–40 cycles). Following amplification, 10 μl of product was analysed by electrophoresis through 3% agarose gels. The gels were stained with ethidium bromide and the bands visualised using a UV transilluminator.

Comparison of cytokine mRNA concentrations

Samples showing reactivity with cytokine primers were subjected to semi-quantitative analysis as previously described [21]. Briefly, the cDNA was adjusted to equal concentrations by co-amplification of a fixed amount of the cDNA with tenfold, and then twofold, dilutions of the control fragment (pMCQ), using primers for β-actin. For quantitation and comparison of cytokine mRNA expression, equal amounts of cDNA were amplified in the presence of tenfold, and then threefold dilutions of the control fragment. The linearized plasmid was used at dilutions ranging from $10^{-2}$ to $10^{-4}$ for the standardization of the cDNA, and from $10^{-3}$ to $10^{-9}$ for comparison of cytokine concentrations. The experiments were repeated three times using mRNA from three different mice. The endpoints for the nude mice, expressed in logarithms, were subtracted from those for the normals, and the resultant gave a factor by which the cDNA concentrations in the nude mice were greater or less than those in the controls. A positive value indicated that the concentrations in the nude mice were greater than in the normals, and vice versa. The hypothesis that the cDNA concentrations in normal and nude mice were not significantly different was tested by determining whether the 95% confidence intervals included zero.

Results

Lesions were detectable in both nude mice and controls on day 1 after systemic infection with $3 \times 10^5$ C. albicans blastoconidia. By the fourth day of infection tissue
damage was pronounced, and comparisons were most easily made at that time point. Detailed histological descriptions of the course of systemic infection in mice have been reported elsewhere [1]. In brief, brain lesions consisted of focal areas of necrosis, which were surrounded by polymorphonuclear leucocytes and macrophages. They were seen somewhat more frequently in grey matter than in white. When stained using the PAS technique, both yeast cells and mycelium were demonstrated within the necrotic foci.

Similar lesions were present in both the cortex and the medulla of the kidney, and tubular atrophy was a consistent finding. Kidney failure associated with acute pyelonephritis is the most probable cause of death after systemic infection [7,22]. As described previously [6], abscesses were both more numerous and more severe in CBA/CaH mice than in BALB/c; however, nude mice of both strains showed a marked reduction in tissue destruction. This was most obvious in the brain (Fig. 1), but was also clearly evident in the kidney (Fig. 2). Furthermore, significantly fewer viable yeasts were recovered from the brains and kidneys of the nude mice when compared with the appropriate controls (Fig. 3).

To determine whether the protective effect observed in the nude mice was associated with changes in the relative concentrations of cytokines known to enhance the candidacidal activity of phagocytic cells, cDNA from the brains and kidneys of three different infected and control nu/nu and nu/+ mice were compared using competitive PCR (Fig. 4). Cytokines recognized to be important in host responses against C. albicans, and that can be produced independently of T lymphocytes, are IFN-γ [23], TNF-α [24,25], and IL-6 [24,26]. The log10 means and 95% confidence intervals of the relative cytokine concentrations were calculated for each experimental group.

The results for BALB/c brain were: IFN-γ, −0.36 ≤ 0.58 ≤ 1.52; IL-6, −0.62 ≤ 0.25 ≤ 1.12; TNF-α, −0.32 ≤ 0.13 ≤ 0.58; for BALB/c kidney: IFN-γ, −1.24 ≤ 0.1 ≤ 1.24; IL-6, −0.86 ≤ 0.08 ≤ 1.02; TNF-α, −0.89 ≤ 0.17 ≤ 0.55; for CBA/CaH brain: IFN-γ, −0.55 ≤ 0.17 ≤ 0.89; IL-6, −1.65 ≤ 0.08 ≤ 1.49; TNF-α, 0 ≤ 0 ≤ 0; and for CBA/CaH kidney: IFN-γ,
Candidiasis in nude mice

Fig. 3 Enumeration of C. albicans in the brains and kidneys of BALB/c and CBA/CaH nu/nu and nu/+ mice, 4 days after intravenous challenge with $3 \times 10^5$ yeast cells. Each bar represents the mean ± SE of duplicate assays from seven mice. The fungal burden in both brains and kidneys of the homozygous nude mice was significantly less than in the heterozygotes ($P < 0.01$).

Discussion

Nude mice having BALB/c and CBA/CaH backgrounds were more resistant to systemic infection by C. albicans than were heterozygous littermates; however, the tissue lesions in the CBA/CaH nu/nu mice were markedly more severe than those in nude mice bred on the BALB/c background. The protective effect of the nude mutation, as indicated by the amelioration of tissue destruction and reduction in colony counts in the infected tissues, was strikingly similar in the two strains.

Nude mice demonstrate enhanced resistance against C. albicans [13–15], as well as other bacterial pathogens [16], and the protective effects are associated with the presence of activated macrophages in these animals [17,18]. However, treatment of nude mice with silica, which selectively alters macrophage functions, did not impair their resistance to systemic challenge [27], whereas comparable treatment of euthymic mice rendered them significantly more resistant to infection. This latter observation led the authors to conclude that silica might induce cytokines that either increased T cell-mediated augmentation of the candidacidal activity of another effector population, or blocked T cell-mediated suppression of these cells. A further possibility is that, in nude mice, the production of bone marrow-derived candidacidal effector cells is increased. Administration of silica to euthymic mice might cause a similar increase, resulting in enhanced resistance to challenge.

Although phagocytic cells express some spontaneous effector activity against C. albicans, the potency of both mononuclear and polymorphonuclear phagocytes is enhanced and regulated by cytokines such as IFN-γ, TNF-α, and IL-6 (reviewed in ref. [11]). However, when cytokine cDNA levels were compared in nu/nu and nu/+ mice, no significant differences could be demonstrated. It is possible that the effector cells mediating the enhanced resistance of nude mice are responding to cytokine activation signals different from those measured; alternatively, as suggested above, the protection seen in these
mice might be associated with a quantitative increase in the production of candididal effector cells by the bone marrow. Immature cells of the monocyte/macrophage lineage have been found to possess strong candidial activity, as well as the ability to lyse an NK-sensitive, but not NK-resistant, cell line [28], so that these precursors may include a cell type that confers protection on the nude mouse. Baccarini et al. [29] have speculated that at least a part of the NK cell compartment might be derived because the enhanced resistance of nude mice to infection independent of the function of T lymphocytes. Second, no evidence that natural killer cells mediate resistance grounds, confirming that the severity of lesions is warranted. First, the strain-specific differences in tissue were reproduced in nude mice bred on the same backlineage have been found to possess strong candidacidal activity, as well as the ability to lyse an NK-sensitive, but

References

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