Special Feature

Innate versus adaptive immunity in *Candida albicans* infection

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**Summary** *Candida albicans* is a common opportunistic pathogen, causing both superficial and systemic infection. Clinical observations indicate that mucocutaneous infections are commonly associated with defective cell-mediated immune responses, whereas systemic infection is more frequently seen in patients with deficiencies in neutrophil number or function. Analysis of mechanisms of host resistance against gastrointestinal and oral infection in mouse models has demonstrated an absolute dependence on CD4+ T cells, although clearance also involves phagocytic cells. Both IL-12 and TNF-α appear to be important mediators, but mouse strain-dependent variations in susceptibility to infection may be related to T-cell enhancement of production of phagocytic cells by the bone marrow. In murine systemic infection, the role of innate and adaptive responses is less well defined. Studies in immunodeficient and T-cell-depleted mice suggest that clearance of the yeast may be predominantly a function of the innate response, whereas the adaptive response may either limit tissue damage or have the potential to cause immunopathology, depending on the host genetic context in which the infection takes place.

**Key words:** cytokines, oral candidiasis, phagocytic cells, systemic candidiasis, T cells.

**Introduction**

The yeast *Candida albicans* is a widespread opportunistic pathogen. Mucocutaneous infections, that manifest as either oral or vaginal ‘thrush’, are commonly encountered in medical and dental practice; and the incidence of systemic candidiasis has risen dramatically over the past decades in parallel with the increasing sophistication of medical technology and widespread use of aggressive therapeutic regimens for transplantation and cancer patients.

Despite the prevalence of *Candida* in the hospital environment – it is now the fourth most common nosocomial infection – the pathways involved in clearance of mucocutaneous and systemic infections with the organism have not been fully delineated. Patients with congenital or acquired defects in cell-mediated immunity are particularly susceptible to mucocutaneous, but not to disseminated candidiasis whereas systemic infections are more commonly associated with neutropenia or congenital defects that affect neutrophil function. Although disseminated candidiasis can often originate from the gastrointestinal tract, it is unclear whether the mechanism(s) that mediate host resistance against the different manifestations of the disease are similar, or significantly different.

**Models of infection**

In inbred mice, both systemic and oral candidiasis closely resemble the human disease. Both neutrophils and macrophages have been shown to exert candidacidal activity, and probably represent the first line of defence against *Candida* infection, but studies of animal models have demonstrated that T cells are essential for recovery from oropharyngeal candidiasis, and cell-mediated immunity has also been implicated in the development of *Candida*-specific protection in murine systemic candidiasis. Activation of phagocytes by T-cell-mediated cytokines appears to be necessary for the full expression of resistance against the disease, and a bias towards a T-helper type 1 (Th1) or type 2 (Th2) cytokine profile after systemic infection with either an avirulent or a virulent strain of the yeast has been linked to the induction of protection, or development of chronic disease, in different mouse strains. Nevertheless, the applicability of this paradigm to other models of the infection has been questioned. There is no increase in the severity or duration of disease after systemic infection of immunodeficient nude or SCID mice, which argues against an essential role for T cells in clearance of the yeast, although depletion studies have indicated that both CD4+ and CD8+ lymphocytes may play a part in limiting the extent of tissue lesions. In addition, analysis of cytokines in lesions of mice that are genetically predisposed to develop either severe or mild tissue pathology has failed to demonstrate any correlation between the extent of tissue destruction and a Th1 or Th2 cytokine profile.

In this paper, models of oro-pharyngeal and systemic candidiasis are described, and the relative importance of innate and adaptive immune responses in each are evaluated.

**Mucocutaneous candidiasis**

In humans, *Candida* species are frequently isolated from various mucosal sites within the oral cavity, oesophagus, and gastrointestinal (GI) tract, but few healthy carriers develop...
clinical signs of disease. It is typically alterations in the integrity of the immune system that ultimately determine whether the fungus remains as a simple commensal, or proliferates and causes disease.

Early studies of children with chronic mucocutaneous candidiasis demonstrated a strong association with defects in cell-mediated immunity, and the importance of T cells, particularly CD4+ cells, in the host response has been reinforced by the high incidence of oropharyngeal candidiasis in patients with HIV-infection with reduced CD4+ cell counts. Salivary cytokine analysis in HIV-negative individuals showed a Th0/Th1 profile, whereas HIV-infected persons with oropharyngeal candidiasis demonstrated predominantly Th2-type cytokines. Salivary cytokine profiles in HIV-negative denture wearers either with or without denture stomatitis demonstrated a mixed Th1/Th2 profile. Mohamed noted the presence of C. albicans inside macrophages in the oral mucosa of humans, suggesting that these cells play a role in resistance to mucosal candidiasis – a conclusion consistent with the reduced capacity of HIV infected monocyte-derived macrophages to phagocytose the yeast. Patients with Candida-associated denture stomatitis also show evidence of an impaired cellular immune response of blood lymphocytes against antigens from C. albicans in vivo (skin test) and in vitro (leucocyte migration test) and may have overactive suppressor T cells or other lymphocyte/phagocyte defects indicating that normal immunoregulatory responses can also be impaired in patients with oral candidiasis.

Mouse models

Understanding the mechanisms of recovery from mucosal candidiasis has been complicated by a plethora of experimental models, and by the extrapolation of results from infection of one particular mucosal site (e.g. the gastrointestinal tract) to other manifestations of the disease. Thus, despite intensive investigation, there is little consensus about the host effectors pathways crucial for recovery from primary infection of the oral mucosa.

Gastrointestinal candidiasis

Mucosal candidiasis of the gastrointestinal tract has been studied in both immunodeficient and immunocompetent mice. Both euthymic and athymic mice develop orogastric candidiasis but only euthymic mice demonstrate Candida-specific lymphoproliferation and delayed-type hypersensitivity (DTH) responses, that correlate with the clearance of C. albicans hyphae from mucosal surfaces. These results substantiated those of Domer who had earlier demonstrated that gastrointestinal infection of immunologically immature neonatal mice primed them for enhanced DTH responses, and protected them from systemic challenge as adults. Treatment of bg/bg nu/+ mice with anti-CD4 monoclonal antibody depleted their CD4+ lymphocytes and increased their susceptibility to Candida infection of the tongue and oesophagus. This suggested that protection of mice from orogastric candidiasis was mediated by CD4+ lymphocytes, possibly via the production of IFN-γ. Further studies of host susceptibility to mucosal candidiasis have been carried out in SCID mice that lack functional T and B cells, and in multiply immunodeficient (bg/bg, nu/nu) mice deficient in both T cells and phagocytic cells. After intragastric or oral inoculation of C. albicans, these mice developed a persistent GI infection whereas mice that lacked only phagocytic cells (bg/bg, nu+/+) cleared the infection efficiency. In contrast, B cell knockout mice, that lack both functional B cells and antibodies, were as resistant to orogastric candidiasis as immunocompetent controls. These data are consistent with a primary role for cell-mediated immunity in host protection against mucocutaneous candidiasis.

Numerous effector pathways of cell-mediated immunity against mucosal candidiasis have been identified, but their relative importance in vivo has not been established. A role for CD4+ T-helper cytokines has been demonstrated in disseminated candidiasis, and parallels have been found in models of gastrointestinal infection. BALB/c mice are resistant to systemic challenge with an avirulent isolate of C. albicans, and the generation of protective host responses is associated with expression of a Th1 cytokine profile by CD4+ T cells. In this mouse strain, systemic infection with a virulent isolate caused early mortality, whereas gastrointestinal colonization with the virulent yeast resulted in the production of both Th1- and Th2-type cytokines by CD4+ cells from Peyer’s patches and mesenteric lymph nodes, at a time when the yeasts were being cleared from the intestine. In contrast, DBA/2Cr mice develop fatal disseminated candidiasis after intravenous infection with the avirulent strain of C. albicans, but intragastric inoculation with the virulent strain was again associated with the induction of Th1-type cell-mediated immune responses and eventual clearance of the infection. IL-12-deficient mice were found to be highly susceptible to primary gastrointestinal C. albicans infection or re-infection, and showed elevated production of IL-4 with defective production of IFN-γ, whereas administration of soluble IL-4 receptor (sIL-4R) to mice with gastrointestinal candidiasis accelerated the clearance of the fungus from the stomach and stimulated Th1-associated resistance. These data suggest that activation of Th1- but not Th2-like responses may be responsible for controlling orogastric candidiasis and generating protective immunity.

A role for T cells in defence against mucosal candidiasis was further demonstrated by studies in multiply immunodeficient mice. Mucosal C. albicans infection in the alimentary tract of bg/bg nu/ mice induced a population of lymphocytes in both the Peyer’s patches and spleen that proliferated and produced IL-2 in response to stimulation with Candida antigens. Depletion of CD4+ cells increased the susceptibility of euthymic mice, but neither IL-2 nor IFN-γ was essential for the host response. Candida-reactive lymphocytes were not found in mice homozygous for the nude mutation (bg/bg nu/nu), and these animals were unable to clear the yeasts. The γδ T cells of the mucosa have also been implicated in the process of host defence. Oral colonization with C. albicans in B-cell-deficient mice, which have a normal T-cell response, increased the number of both αβ and γδ T cells in the gastrointestinal mucosa and intraperitoneal infection of immunocompetent and knockout mice with C. albicans resulted in an accumulation of γδ T cells in the peritoneal cavity. The γδ T cells produced IFN-γ, that enhanced
macrophage candidacidal activity and nitric oxide production. Interestingly, mice lacking both α/β and γδ T cells were found to be susceptible to orogastric candidiasis, but not to acute systemic candidiasis.35

As neither α/β nor γδ T cells have any candidacidal or candiastatic effect per se, clearance of the yeast from the infected mucosa is probably mediated by phagocytic cells. Treatment of SCID mice with cyclophosphamide, which causes severe neutropenia and further impairment of innate immune mechanisms, enhanced their susceptibility to mucosal candidiasis36 and impairment of macrophage function by administration of poly(I-C) increased susceptibility to disseminated candidiasis of endogenous (gastrointestinal tract) origin.37,38 The resistance of immunocompetent controls to mucosal candidiasis was not altered by treatment with poly (I-C) alone, and interference with both macrophage and neutrophil function was necessary to render these mice susceptible to the disease.39 In the absence of activation, Candida killing by phagocytic cells (both neutrophils and macrophages) is relatively inefficient, but the candidacidal potential of both cell types is significantly enhanced by exposure to Th1-type cytokines, such as IFN-γ and TNF-α.

Gastrointestinal colonization of athymic34 and SCID40 mice with C. albicans stimulated the expression of inducible nitric oxide synthase (iNOS) in the gastric and oral mucosa. Inhibition of NO production in SCID mice enhanced their susceptibility to orogastric candidiasis, but in athymic mice, the expression of iNOS was controlled by γδ T cells34 because depletion of these cells not only abrogated the expression of iNOS in the tongue and stomach but also increased susceptibility to orogastric candidiasis. In contrast, immunocompetent mice did not express iNOS in the orogastric mucosa after they were monoassociated with C. albicans, and mucosal candidiasis was not exacerbated after treatment with NOS inhibitors.35 However, there was no direct correlation between nitric oxide production and Candida killing.41 These authors suggested that nitric oxide was candiastatic rather than candidacidal, and was associated with or induced other macrophage candidacidal mechanisms.

Oral candidiasis

There are few models of oral, as distinct from mucosal, infection. As expected, Candida infection of the oral mucosa in mice triggers an inflammatory response and stimulates cellular immunity.42 A comparison of oral infection in BALB/c and DBA/2 mice, that carry the same MHC haplotype (H-2δ), demonstrated an increase in MAC-1+ cells in both strains, and a similar recruitment of CD4+ and CD8+ T lymphocytes into the mucosal tissue.43 The presence of intraepithelial CD4+ T cells was also five- to sevenfold greater in infected animals compared to control mice.44 Infection was associated with an influx of γδ T cells, on day 3 in BALB/c mice but on day 5 in the DBA/2 strain, that correlated with a substantial decrease in the number of viable organisms recovered from the mucosal tissue.45

Oral infection resulted in the development of systemic immunity, demonstrable as Candida-specific DTH responses in the susceptible DBA/2, but not in the more resistant BALB/c mice.31 Humoral immune responses could be detected after infection had resolved46 but levels of serum IgG and salivary IgA antibodies were higher in BALB/c than in DBA/2 mice. Mice infected via the oral cavity, but not intravenously, were protected against a second oral infection (Farah, unpubl. data). IgM was the predominant immunoglobulin present in the serum of these mice. IgG1 was present in BALB/c mice and, to a lesser extent, in CBA/CaH, but only very small amounts of IgG2 were detected. However, protection could not be transferred to naive recipients by transfer of either serum or cells from orally or systemically immunized mice.

Further studies of oral infection in BALB/c and DBA/2 mice confirmed expansion of the γδ T-cell population after oral infection45 and demonstrated significantly higher Candida-specific T-cell proliferation in BALB/c mice as compared to DBA/2 mice. In the resistant BALB/c mice, rapid clearance of C. albicans from the oral mucosa was associated with an early increase in levels of IL-4, IL-12 and IFN-γ in cells from the cervical lymph nodes, whereas in infection-prone DBA/2 mice that cleared the infection more slowly, expression of message for IL-4 was delayed, and the levels secreted were lower.45 In BALB/c mice, monoclonal antibody neutralization of IL-4 increased the fungal burden and delayed clearance of the yeast. Thus, in contrast to results in systemically infected mice,46 IL-4 appears to be an important mediator of protection in oral candidiasis.

As in the gastrointestinal models described above,40,47 NO was detected in the effector phase of the response against oral infection48 and has been shown to be an important component of the host response. In mice, concentrations of NO in saliva increased after infection and saliva from infected mice inhibited the growth of yeast in vitro. In patients with denture stomatitis, there was an inverse correlation between levels of NO in saliva and a number of yeasts recovered from the oral cavity (Clancy, unpubl. data). In mice, neutralization of IL-4 in vivo caused a marked reduction of NO levels in saliva and in cultures of cervical lymph node cells after stimulation with C. albicans antigen.48 Conversely, treatment with NG-nomethyl-L-arginine (NMLA), which inhibits NO synthesis, led to an increase in C. albicans in the oral cavity and a concomitant abrogation of expression mRNA for IL-4, but not IFN-γ, in lymphocytes from the draining lymph nodes. Paradoxically, inhibition of IL-4 production was accompanied by an increase in IFN-γ production in susceptible DBA/2 mice49 which tends to argue against a strict Th1/Th2 dichotomy as a determinant of resistance and susceptibility in oral candidiasis. However, in a different model, treatment of orally infected mice with aminoantiguanidine (AG), that abrogates production of NO, had no effect on the magnitude or duration of infection (Farah, unpubl. data).

Cellular immunodeficiency, particularly infection with HIV, substantially increases the risk of mucosal infections with C. albicans, and the nude mouse has been used as a model of oropharyngeal infection. These mice displayed increased levels of oral colonization compared to eutrophic controls, and developed a chronic infection.7 There was extensive hyphal penetration of the epithelium, associated with infiltration of polymorphonuclear leucocytes and the formation of microabscesses. CBA/CaH mice, that are predisposed to severe tissue pathology,49 developed a greater fungal burden and more severe lesions after oral infection than the ‘low pathology’ BALB/c mice. This is consistent with the regulation of tissue susceptibility by the Carg1 gene.50
Established infections in nude mice could be cleared by the adoptive transfer of syngeneic lymphocytes\textsuperscript{7} and the protective effect was mediated by the CD4\textsuperscript{+}, but not the CD8\textsuperscript{+} lymphocyte subset. Recovery was associated with the presence of IFN-\(\gamma\) and IL-12 in the cervical and submaxillary lymph nodes of the reconstituted mice.

Initial attempts to confirm the essential role of T cells in host resistance by antibody depletion of CD4 or CD8 cells from normal (euthymic) mice were unsuccessful; however, when mice were given irradiation to the head and neck, depletion of CD4\textsuperscript{+}, but not CD8\textsuperscript{+}, lymphocytes caused a prolongation of infection and increased severity of oral lesions with hyphae penetrating the oral mucosa.\textsuperscript{51} In the irradiated mice, recovery from infection was associated with production of high levels of IL-12 by lymph node cells, but concentrations of IFN-\(\gamma\) in infected mice were comparable to those in controls. Either depletion of neutrophils or inactivation of macrophages/monocytes increased the severity of infection in BALB/c mice, but had a lesser effect in CBA/CaH.\textsuperscript{32} Depletion of both cell populations further increased infection in BALB/c mice, but dramatically exacerbated the fungal burden in the CBA/CaH strain. It therefore seems that phagocytic cells play a more dominant role in the susceptible CBA/CaH mice than in the resistant strains, although there was no significant difference in clearance of chronic infections in nude mice of either strain after reconstitution with lymphocytes.\textsuperscript{7}

The identity of the mediator(s) that link T cells and phagocytic effector cells remains elusive, and the relationship seems quite complex. In general, IL-12 and IFN-\(\gamma\) were the dominant cytokines produced by lymphocytes from the draining lymph nodes of recovering animals\textsuperscript{53} but levels of IL-4 and IL-10 did not show any association with recovery from oral infection. In contrast, TNF-\(\alpha\) was the only cytokine that was unique to infected oral mucosa.\textsuperscript{53} Studies of candidiasis in knockout mice has confused, rather than clarified the issue.

In one study, IFN-\(\gamma\) KO mice showed increased susceptibility to both gastric and systemic candidiasis,\textsuperscript{54} whereas another reported no effect on either form of the disease,\textsuperscript{35} and a third found increased mortality of the KO mice although the increased susceptibility did not correlate with the extent of organ colonization.\textsuperscript{56} Ablation of IL-10 increased resistance against both gastrointestinal\textsuperscript{37} and systemic\textsuperscript{58} candidiasis, but deletion of the gene for IL-4 had no effect.\textsuperscript{56} Recent studies of oral candidiasis in IFN-\(\gamma\), IL-4, IL-10 and iNOS KO mice have failed to demonstrate any alteration in the severity or course of the disease (Farah, unpubl. data). In TNF-\(\alpha\) KO mice, there was an early increase in the fungal burden in the oral cavity, but the duration of the infection was not different from controls. Infection in IL-12 KO mice, however, was similar to that in T-cell-deficient nude mice (Fig. 1). The fungal burden in the oral cavity increased steadily, and became chronic, persisting undiminished for at least 3 months (Farah, unpubl.).

The precise role of IL-12 as a mediator of host resistance, and the anomalous position of IFN-\(\gamma\), remain to be resolved. Our failure to identify a particular cytokine or set of cytokines as a crucial link in the effector pathway against the yeast may be due to a redundancy among the cytokines that masks the effect of gene deletion in knockout mice; however, it is difficult to evaluate the significance of such functional overlap in vivo. Alternatively, there may exist additional effector pathways or differences between the experimental models. Recent studies in nude mice have demonstrated that bone marrow colony formation in vitro is significantly depressed in the ‘high pathology’ CBA/CaH mice compared to BALB/c (Wanasaengsakul, unpubl. data), and reconstitution of the nude mice with T cells significantly increases the colony-forming response in infected BALB/c, but not in infected CBA/CaH mice (Fig. 2). These results suggest that

![Figure 1](image1.png)  
**Figure 1** Fungal colonization of thymus-deficient ‘nude’ and IL-12 knockout mice compared to mice of the parental strains after oral infection with 10\(^8\) *Candida albicans* yeasts. ■, IL-12 KO; ▲, nude; ▼, C57/BL6; ●, BALB/c.

![Figure 2](image2.png)  
**Figure 2** Colony-forming units in the bone marrow of nude mice reconstituted with lymphocytes from syngeneic normal mice after oral challenge with 10\(^8\) *C. albicans* yeasts. The response of cells from BALB/c mice is significantly greater than those from CBA/CaH mice (\(P < 0.001\)). □, CBA/CaH; ■, BALB/c.
one effector pathway may be T-cell-mediated enhancement of phagocytic cell production by the bone marrow.

Although using the same isolate of yeast, ‘traumatic’ and ‘atraumatic’ modes of infection showed substantial differences between the experimental models. After oral infection, DBA/2 mice showed a bimodal pattern of colonization, a feature reproduced by Elahi whereas Farah demonstrated only a single peak (Fig. 3). As both studies were carried out using the same yeast isolate (3630, from the Mycology Reference Laboratory at the Royal North Shore Hospital), it seemed probable that the course of oral infection in this mouse strain was influenced by the actual technique used for infection. Elahi pressed the yeast onto the gums using a small swab, whereas Farah introduced a suspension of yeasts into the oral cavity without damaging or traumatizing the oral mucosa. Thus, in the former case, microtrauma may have facilitated both deeper penetration of the yeast and the rapid elicitation of protective cell-mediated immune response. When inoculation is atraumatic, adhesion of the yeast to the oral mucosa may be more difficult to establish, and innate immune mechanisms may be more directly involved and more effective in eradication of the yeasts.

This observation led us to test the hypothesis that there was a gradation in host responses from innate to adaptive immunity that was determined by the severity of the infectious challenge. Nude mice develop chronic infections after oral challenge with 10⁸ yeasts. However, as the doses used for infection were progressively decreased, the magnitude of the fungal burden in the chronically infected mice decreased proportionally (Fig. 4), until eventually the nude mice were able to clear the infection completely. Thus, the requirement for CD4⁺ T cells for clearance of the infection in these mice apparently relates to the immunological deficit that permitted the infection to become established – a situation comparable to the susceptibility to oropharyngeal candidiasis of HIV/AIDS patients. It is noteworthy that in neither the human nor the experimental model is there any systemic dissemination of the mucosal infection, suggesting that innate immunity may also play a major role in the response against systemic disease.

**Figure 3** Representation of patterns of colonization that develop in ‘infection-prone’ DBA/2 mice using different techniques of infection. Atraumatic infection involves direct inoculation of a suspension of yeasts into the oral cavity; in the traumatic method, the pelleted yeast is pressed onto the gums and oral mucosa using an ear, nose and throat (ENT) swab. ▲, traumatic; ■, atraumatic.

**Figure 4** Oral colonization of nude mice after infection with differing doses of *C. albicans* yeasts.

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**Systemic candidiasis**

Considerable insight into the pathogenesis of the systemic disease has been obtained by comparing inflammatory and immune responses in inbred strains of mice that exhibit different patterns of susceptibility to infection with the yeast. Susceptibility to systemic candidiasis has been evaluated by gross measures such as mortality, by colony counts in infected tissues, and by histological evaluation of the severity of lesions in the tissues.

The most informative was histopathological assessment, which revealed two distinct patterns of tissue destruction in the brains of inbred mice of different strains. Lesions in mice with high pathology were characterized by numerous large abscesses containing yeast and mycelial growth forms within the necrotic debris, together with an inflammatory infiltrate consisting of mononuclear and polymorphonuclear leukocytes. Mice with mild lesions (low pathology) showed similar characteristics, but the abscesses were small and infrequent.
Mild and severe tissue damage can also be discerned in other organs, but these patterns do not show a strong correlation with either mortality or with the fungal burden in infected tissues. The mild and severe patterns of lesion severity are inheritable and controlled by a single co-dominant gene. The variables that determine the expression of mild or severe tissue damage have not yet been defined but, as the phenotypes are expressed in nude mice, they are probably related to qualitative or quantitative differences in the effector functions of bone marrow-derived phagocytic cells.

Mortality correlates poorly with tissue damage; however, mice deficient in the fifth component of complement die after challenge with substantially lower doses of C. albicans than complement sufficient mice, typically from acute fungal pyelonephritis. An evaluation of the role of C5 in normal and immune congenic resistant B10.D2 ‘old’ and ‘new’ mice demonstrated that an intact complement system contributes to inhibition of the growth of C. albicans, but the presence or absence of C5 did not affect the development of specific immune responses or the ultimate outcome of challenge. The presence of C5 appears to be crucial in limiting the initial fungal growth in the kidney and, as the candidacidal activity of phagocytic cells from normal and C5-deficient mice is equivalent, it is clear that the opsonizing and/or chemotactic properties of C5 are critical factors in the early containment and elimination of the yeast. However, neither the severity nor the duration of kidney infection in C5-deficient mice is influenced by depletion of T cells. Although there are many reports of the T-cell-dependence of host responses against disseminated Candida infection (reviewed in ) these have been carried out in one experimental system, and the paradigm that has evolved to explain susceptibility and resistance to infection has yet to be independently confirmed. In brief, this model used various combinations of yeasts (attenuated and virulent), and mouse strains (DBA/2 and BALB/c), to produce healing infections that resolved with the development of resistance to re-infection, or non-healing infections that led to chronic disease and death. Cytokine production in healing combinations was associated with the generation of a Th1 cytokine profile by CD4+ splenic cells in vitro, whereas a Th2 profile developed in lymphocytes from animals with chronic disease. Neutralization of IFN-γ in vivo prevented the development of protective Th1 responses whereas neutralization of IL-4 reduced mortality with a concomitant induction of a Th1 cytokine profile and development of protective immunity.

Other models have demonstrated alternative pathways of host resistance. Neither nude nor SCID mice show any increased susceptibility to systemic infection, indicating that cells of the innate immune system are competent to clear the yeast in the absence of activation by T cells or their products. It had been thought that the resistance to disseminated candidiasis of T-cell-deficient mice might be attributable to non-specific activation of macrophages known to occur in these animals; however, macrophage activation by treatment with Bacille Calmette-Guerin (BCG) did not enhance resistance. The contrast between the resistance of T-cell-deficient nude mice to disseminated infection, and their acute susceptibility to oral infection, strongly suggests that the dominant effector response is different in the two types of infection. Furthermore, mice in which the T-cell receptor δ- and α-chains had been genetically deleted were highly susceptible to orogastric candidiasis, but remained resistant to disseminated candidiasis of endogenous origin and to acute systemic candidiasis induced by intravenous challenge. The differences in host responses exhibited by the various models of systemic candidiasis have not yet been addressed, and the precise role of T cells is still incompletely understood.

Mice that are genetically susceptible or resistant to tissue damage display substantial differences in both early immune and inflammatory responses, and in the nature and evolution of immunological memory. BALB/c (H-2d) mice, that develop mild lesions, show a significantly greater recruitment of immune/inflammatory cells to the draining lymph node after subcutaneous challenge with live Candida yeasts than do CBA/CaH (H-2k) mice, in which the lesions are more severe. However, these responses do not correlate with the severity of tissue damage as BALB/k (H-2k) mice, that develop only mild lesions, are low responders. In contrast, both BALB/c and BALB/k mice make strong proliferative and DTH responses when challenged after recovery from a primary systemic infection, whereas the response of CBA/CaH lymphocytes remains low. The contrast between the both primary and secondary immune responses of MHC-identical CBA/CaH and BALB/k mice suggests that infection in the genetically tissue-susceptible strain may elicit some form of regulatory activity that inhibits the development or expression of Candida-specific T-cell activity.

Although there is an inverse correlation between the magnitude of the cell-mediated immune response and the extent of tissue destruction in the susceptible and resistant mice, yeast is cleared from the spleen of the susceptible CBA/CaH strain more rapidly than from the resistant BALB/c mice. A similar disjunction between immune responses in spleen and other organs has recently been reported. A Th1-type cytokine response developed in the kidney after sub-lethal infection, whereas a Th2 response was seen after lethal challenge. The spleen showed Th2 responses only. Thus, it was considered that the Th subset response in infected organs, rather than in the lymphoid tissue, was associated with mortality or survival. The role of T cells in tissue resistance against disseminated infection has also been evaluated directly by depletion of T cells in vivo. Overall, depletion of CD4+ cells did not influence fungal colonization or outcome of infection in BALB/c or CBA/CaH mice, although there was a significant, but transient, decrease in the fungal burden in the brain of the latter strain.

The effect of T-cell depletion on lesion severity was strikingly different. Depletion of CD4+ cells from BALB/c mice caused a marked increase in tissue damage associated with infection. In contrast, depletion of CD4+ cells from CBA/CaH mice delayed the onset of tissue damage by about 4 days when compared with BALB/c mice. After this time, tissue damage was exacerbated in the same way, and to the same extent, as in the BALB/c mice. After passive transfer into infected syngeneic recipients, spleen cells from infected CBA/CaH mice markedly increased tissue damage when compared to controls, and also caused a significant increase in fungal colonization in the brain. A similar transfer in BALB/c mice increased the number of inflammatory cells in and around the lesions, but had no effect on the fungal burden in brain and kidney. The data suggest that systemic infection
results in the generation of CD4+ T-cell populations that limit tissue damage and/or enhance healing, but that do not directly augment clearance of the organism from infected tissues.

The phenotype of these lymphocytes has not yet been determined; however, there is a parallel between the activities of these T cells and those of a population of regulatory CD4+CD25+ T cells that have been demonstrated in mice with candidiasis. This cell population produced IL-4, IL-10 and TGF-β, required an IL-10-enriched environment for its generation, and was not present in B7-2- or CD28-deficient mice which, although capable of efficiently restricting fungal growth, experienced increased inflammatory pathology. There is an early abundance of IL-10 in CBA/CaH compared to BALB/c mice (Wanasaengsakul, unpubl.), and this might favour the generation of these regulatory cells and the production of Candida-specific IgG1 and IgG2a antibodies (Hu, unpubl. data) in CBA/CaH mice, whereas high levels of IFN-γ and a relative lack of IL-10 would drive antibody production by BALB/c mice towards a predominantly IgG1 isotype.

Downregulation of cell-mediated immune responses in CBA/CaH mice correlates with the severe pattern of tissue damage and the presence of the susceptible allele of the Carg1 gene in this strain. In addition, CD8+ lymphocytes from primed CBA/CaH, but not BALB/c, mice can be activated for anti-self recognition and reactivity although the relevance of this to the specific response against the organism, or to the general biology of the animal, remains unknown. Nevertheless, these experiments suggest that the genetic context in which the immune response evolves influences the effector function of both T cell subsets.

Conclusion
A key issue in the study of Candida infection is the complex relationship between innate and adaptive immunity, and the role played by each in different manifestations of the disease. Both clinical and experimental data are consistent with a central role for T cells and cell-mediated immunity in the host response against oropharyngeal candidiasis, but further research will be needed to understand the way in which the presentation of the infectious challenge triggers the transition from an innate to a specific adaptive immune response.

Understanding the systemic disease remains a challenge, with formidable cases having been made for a dominant role of both adaptive and innate immunity. Patterns of cytokine production may be determined not only by the characteristics of the yeast used for infection, but also by the genetic makeup of the host, with both MHC and non-MHC genes clearly influencing the evolution of the immune response; but how, or indeed whether, this is reflected in efficiency of clearance of the yeast, or in the extent of the associated pathology, is a question that remains to be resolved.

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