

The Role of the Gastrointestinal Tract in Hematogenous Candidiasis: From the Laboratory to the Bedside

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The gastrointestinal (GI) tract is a frequent source of hematogenous candidiasis in humans. Animal models of GI and hematogenous candidiasis have provided insights into the nature of candidal infection of host mucosal tissue, mechanisms of fungal dissemination to body organs, and features of host response to candidal infections. Biological systems such as these that simulate human candidiasis can be used for testing novel antifungal drugs. We have focused on two murine models of candidiasis with similarities to this fungal disease in humans. The first model simulates a commensal association of *Candida albicans* with the GI tract of immunocompetent hosts; it has permitted studies of innate and immune cell response to long-term (>60 days) infection of the esophageal, gastric, and intestinal mucosa. The second model simulates candidal infection in granulocytopenic patients with invasive candidiasis that originated from sites of colonization in the gut. Both models are well suited for investigating new approaches to prevention and treatment of hematogenous candidiasis. A review of the data on the role of GI candidiasis in hematogenous candidal infections is presented.

Candidiasis is a disease of immunocompromised patients, and *Candida albicans* is the most frequent causative agent of this mycosis [1]. Paradoxically, *C. albicans* is a common component of the oral and gastrointestinal (GI) flora of immunocompetent humans [2, 3], and the yeast has apparently evolved as a successful commensal in healthy persons. The fungus may be acquired at an early age, even by the fetus during its passage through the birth canal [4]. Evidence derived from experimental animal studies has indicated that impairment of neutrophil, T cell, and B cell functions permits the opportunistic pathogen to invade the submucosal tissue of the GI tract and to disseminate hematogenously to other organs [5]. The GI mucosa of immunocompromised patients is most probably a common portal of entry of *C. albicans* into the body [6]. Yeast colonization of the gut, therefore, poses a potential threat to a person's health, especially if mucosal immunity becomes impaired.

As in the murine host, clinical observations of humans indicate that multiple immune defects are associated with disseminated candidiasis. Such defects are seen in patients with cancer who undergo aggressive chemotherapy with cytotoxic drugs that can influence several pivotal immune functions. Particularly disturbing is the observation that *Candida* species that

are resistant to commonly prescribed candidacidal drugs have been increasingly isolated from patients who have cancer, and outbreaks of infections caused by these less susceptible organisms have occurred in several centers [7].

Unlike most patients with cancer, those with AIDS do not show an increased tendency toward development of disseminated candidiasis of endogenous origin [8]. Apparently, the characteristic CD4⁺ T-cell depletion in patients with AIDS does not sufficiently diminish mucosal immunoprotection to predispose the host to candidal invasion of the GI tract and subsequent hematogenous dissemination. Oropharyngeal and esophageal candidiasis, on the other hand, occur at high frequency among these patients [9] and have been cited by the World Health Organization as diagnostic criteria for the HIV staging system [10].

As mentioned above, the emergence of candidacidal drug-resistant strains in patients with HIV infection who have recurrent oropharyngeal or esophageal candidiasis has been reported [11, 12]. With any therapy, relapses tend to occur and progressively recalcitrant disease often develops. Current evidence suggests that prolonged azole treatment of mucosal candidiasis in immunocompromised patients should be avoided [13]. There is clearly a need to address the factors that contribute to candidal resistance and the risk for candidal infection and to develop more effective approaches to management of recurrent mucosal candidiasis [14].

Murine models of GI candidiasis have been used to explore events of mucosal colonization and invasion by *C. albicans* under conditions that simulate the predisposition of the human GI mucosa to candidiasis. These models include mice with retrovirus-induced immunodeficiency syndrome, which have been used to explore dysfunctions of the host's innate and

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cellular immune responses to *C. albicans* infection [15–17]. Development of a mouse strain with severe combined immunodeficiency syndrome [18] has permitted studies of the susceptibility of a host that lacks functional B- and T-cell protective mechanisms against GI candidiasis [5]. Other genetically immunodeficient mice that manifest defects in innate cellular responses and/or in cell-mediated immunity have also been used to clarify the relative importance of nonspecific and specific host-defense mechanisms in resistance to *C. albicans* infections [19, 20]. Immunocompetent mice with preexisting GI candidiasis established by yeast inoculation at infancy [21] or in adulthood [22–24] have been used to examine the influence of immunocompromising drugs (e.g., cyclophosphamide and cytarabine) administered as chemotherapy on the development of hematogenous candidiasis originating from the gut [25]. Some of these same animal models have been used to test the efficacy of candidacidal drugs administered parenterally or orally for clearance of *C. albicans* from the GI tract [26]. Prophylactic therapy with anti-*Candida* drugs alone or in combination with recombinant cytokine therapy may provide a rational approach to the prevention of disseminated candidiasis of endogenous origin [27–30].

In this article we review certain features of murine models of GI and hematogenous candidiasis that have provided an understanding of the mechanisms of candidal infection and the nature of host response, as well as insights into the means of successful management of this mycosis in humans. We also discuss the pathogenesis of hematogenous candidiasis in humans and attempt to correlate the laboratory findings in animal studies with clinical observations in studies of patients.

GI and Hematogenous Candidiasis in Animals

Murine models of GI candidiasis. This review focuses on two murine models of GI candidiasis that address different aspects of the interplay between a fungal pathogen and a host [31]. The first model is of persistent GI candidiasis in mice that were challenged by the oral-intragastric route when they were neonates [32]. The second murine model is of GI and hematogenous candidiasis in immunosuppressed, drug-treated animals [23, 33]. Two strains of mice were used: an outbred strain of Swiss White mice (CrI:CFW[SW]BR; Charles River Farm, Wilmington, MA) and an inbred strain (C57BL/6; Jackson Laboratories, Bar Harbor, ME). In each case a breeding colony was established with mating pairs. Infants (6 days old, mixed sexes) derived from these mice were isolated from their mothers and held in an incubator at 35°C for 3–4 hours before inoculation with *C. albicans*. Clearance of most of the milk from the stomachs and intestines of the neonates during this preinoculation period enhanced the ability of *C. albicans* to interact with the mucosal surface of the host. The inoculum, delivered by the oral-intragastric route with a 24-gauge feeding needle attached to a 1.0-mL syringe [32], consisted of 2.0×10^8 yeast cells suspended in 0.05 mL of sterile saline [33].

Colonization of the GI tract of immunocompetent mice by *C. albicans*. *C. albicans* is not a component of the indigenous microflora of the rodent gut. Colonization by this fungus in the GI tract of mature mice apparently is inhibited by the host's innate defenses and mucosal barrier, as well as by competition between the yeast and existing microbial flora [34]. Kennedy [35, 36] has suggested that the indigenous microflora may directly influence candidal colonization of the alimentary tract by at least two mechanisms: (1) antagonism of *C. albicans* growth and (2) blockage of host receptor macromolecules that could otherwise bind with *C. albicans* adhesins.

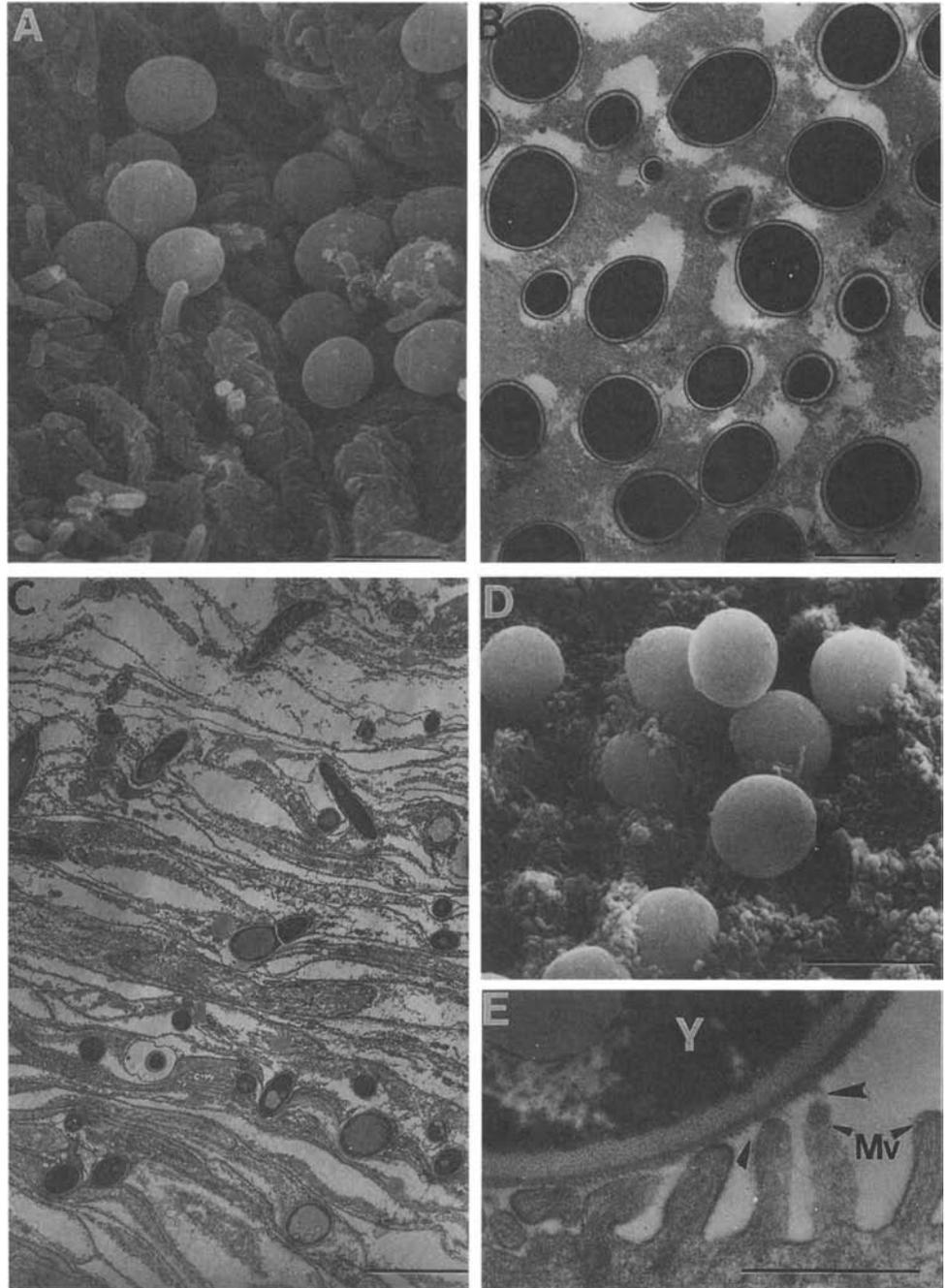
The neonatal mouse model was developed primarily to avoid these problems of establishing GI colonization by *Candida* species [21]. Innate defenses are minimal in the immunoincompetent animals. The microbial flora consisted almost solely of lactobacilli, and the yeast were able to compete effectively with these indigenous microbes for sites of adherence and colonization (figure 1A). The mucin barrier in the GI tract appeared to be breached by yeast cells within 1 hour after oral-intragastric inoculation (figure 1B). A mucinolytic enzyme has not yet been reported for *C. albicans*.

Over a period of 2–4 days after challenge, the keratinized epithelium of the neonatal alimentary tract became colonized with *C. albicans* (figure 1C). The keratinized epithelium is continuous through the esophagus to the cardiac-atrium fold. The keratinized surface of the alimentary tract is a formidable barrier against fungal invasion. Although it is evident that *C. albicans* can penetrate this barrier and reach the epithelial cell surface, it is still controversial whether this opportunistic pathogen produces a keratinase [37]. Secreted aspartyl proteinases (SAPs) of *Candida* species [38] have been shown to utilize a wide range of substrates, including keratin and collagen [39]. For example, Hattori et al. [40] demonstrated that a putative keratinolytic proteinase of *C. albicans* was able to release amino acids from the host's stratum corneum. The optimum pH for activity of the secreted hydrolase was 4.0 and it was inhibited by pepstatin, a finding that suggested the enzyme was actually a SAP.

A portion of the oral-intragastric inoculum of *C. albicans* passes through the stomach of the neonate and associates with the mucosal surface of the jejunum and ileum. As in the upper alimentary tract, yeast cells were visible within the mucin layer [21], but they were also frequently observed in association with the columnar epithelial cell surface in cavities apparently formed by digestion of the microvillus layer (figure 1D) [32]. Ray and Payne [41] showed that *Candida* yeasts that adhered in vitro to skin corneocytes formed depressions, or cavitations, at sites of contact with the host cells. The authors suggested that this phenomenon was at least partly due to extracellular proteinases secreted by the adherent *C. albicans* cells.

Argon-laser confocal imaging of experimental cutaneous candidiasis was performed with use of fluorescein isothiocyanate-conjugated SAP-specific antisera [42]. The antigen was shown to be deposited between adherent *Candida* organisms

Figure 1. Histopathologic evidence of GI candidiasis in neonatal CFW (Charles River Farm; Swiss White, outbred strain) mice killed 1 hour after oral-intragastric inoculation with *C. albicans* yeast. *A*, scanning electron micrograph of keratinized region of gastric mucosa and associated yeast cells. *B*, yeast in mucin layer of gastric mucosa showing zones of apparent clearance. *C*, yeast and germ tubes associated with keratinized layer of gastric mucosa showing sites of apparent fungal penetration. *D* and *E*, yeast cells associated with microvillus layer of the jejunum showing cavitation (*D*) and fibrillar connections (*arrowheads*) between microvilli (Mv) and yeast (Y) cell surface layer. (Bars in *A–E* = 5, 5, 10, 5, and 0.5 μ m, respectively.)



and host cells in the stratum corneum. SAPs have been implicated in both adherence and invasion of the epithelial barrier [38]. Studies of *C. albicans* SAPs have now revealed that at least seven isoenzymes exist and are correlated on the basis of the presence of two active sites containing aspartic proteinases [43]. SAP expression in *Candida* species is apparently under the control of a multigene family (*sap1–sap7*). Two additional genes, *sap8* and *sap9*, are now known to exist [43]. Hube et al. [44] have shown that *sap1–sap6* are regulated differentially at the mRNA level, a finding which supports the contention

that the various isoenzymes have different and specific functions during growth and infection of the host. The authors demonstrated that *sap2* was the most abundant transcript of the yeast phase under a wide range of growth conditions. They suggested that to date, most biochemical studies of partially purified aspartyl proteinases of *C. albicans* probably reflect characterization of the *sap2* gene product.

Examination of thin sections of the host's jejunum just 1 hour after oral-intragastric inoculation with *C. albicans* revealed an intimate association between the yeast cell surface and the

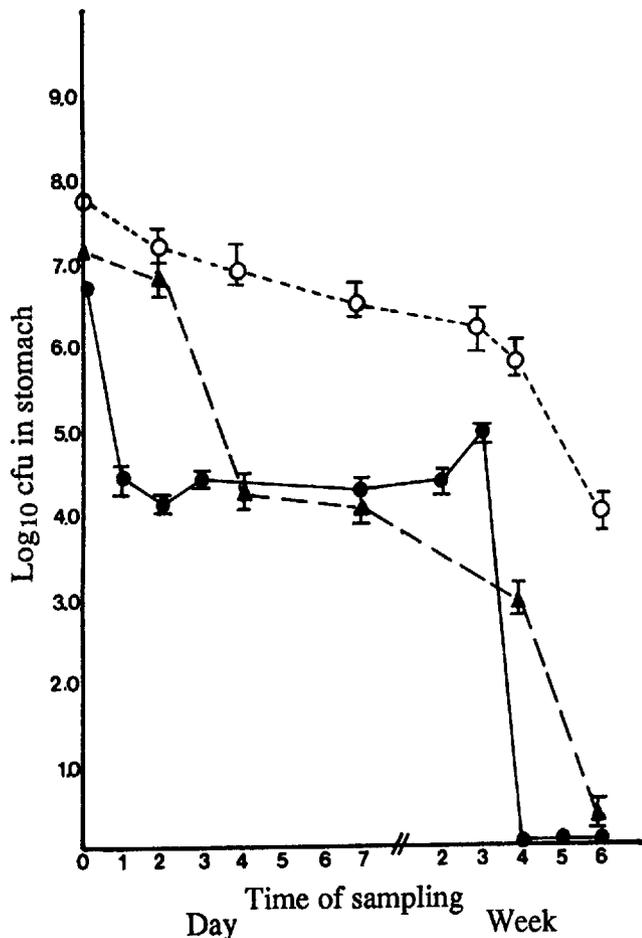


Figure 2. Persistence of *C. albicans* strains NS33, CA30, and CA87 (see [32] for source of strains) in the stomach of infant CFW mice. The mice were inoculated by the oral-intragastric route with 8.8×10^6 , 7.0×10^7 , or 2.0×10^8 yeast cells, respectively, and then killed at different times after inoculation. The first sampling was taken at 30 minutes after challenge (day 0). Each point represents the mean value (\pm SD) for five mice. ● = NS33; ▲ = CA30; ○ = CA87.

microvillus membrane (figure 1E). The outermost layer of the yeast cell wall consists of a dense fibrillar coat and has been reported to be composed of mannan or mannoprotein [45]. Figure 1E shows thin fibrils that extend between the yeast surface and the glycocalyx of the host cell. Studies of bacterial attachment to host cell membrane surfaces have revealed that gene products expressed on the bacterial surface (i.e., adhesins) interact with carbohydrates or proteins on the eukaryotic cell (i.e., integrins) to mediate attachment and invasion [46, 47]. Exceptions are noted in the pathogenesis of some bacteria (e.g., *Shigella* species) in which host integrins apparently are not involved in the initial attachment and subsequent invasion of the mucosa [48].

Integrins are expressed as heterodimeric transmembrane proteins and classified according to the structure of their β -chains [49]. In *C. albicans*, cell surface integrins are expressed by the pathogen rather than by the host, and they mediate adhesion by recognition of extracellular matrix proteins synthesized by

Table 1. Evidence regarding persistence of *C. albicans* strain CA30 in CFW mice, on the basis of the number of cfu in plated fecal-pellet homogenates.

Time after inoculation (w)	No. of mice positive for <i>C. albicans</i> /total no. of mice	cfu/fecal pellet
3	14/14	2.1×10^3
4	17/18	2.6×10^3
5	19/19	8.6×10^2
6	14/18	5.7×10^2
7	10/19	3.4×10^2
8	10/24	9.2×10^1
10	4/15	1.4×10^1

the mammalian epithelial or endothelial cells. Bailey et al. [50] identified specific proteins synthesized by *C. albicans* in vitro when blastoconidia adhered to human buccal epithelial cells. They suggested that host-induced synthesis of yeast signal proteins was a pivotal event of the adherence process.

Furthermore, evidence exists that expression of such cell surface ligands of *C. albicans* is growth-phase specific. For example, hyphal and pseudohyphal forms of the organism bind fibrinogen, laminin, and the complement (C3) conversion product C3d, whereas yeast cells do not [45]. A C3d-binding glycoprotein of *C. albicans* was shown to be expressed in vivo [51] and was purified and characterized [52]. Mutant strains were isolated that are deficient in binding complement ligands iC3b and C3d [53]. An intriguing hypothesis is that yeast integrin analogs of mammalian integrin proteins (e.g., CR3 and CR4) may be evolutionary precursors of the host receptor macromolecules [46, 54]. If such an evolutionary progression indeed occurred, it would help explain the occurrence of *C. albicans* as a successful commensal in the human gut and may provide insights into novel therapeutic approaches to mucosal candidiasis.

Host cell response to C. albicans infection of the GI tract of immunocompetent mice. *Candida* species organisms were recovered from the esophagus, stomach, and intestines of CFW and C57BL/6 mice up to 60 and 120 days, respectively, after the animals were inoculated with 2×10^8 yeasts at infancy via the oral-intragastric route [55]. Thus, the degree of persistence of GI candidiasis varied with the mouse strain. Differences in the numbers of organisms recovered and the duration of colonization of selected regions of the GI tract were also observed with use of various clinical strains of *C. albicans* (figure 2) [32]. The rate of clearance of strain CA30 from CFW mice was monitored on the basis of the number of cfu of *C. albicans* detected in fecal pellet homogenates >10 weeks after inoculation (table 1). Our interest in this phenomenon was piqued by results of histopathologic examination of *C. albicans*/host interactions during progressive stages of clearance of the organism from the GI tract of immunocompetent mice (figures 3–5) [32].

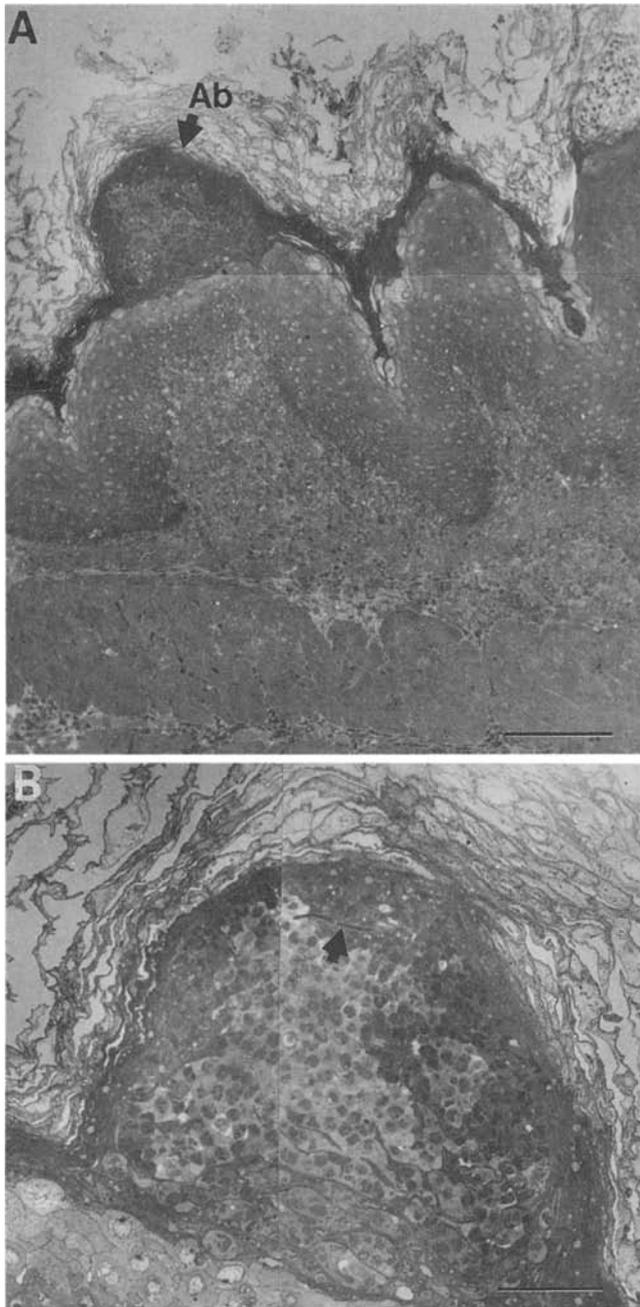


Figure 3. Intraepithelial abscesses (Ab) in the gastric mucosa of mice infected with *C. albicans* and killed at 4 weeks (A) or 12 weeks (B) after oral-intragastric inoculation. Arrow in (B) identifies a hyphal element. Note dense cluster of neutrophils within abscess. (Bars = 100 μm [A] and 50 μm [B].)

We focused our studies on the gastric mucosa in the region of the cardiac-atrium fold, because this is a major site of colonization by *C. albicans* in the GI tract. The cardiac-atrium fold of the murine stomach is at the junction of the keratinized and glandular epithelium. In humans, the keratinized layer of the upper alimentary tract extends only to the cardiac sphincter. Histologic sections of the stomachs of infected mice (CFW

or C57BL/6) have revealed multiple intraepithelial abscesses associated with yeast and hyphae in the region of the cardiac-atrium fold (figure 3A, B). The abscesses were found adjacent to regions of *C. albicans* colonization of the gastric mucosa and consisted of a thickened keratinized outer layer that partly enveloped a dense aggregate of polymorphonuclear neutrophils. The majority of neutrophils in the central core of the abscess were degranulated (figure 4A) and the *C. albicans* cells within the abscess were highly vacuolated. Products released by the aggregation of degranulated neutrophils, such as oxygen radicals and associated myeloperoxidase, may contribute to the death of *C. albicans* cells trapped within the abscesses [56].

Both candidacidal and candidastatic activity may occur within and adjacent to the mucosal abscesses, and these activities may contribute cooperatively to the gradual clearance of GI candidiasis in CFW and C57BL/6 mice. Packets of neutrophils were commonly visible at the base of the abscess (figure 4A, arrows), and these were surrounded by layers of keratin, keratinocytes, and remnants of epithelial cells. The neutrophils appeared to have migrated to these sites of infection via the lamina propria and submucosal regions of the gastric tissue [32].

Large numbers of mononuclear cells were also detected in histologic sections of the submucosal region of the stomach adjacent to sites of infection [32]. It appears that contributions of monocytes and macrophages to protection against candidal infection are minimal in comparison with the protection provided by neutrophils, unless the former are activated by cytokines such as IFN- γ and granulocyte-macrophage colony-stimulating factor [57]. The mechanisms of killing by monocytes and macrophages have been reported to be both oxidative and nonoxidative in nature [56]. Granulocyte-macrophage colony-stimulating factor apparently augments both superoxide anion and the level of mannose receptors of treated monocytes, which in turn increase the killing capacity of these cells. The precise mechanisms of killing of *Candida* organisms by monocytes and macrophages may be different [57]. A diagrammatic summary of the histologic data on host cellular response to gastric candidiasis in immunocompetent mice is presented in figure 5.

It has long been appreciated that T lymphocyte-dependent, cell-mediated immune response and mobilization of phagocytic cells (neutrophils and macrophages) are pivotal for resistance to candidiasis [58, 59]. Neutrophils appear to respond chemotactically to the presence of *C. albicans* and accumulate in intraepithelial abscesses [32]. Cell-wall preparations of *C. albicans* that contain mannan and/or mannoproteins have been shown to be potent modulators of neutrophil function [60, 61] and probably contribute to the apparent chemotaxis of these host cells to sites of infection, as shown in figures 3 and 4.

To examine the mobilization of host cells to sites of *C. albicans* infection in the gastric mucosa, we combined histopathology with immunofluorescence labeling of sectioned tissue [32]. Murine granulocytes, macrophages, and T lymphocytes were separately labeled with specific, cell surface-binding antibodies and then localized in sections of gastric

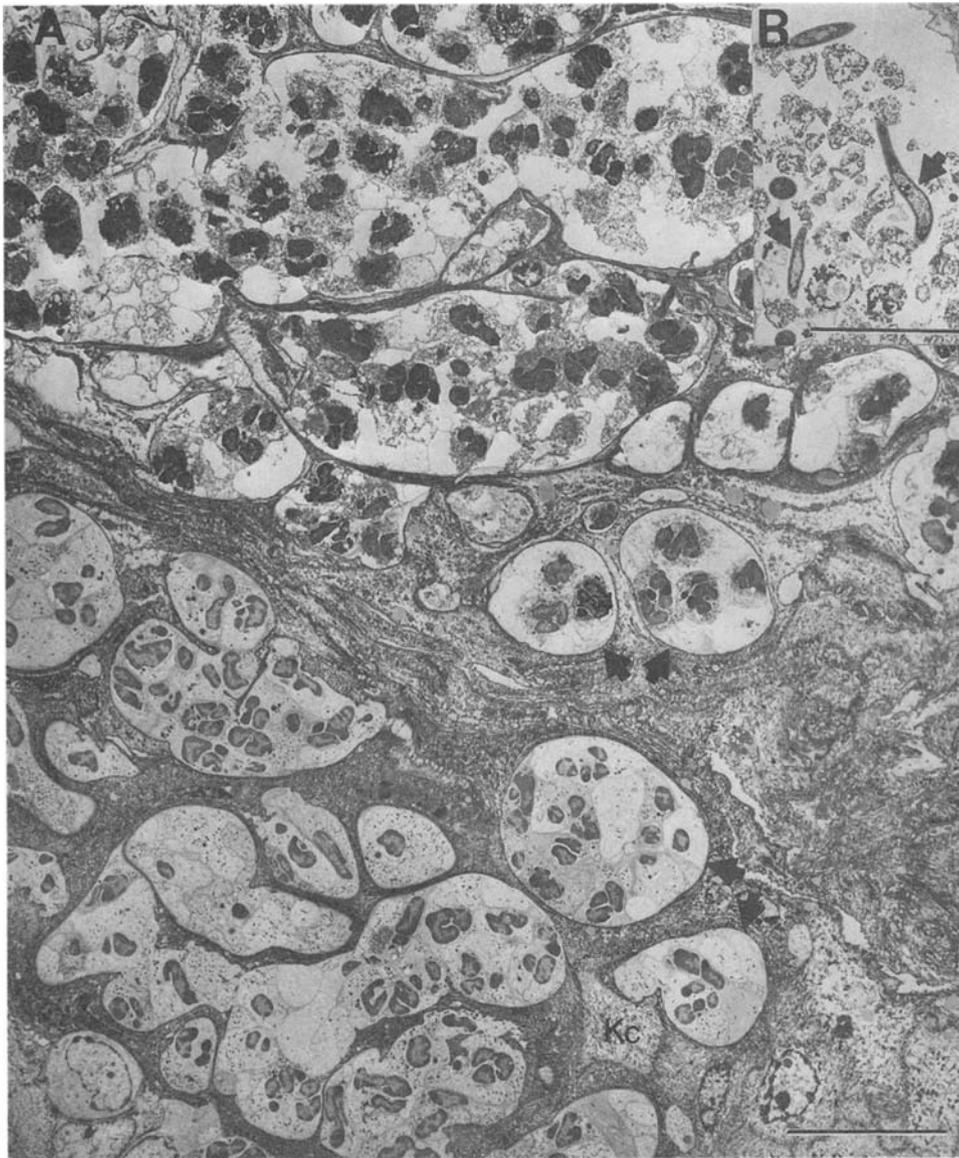


Figure 4. Intraepithelial abscess in gastric mucosa of a mouse infected with *C. albicans* and killed 3 weeks after inoculation. *A*, packets of neutrophils (arrows) are visible at the base of the abscess juxtaposed to keratinocytes (Kc) and remnants of epithelial cells. A thin layer of keratin encompasses the packets of neutrophils. *B*, the upper region of the abscess contains degenerated neutrophils and entrapped *C. albicans* cells (arrows). (Bars = 20 μm .)

tissue, after their reaction with the secondary antibody/fluorescein isothiocyanate conjugate and examination by fluorescence microscopy. Murine granulocytes were localized with use of a monoclonal antibody (clone RA 6-8C, raised in rats) to granulocyte-specific surface antigen (Caltag Laboratories, San Francisco). Macrophages were localized with a monoclonal antibody raised in rats to Mac-1 surface antigen (Caltag). T lymphocytes were localized with a monoclonal antibody (clone 53-2.1, raised in rats) to Th 1.2 surface antigen (PharMingen, San Diego).

Observations of the distribution of labeled granulocytes were conducted by fluorescence microscopy of histologic sections through the lamina propria of the gastric mucosa adjacent to and continuous with intraepithelial abscesses. Table 2 shows the results of analysis of the relative numbers of T lymphocytes and macrophages in selected regions of the murine gastric mu-

cosa 20 days after challenge with *C. albicans* strain CA30. Quantitative analyses of these same host cell types in labeled sections of noninfected mice were provided for comparison.

The data were compiled from examinations of segments of the gastric mucosa that were of equal width (0.16 mm). The location of each segment is indicated in the diagram above table 2. Ten sections (each 5 μm thick) were stained for either T lymphocytes or macrophages in each murine group (eight infected and eight noninfected CFW mice). The number of specific host cells, based on counts of fluorescent antibody-labeled T lymphocytes and macrophages, was determined for the designated area of the lamina propria and submucosa. The mean numbers of both host cells were significantly higher in infected mice than in noninfected mice for all segments in regions of both the lamina propria and submucosa. The greatest difference in number of host cells between infected mice and noninfected

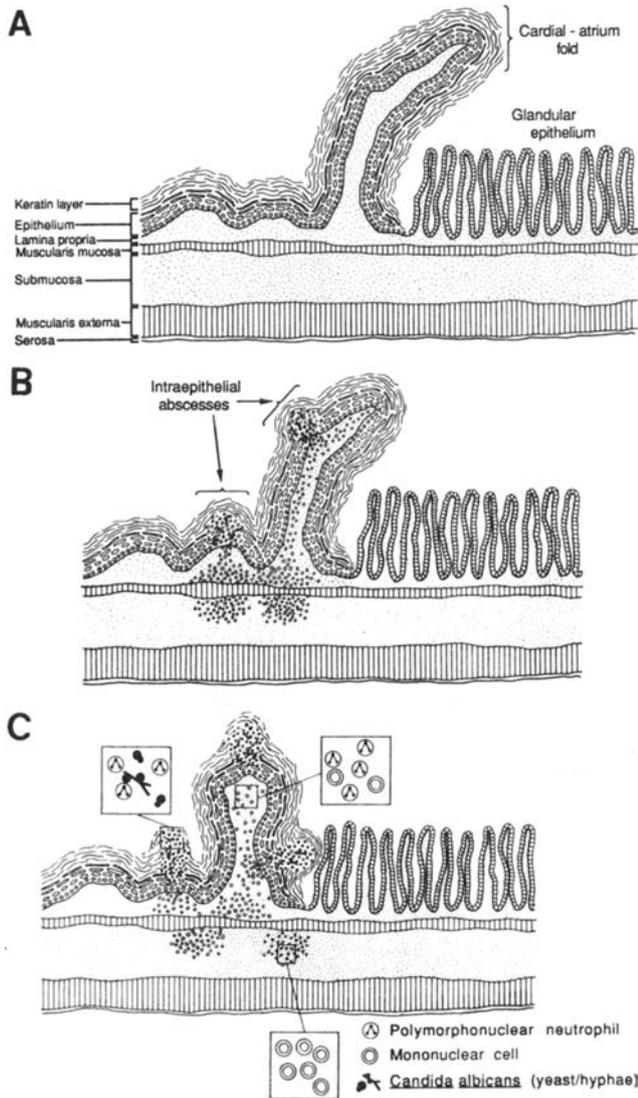


Figure 5. Comparison of histologic appearance of the gastric mucosa in the region of the cardiac-atrium fold in an untreated control mouse (A) and that of mice challenged with *C. albicans* strain CA30 by the oral-intragastric route at infancy and killed at 3 weeks (B) or 5 weeks (C) after inoculation.

mice was found in sections through the cardiac-atrium fold. On the basis of these data and results of conventional histopathology, it appears that cell-mediated host response to persistent GI candidiasis marshals the cooperative efforts of neutrophils, T lymphocytes, macrophages, and monocytes [32].

An attempt to summarize the coordinated responses of the host cellular defense network to *C. albicans* insult of the GI mucosa is presented in figure 6. Prominent in this scheme is the neutrophil, which directly interacts with the pathogen and apparently is the first cell to arrive at sites of infection. Neutropenia has been well documented as a condition that predisposes the host to candidiasis [58]. Considerable research is now focused on identification and characterization of neutrophil-acti-

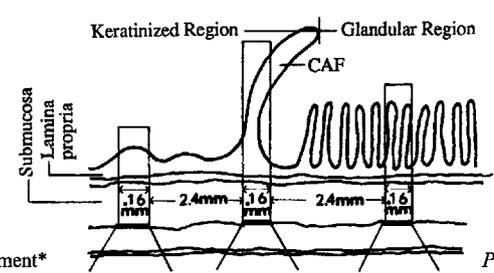
vating factors released from lymphocytes, monocytes, and macrophages that enhance the microbicidal function of neutrophils. Recombinant DNA technology has contributed significantly to the functional analyses of these macromolecules [65]. The availability of purified and recombinant cytokines has permitted critical assessment of the influence of these host products on chemotaxis and microbicidal activity of phagocytes. Clinical evaluations of the efficacy of recombinant cytokines as immunomodulators in therapeutic protocols for infectious diseases are underway.

GI and hematogenous candidiasis in immunocompromised mice. A murine model of hematogenous candidiasis was developed that has been suggested to simulate conditions of this disease in patients with leukemia and other highly immunocompromising disorders [25, 33]. Outbred CFW mice were inoculated with *C. albicans* by the oral-intragastric route as infants and then immunocompromised by intraperitoneal administration of cyclophosphamide and cortisone acetate at 11 and 14 days after inoculation. Systemic spread of the opportunistic pathogen to the liver, lungs, spleen, and kidneys occurred. Treatment of the animals with these immunosuppressive drugs resulted in alterations of the normal integrity of the epithelium of the gut as well as severe neutropenia. Approximately 55% of the animals with hematogenous candidiasis were found to have hepatic abscesses.

In accord with these observations, clinical studies have shown that for patients in various stages of recovery from neutropenia after induction and/or consolidation chemotherapy for acute leukemia and other malignant neoplasms, there is a high probability of development of hematogenous candidiasis localized to the liver and spleen [70–72]. Bodey and Anaissie [73] have suggested that certain patients with leukemia who are treated with cytarabine are at particularly high risk for development of hematogenous candidiasis because of the GI toxicity of this antileukemic drug. Different immunocompromising procedures were compared with use of the murine model to determine the relative risk of systemic infection by *C. albicans* originating from sites of colonization in the gut. The CFW mice were inoculated at infancy with *C. albicans* strain CA30, immunocompromised at 11–17 days, and killed 20 days after challenge.

A summary of the protocols that involved the use of different immunocompromising agents is presented in figure 7. Table 3 summarizes the effects of each agent on GI and hematogenous candidiasis. The most severe leukopenia resulted from the administration of cyclophosphamide plus cortisone acetate: 90% of the mice had hematogenous candidiasis, and the highest yeast counts were detected in their livers. No indication of invasion of the GI mucosa by *C. albicans* was observed in mice that received 5-fluorouracil (200 or 300 mg/kg) or cytarabine (arabinosyl cytosine) (20 mg/[kg · d]). Only when cytarabine was administered by continuous subcutaneous infusion over a 3-day period (35 mg/[kg · d]) (figure 7) was GI mucosal invasion detected, and then only in ~30% of the mice.

Table 2. Relative numbers of T lymphocytes and macrophages detected in the lamina propria and submucosa of the stomachs of *C. albicans*-infected and noninfected mice.



Immune cell type/region of gastric mucosa	Animal treatment*	1.6 mm	2.4 mm	1.6 mm	2.4 mm	1.6 mm	P value†	
T lymphocytes/lamina propria	Infected	11.6 ± 2.6	53.9 ± 12.8	29.4 ± 5.9				<.001
	Noninfected	3.1 ± 0.9	8.4 ± 1.7	3.2 ± 0.9				
Macrophages/lamina propria	Infected	27.3 ± 6.6	86.4 ± 15.0	27.8 ± 5.3				<.001
	Noninfected	6.4 ± 2.4	17.0 ± 6.0	5.9 ± 1.6				
T lymphocytes/submucosa	Infected	12.8 ± 3.3	30.5 ± 7.8	11.8 ± 2.1				<.001
	Noninfected	4.5 ± 1.1	3.9 ± 1.2	4.4 ± 1.2				
Macrophages/submucosa	Infected	29.8 ± 6.5	97.5 ± 27.5	14.6 ± 5.1				<.001
	Noninfected	6.4 ± 2.6	7.4 ± 3.1	2.3 ± 0.9				

NOTE. T lymphocytes and macrophages were identified in cryostat sections of the gastric mucosa by immunofluorescence with use of specific monoclonal antibody/fluorescein isothiocyanate probes, as described in the text. With regard to the diagram above: three areas of each histologic section through the region of the cardial-atrium fold (CAF) indicated in the diagram were examined. The same set of parameters (location of area examined relative to the CAF, width of segment, approximate total area, distance between segments examined) was used for enumeration of host cells in the gastric mucosa of infected and noninfected mice. Values are expressed as the mean (\pm SD) for 10 sections examined for each animal.

* Cri:CFW(SW)BR mice inoculated with *C. albicans* strain CA30 (2×10^8 yeast cells) by the oral-intragastric route (at age 6 days) were killed by asphyxiation with CO₂ at 20 days post-inoculation. Uninfected control animals were inoculated with saline alone by the oral-intragastric route at age 6 days and killed (as above) 20 days later.

† Statistical comparisons between infected and noninfected groups (eight mice each) were performed by the Student's *t*-test. *P* values of <.05 were considered significant.

Cyclophosphamide is known to be toxic to dividing cells in the GI tract and can alter the normal integrity of the mucosal epithelium of the gut [74, 25]. Corticosteroids cause impaired cell-mediated immunity that may encourage the development of GI and respiratory infections [75]. Oropharyngeal candidiasis is a common complication of the use of corticosteroids for pain relief, hormone therapy, and stimulation of appetite in patients with advanced cancer [76]. The toxic effects combined with severe leukopenia induced by the administration of cyclophosphamide plus cortisone acetate [77] probably accounted for the proliferation of *C. albicans* and their ability to invade the GI mucosa.

This murine model has been suggested to simulate patients with leukemia who have *C. albicans* in their GI tracts as a recently acquired or previously established indigenous microbial component of the gut flora and who are subjected to aggressive chemotherapy. In both humans and the murine model, leukopenia and GI mucosal damage resulting from chemotherapy predispose the host to hematogenous candidiasis of endogenous origin [78, 79].

Identification of noninvasive strains of C. albicans: Implications of defective virulence factor(s). Two clinical strains of *C. albicans*, identified as CA30 (from a patient with vaginal candidiasis) and CA87 (from a patient with severe oral thrush),

were tested for their ability to persist in the murine model (figure 2). It was evident that CA87 maintained higher numbers of cfu in the stomach and other parts of the alimentary tract (tongue and esophagus) [80] and remained longer in the GI tract of immunocompetent mice than did CA30. Mice with GI colonization established at infancy were compromised by intraperitoneal administration of cyclophosphamide plus cortisone acetate, as described in figure 7.

The histopathologic appearance of the gastric mucosa of mice infected with CA87 was distinctly different from that of CA30-infected mice (figure 8). Twenty days after challenge, it appeared that CA87 filaments were unable to penetrate the epithelial barrier of the gastric mucosa, and minimal neutrophilic response to the infection was evident (figure 8A, B). No hematogenous candidiasis was established in severely immunocompromised mice whose GI tracts were originally colonized by strain CA87 [32]. Strain CA30, on the other hand, invaded the submucosal tissue of the stomach, elicited a neutrophilic response (figure 8C, E), and established hematogenous candidiasis.

Three other clinical strains of *C. albicans* were tested in this same murine model for their ability to invade the gastric mucosa and reach the liver, presumably by dissemination via the hepatic portal vein [33]. Two additional, noninvasive strains

Figure 6. Proposed pathways of stimulation of murine-specific and nonspecific cellular responses in the gastric mucosa to *C. albicans* infection that contribute to clearance of GI candidiasis (data based on sources from [57] and [62–69]) (GM-CSF = granulocyte-macrophage colony-stimulating factor; MDNCF = monocyte-derived neutrophil chemotactic factor).

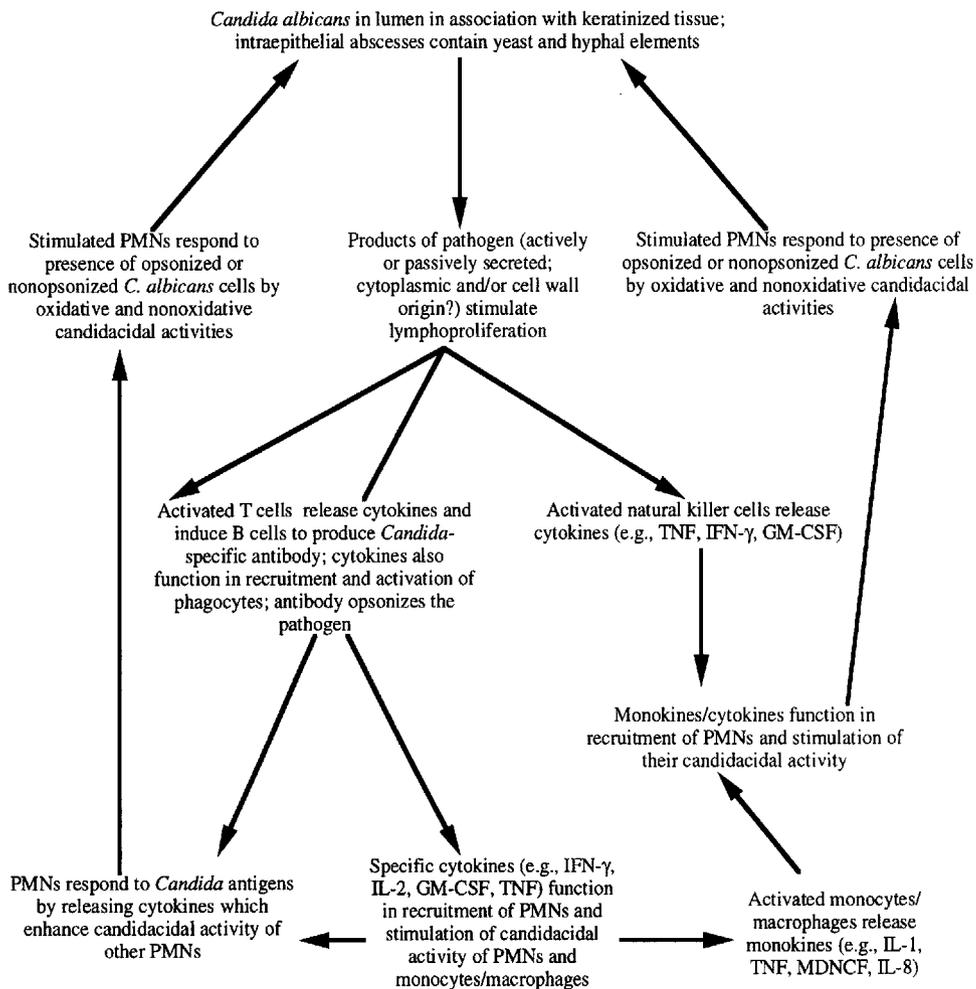


Figure 7. Summary of different immunocompromising procedures used after oral-intragastric inoculation of CFW mice with *C. albicans*. Wt = weight.

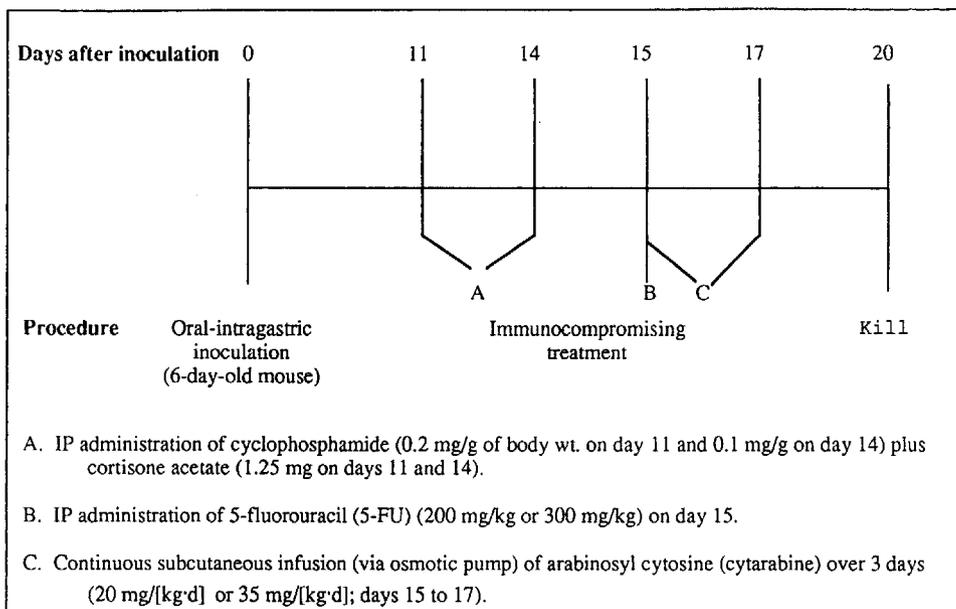


Table 3. Comparison of effects of cyclophosphamide/cortisone acetate, 5-fluorouracil, and arabinosyl cytosine treatment of mice with established GI colonization by *C. albicans*.

Treatment group	Mean cfu of <i>C. albicans</i> in homogenates of indicated organ*				No. of leukocytes per μL of peripheral blood	Histologic observations of gastric mucosa
	Esophagus	Stomach	Intestines	Other		
Control [†]	0.30×10^1 (4/15)	1.69×10^5 (10/10)	1.34×10^5 (0/10)	0 (0/20)	7,170 (± 891)	Hyphae + yeast but no invasion
Cyclophosphamide (0.2 mg/g + 0.1 mg/g) [‡] + cortisone acetate (1.25 mg twice)	1.79×10^2 (10/10)	1.57×10^7 (10/10)	5.67×10^5 (10/10)	7.31×10^3 (9/10 [Li])	1,270 (± 402)	Invasion
5-Fluorouracil [§] 200 mg/kg	4.51×10^2 (9/10)	1.14×10^7 (10/10)	4.25×10^6 (10/10)	100×10^1 (1/10 [Li])	3,050 (± 854)	Hyphae + yeast but no invasion
300 mg/kg	4.66×10^1 (6/10)	7.60×10^6 (8/8)	4.95×10^6 (10/10)	2.00×10^1 (1/10 [Lu])	1,550 (± 772)	Hyphae + yeast but no invasion
Arabinosyl cytosine 20 mg/(kg · d)	1.00×10^1 (1/2)	ND	1.20×10^6 (2/2)	3.80×10^1 (1/2 [Lu])	2,300 (± 424)	Hyphae + yeast but no invasion
35 mg/(kg · d)	3.60×10^2 (7/7)	8.94×10^6 (5/5)	5.49×10^4 (7/7)	9.55×10^2 (2/7 [Lu])	2,133 (± 611)	Invasion

NOTE. The mice were killed at 20 days post-inoculation. [Li] = (liver); [Lu] = (lungs); ND = not determined.

* In parentheses: no. of *C. albicans*-positive mice per no. tested.

[†] Infected with *C. albicans* by oral-intragastric inoculation at age 6 days but given no immunocompromising drug treatment.

[‡] Intraperitoneally administered (0.2 mg per gram of body weight on day 11 and 0.1 mg/g on day 14), plus cortisone acetate (1.25 mg on days 11 and 14 post-inoculation).

[§] Intraperitoneally administered on day 15 post-inoculation.

^{||} Continuous subcutaneous infusion (via osmotic pump) over 3 days (days 15–17 post-inoculation).

were identified in this cursory survey of our collection of clinical isolates (table 4).

Strains CA87 and CA30 were compared in vitro with use of a series of assays proposed to evaluate their relative virulence in a mammalian host [81–84]. The only major difference detected was that CA30 (invasive strain) had an approximately 3.6-fold higher level of phospholipase production in vitro than did CA87 (noninvasive strain). The other noninvasive strains of *C. albicans* cited in table 4 (CA88, CA77) showed comparable low levels of phospholipase production. In collaboration with B. Hube (University of Aberdeen, Scotland), we also found that strains CA87, CA88, and CA30 showed expression of *sap2*, *sap4*, and *sap5* genes in vitro by northern blot analyses. These data suggested that the noninvasive strains were not defective in aspartyl proteinase production.

Results of early assays of phospholipase secretion by *C. albicans* during infection of chick chorioallantoic membranes revealed that both phospholipase and lysophospholipase are released at the surface of yeast cells and tips of hyphae during tissue invasion [85]. If the activities of these hydrolases are essential for crossing the bowel wall from the lumen of the gut, it follows that strains with very low levels of phospholipase

activity may not be able to establish hematogenous candidiasis in mice challenged via the oral route. Efforts are under way to identify and clone the genes that encode these enzymes, which will ultimately permit evaluation of the significance of secreted phospholipase and lysophospholipase in the pathogenesis of *Candida* species [81].

Applications of murine models of GI and hematogenous candidiasis to evaluation of candidacidal drugs. Two protocols have been used to test the efficacy of antifungal drugs in treatment of murine GI and hematogenous candidiasis. In the first protocol, mice were immunocompromised with cyclophosphamide plus cortisone acetate, as described in figure 7. The data presented in table 3 indicate that in 90% of these mice, hematogenous candidiasis had developed by 24 days after inoculation. Antifungal drugs were administered by gavage twice per day for 8 days (24–31 days after inoculation). The mice were killed 13 days later, and aliquots of tissue homogenates of their esophagus, stomach, intestines, and liver were dilution-plated to determine cfu of *C. albicans* [25, 33]. To exemplify the application of this immunocompromised murine model to antifungal drug evaluation, the results of tests with two concentrations of fluconazole follow. Fluconazole (10 mg/kg twice per

Figure 8. Histopathologic appearance of gastric mucosa of CFW mice infected with *C. albicans* strain CA87 (A, B) or CA30 (C–E), 20 days after oral-intragastric inoculation at infancy. The mice were severely compromised by immunosuppressive drug treatment between 11 and 14 days after challenge. Note the absence of deep hyphal invasion of the mucosa by strain CA87. Arrow in B shows hyphal association with the epithelial cell membrane. Note contrasting submucosal invasion and neutrophil response after challenge with strain CA30 (C) and apparent necrosis of host tissues (D, E) (CLC = chlamydospore-like cell of strain CA30). (Bars in A–D = 50 μm; bar in E = 5 μm.)

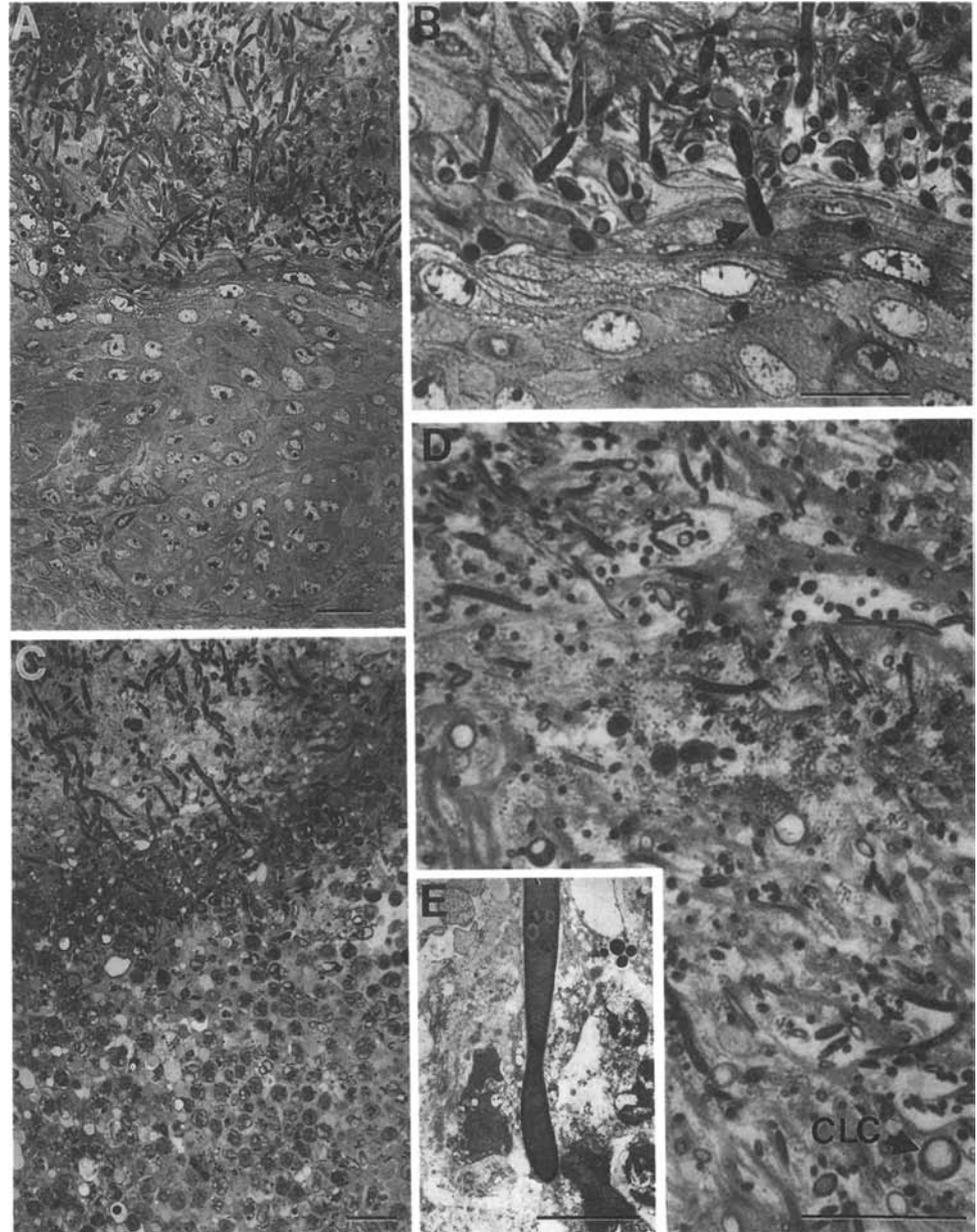


Table 4. Identification of invasive and noninvasive strains of *C. albicans*.

Strain of <i>C. albicans</i>	Detection of cfu in liver: No. positive/total no. of mice	Histopathologic evidence of invasion
CA87	0/7	–
CA88	0/11	–
CA77	0/7	–
CA30	7/7	+
CA85	1/6	±

NOTE. Neonatal CFW mice were inoculated by the oral-intragastric route with 10^8 yeasts and immunocompromised at 11 and 14 days after inoculation with cyclophosphamide plus cortisone acetate. – = no gastric mucosal invasion detected; + = extensive invasion detected; ± = minor invasion detected.

day) was delivered by gavage in distilled water and resulted in clearance of the esophagus and liver of the 18 mice examined. However, residual organisms were detected in the stomach of five mice (mean, 1.4×10^3 cfu; range, 6.0×10^1 – 2.8×10^3 cfu) and in the intestines of five (1.1×10^2 cfu; 2.0×10^1 – 2.4×10^2 cfu). When fluconazole (20 mg/kg) was administered by the same regimen, the GI tract and liver of 15 mice tested were cleared of *C. albicans* by 44 days after inoculation.

The second protocol was designed to test for relapse of GI and hematogenous candidiasis after treatment with a candidacidal drug. The mice received two separate doses of cyclophosphamide plus cortisone acetate: the first at 15 and 18 days (as described in figure 7) and the second at 37, 39, and 41 days after *C. albicans* inoculation. The concentration of cyclophos-

phamide used at 37, 39, and 41 days was 0.1 mg per gram of body weight. As above, the antifungal drug was administered for 8 days (24–31 days after inoculation). It was expected that if some organisms remained in the GI tract after the antifungal drug treatment, then cfu of *C. albicans* would be detected in liver homogenates after the second severe immunocompromising procedure.

In this murine model, we tested the efficacy of an early echinocandin preparation (Cilofungin; Lilly Research Laboratories, Indianapolis) for clearance of *C. albicans* CA30 from the esophagus, stomach, intestines, liver, and kidneys. The antifungal drug was delivered by infusion over an 8-day period by means of a subcutaneously implanted osmotic pump (Alza, Palo Alto, CA). The pump was surgically implanted 24 days after inoculation [25] and removed on day 31. The initial concentration of antifungal drug in the 7-day osmotic pump was 50 mg/kg.

In the 15 Cilofungin-treated mice that had received only a single dose of immunocompromising drugs (i.e., first protocol), the liver and kidneys were cleared of *C. albicans*, but cfu were detected in plated homogenates of the esophagus of 2 mice (mean, 1.0×10^1 cfu), the stomach of 15 (2.6×10^3), and the intestines of 13 (2.4×10^3). Mice subjected to a second immunocompromising treatment after administration of Cilofungin acquired hematogenous candidiasis; organisms were detected in the liver of ~50% (7) of the mice (mean, 5.4×10^2 cfu), and proliferation of *C. albicans* was detected in the esophagus of 11 (1.7×10^2), the stomach of 15 (9.7×10^5), and the intestines of 15 (1.6×10^5).

This model simulates the patient with cancer (e.g., leukemia) who presents with hematogenous candidiasis following an original regimen of chemotherapy, whose condition is successfully managed with antifungal treatment, and who then has a relapse with severe disseminated candidiasis following subsequent chemotherapy. These and other animal models of hematogenous candidiasis can provide a “scientific foundation and guide for development of clinical protocols investigating new approaches to prevention and treatment of invasive candidiasis in granulocytopenic patients” [86].

A third untested application of the murine models described in this review is use of immunocompetent mice with persistent GI candidiasis (e.g., C57BL/6 mice) to evaluate the effects of immunomodulators and prophylactic antifungal agents in the clearance of *C. albicans* before immunosuppressive drug treatment. Other animal models have been used to explore the effects of preventive, early, and late antifungal therapy in immunocompromised hosts with established candidiasis [87–89]. This is a promising area of clinical research that focuses on the management of candidal infection in the severely immunocompromised host.

Gastrointestinal and Hematogenous Candidiasis in Humans

The clinical spectrum of GI candidiasis in humans. GI candidiasis in humans can involve practically any site of the

alimentary tract. Candidal infection of the oral cavity can manifest as oral thrush, acute atrophic candidiasis, chronic atrophic candidiasis, or angular cheilitis [8, 37, 90]. Esophageal involvement can be part of thrush extending from the oral cavity to the anus, but in two-thirds of all cases, the esophagus is the only site involved [8, 9, 37, 90, 91]. The stomach is the second most commonly involved site [37, 91]. Gastric involvement can take the form of thrush or, more commonly, mucosal ulceration. Most cases of gastric candidiasis are diagnosed at autopsy. Involvement of the small and large bowels by *Candida* species occurs much less frequently than does involvement of the esophagus or the stomach [2, 37, 90–92]. Ulceration and pseudomembrane formation are the most frequent presentations of intestinal candidiasis. As with gastric candidiasis, intestinal involvement is most commonly diagnosed at postmortem examination.

Three commonly occurring species of *Candida*—*C. albicans*, *C. albicans* var. *stellatoidea*, and *C. tropicalis*—have been shown to be responsible for serious, potentially life-threatening opportunistic infections in immunocompromised or otherwise debilitated patients. Rüchel and co-workers [38] reported that *Candida glabrata* ranked second to *C. albicans* in frequency among *Candida* yeasts isolated from clinical specimens. These investigators indicated that coisolation of *C. albicans* and *C. glabrata* was common. Little is known about the nature of interaction between the host GI mucosa and *Candida* species other than *C. albicans*.

The role of the GI tract in disseminated candidiasis. Endogenous *Candida* organisms in the GI tract seem to be the most important source for dissemination of the opportunistic pathogen [6, 37, 93]. In a prospective study of 139 neutropenic patients with hematologic malignancies, Martino et al. [94] showed that invasive candidiasis was documented in 22.2% of patients who had been colonized by *Candida* species at multiple sites, 4.8% of patients who had been colonized at a single site, and none of the patients who had not been colonized. Similar results were reported in a retrospective study of patients with cancer [95]. In that study, hematogenous candidiasis had developed in 0.5% of patients who had not been colonized and in 32% of those who had been colonized at multiple sites. Nosocomial transmission of candidal infection via the hands of health care workers has also been described [96, 97]. Tollemar et al. [98] reported similar findings in recipients of bone marrow. Hence, candidal colonization of the GI tract is a prerequisite for the development of invasive candidiasis.

GI tract involvement has been documented in autopsy studies of disseminated candidiasis. Eras et al. [90] noted that of 2,517 autopsies performed, 109 revealed histologically documented candidal involvement in the GI tract. GI candidiasis was found in 76 (13%) of 586 cases of leukemia or lymphoma, and GI candidal involvement was found in 33 (1.6%) of 1,931 cases that were not associated with leukemia or lymphoma [91]. Similar results have been reported by Walsh and Merz [92]. Contrary to common belief, the stomach and esophagus are

more commonly involved than the rest of the GI tract, and the gastroesophageal junction is the site most frequently colonized [37, 90, 91].

Colonization is an essential first step in the pathogenesis of disseminated candidiasis [37, 94, 95, 99, 100]. Antibiotics, corticosteroids, H₂-blockers, antacids, T cell dysfunction, burns, GI surgery, oral contraceptives, diabetes mellitus, irradiation, and chronic renal disease are most frequently cited as risk factors for colonization by *Candida* species [24, 37, 96, 101–108]. Many of these factors may coexist in the same patient, making evaluation of the contribution of each factor a difficult task.

Once colonization has been established, *Candida* species organisms are able to cause a localized infection, particularly if there is a disruption in the integrity of the GI mucosa. Autopsy studies seem to support this notion [91, 92]. Disruptions of the mucosal lining of the GI tract aid the organisms in establishing a localized infection and predispose to dissemination. The most commonly recognized factors that disrupt the GI barrier include cytotoxic chemotherapy, especially with cytarabine [37, 73, 100], as well as graft-versus-host disease [109] and hypotension [96]. *Candida* species organisms have been demonstrated in blood vessels, lymphatics, and macrophages within 30 minutes to a few hours following intestinal inoculation [78, 110, 111]. The mechanism by which the organisms reach the circulatory system is not clear. Translocation to the mesenteric lymph nodes has been proposed [37, 78, 93, 111–113]. There seems to be a critical inoculum size, above which *Candida* species organisms will readily cross the GI tract barrier [37, 93, 99, 110, 111]. Another possible mechanism of dissemination of *C. albicans* is direct hyphal invasion, especially if localized mucosal infection is established [37, 92, 114].

Candidiasis in the immunocompromised host. For disseminated infection to be established, the GI tract must be confronted with a very large inoculum [110] or the host's immunity must be impaired [37, 100, 114]. Studies in humans have shown a direct correlation between the level (and duration) of neutropenia and the host's susceptibility to disseminated candidiasis [37, 114]. Patients with impaired T-cell function (e.g., those with AIDS) have increased susceptibility to superficial candidal infection, but dissemination occurs very rarely in this subset of patients [8, 37, 114]. As discussed in relation to our murine models, this demonstrates the importance of neutrophil function in prevention of candidal dissemination.

Conclusions

Because of our reliance on experimental models of candidiasis, considerable progress has been made in understanding the pathogenesis of *Candida* species and the host's response to these fungi. Evidence derived from animal and clinical studies supports the contention that the GI mucosa is a common portal of entry of *Candida* species organisms into the bloodstream and organs. These studies have also shown that in both animals

and humans the gastroesophageal junction is the most commonly colonized site of the GI tract. Human and animal host responses are characterized by the development of microabscesses in which there is a predominance of neutrophils.

Factors that predispose both humans and animals to candidal infection and dissemination include increased colonization by *Candida* species because of prolonged use of broad-spectrum antibiotics, disruption of the GI barrier resulting from the effects of cytotoxic drugs or hypotension, and immunosuppression resulting from neutropenia or T cell dysfunction. Candidiasis in severely immunocompromised patients is sometimes overwhelming because of preexisting colonization of portions of the alimentary tract by *C. albicans* as a component of the indigenous flora. Management of mucosal candidiasis is often problematic because of recurrent infections and the appearance of drug-resistant strains. Prophylactic drug therapy is a rational approach to the prevention of candidiasis in patients who are predisposed to infection as a result of chemotherapy. Prevention of mucosal infection through use of a vaccine against *Candida* species is a more ambitious approach that is currently under investigation in animal models.

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