Adherence of germ tubes of *Candida albicans* to tissues from immunocompromised mice

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Abstract

The influence of immune status of the host on binding of germ tubes of *Candida albicans* to murine tissue sections in an ex vivo assay was examined. Generally, germ tubes appeared randomly adhered to the tissues examined and binding was unaffected by immunodeficiency induced by treatment with cyclophosphamide and cortisone acetate. Adherence was somewhat reduced in spleen and kidney sections or increased in liver sections and unchanged in lymph node sections from treated mice compared to sections from control animals. Scanning electron micrographs showed organisms appeared to be loosely or tightly bound to the surface or partially embedded in spleen sections from both control and treated mice. These observations suggested that qualitative and quantitative difference in adhesion of germ tubes to various tissues may contribute little to the susceptibility of the immunodeficient animal to candidal infection.

Keywords: *Candida albicans*; Immunocompromisation; Adherence

1. Introduction

*Candida albicans* is both a commensal and an agent of opportunistic disease. A number of factors, including immune status, affect the susceptibility of the host to disease. Infection of the oropharynx and esophagus has frequently been reported among patients with acquired immunodeficiency syndrome (AIDS) [1], while disseminated disease is more commonly encountered in immunosuppressed individuals with reduced neutrophil function [2]. The role of the fungus in this interaction is not completely passive and a number of virulence factors have been postulated [3]. The ability of the organism to undergo a yeast-to-hyphal transition is one of the most commonly attributed virulence traits [3]. The cell wall of germ tubes appears to acquire special features including new surface antigens [4–6], induction or enhanced expression of surface proteins, some of which promote interaction with host tissue and proteins [7–11], and increased expression of cell surface hydrophobicity (CSH) [12–14]. Adherence of the microbe to tissue is a parameter in colonization and infection which has received much recent attention [3,15,16].

The ex vivo assay has been used to examine both host and fungal parameters of adhesion [17–25]. Yeast cells with hydrophilic surfaces utilize mannan
structures to adhere primarily to macrophages in the marginal zones of spleen sections and subcapsular regions and trabecular sinuses of lymph node sections [17–21]. Adhesion to kidney sections is associated with several morphological features and adhesion appears random in liver. Hydrophobic yeast cells and germ tubes bind randomly throughout the tissues with little morphological specificity [22,23]. Host factors which may influence binding have also been examined [24,25]. Adhesion of hydrophilic yeast cells to tissues from mice rendered immunodeficient due to genetic alteration, irradiation or treatment with cyclophosphamide and cortisone acetate is generally reduced to spleen but not to lymph node and kidney sections compared to sections from immunocompetent mice [24,25]. Binding of yeast cells to macrophages has been suggested to be a clearance mechanism for the normal host. In this report we have investigated the effect of cortisone acetate and cyclophosphamide induced immunodeficiency on adhesion of germ tubes to tissue sections from spleen, lymph node, kidney and liver.

2. Materials and methods

2.1. Organisms and culture conditions

C. albicans 3153A (serotype A) was grown and germ tubes prepared as previously described [23]. Germ tubes induced in the medium of Lee et al. [26] were harvested, washed in sterile saline and Dulbecco’s modified Eagle medium (DMEM), and resuspended at approximately 5 x 10⁶ organisms per ml in DMEM with 5% calf serum. The organisms were maintained on ice and sonicated for 2 min in a water bath sonicator prior to use.

2.2. Ex vivo assay

Male BALB/c mice (23–28g) (3–6 per group) were compromised by treatment with cyclophosphamide (0.2 mg per g body weight) and cortisone acetate (1.25 mg) injected intraperitoneally on day 1 as described previously [20,27]. A second injection of cyclophosphamide (0.01 mg per g body weight) and cortisone acetate (1.25 mg) was administered on day 4. The animals were sacrificed on day 9 and the organs rapidly removed and immediately frozen. Control animals (3 per group) received injections of equal volumes of sterile saline. Total leukocyte counts and neutrophil proportion were determined on blood samples drawn from the tail vein on day 0 and day 8. Cryostat sections (10 μm) of each organ were obtained and transferred to slides for the assay [17]. Tissue sections from 2–3 animals were prepared on each slide and 2–3 slides were prepared for each assay. The binding assay was performed in triplicate for each tissue.

Tissue sections were incubated with 120 μl of germ tube suspension prepared as above for 15 min at 4°C as we described previously [23]. Sections were washed in Dulbecco’s phosphate-buffered saline, fixed in 1.5% glutaraldehyde, rinsed in water and stained with periodic acid, Schiff’s reagent and hematoxylin.

Slides were coded and adherent germ tubes quantified by light microscopy. Slides were counted by three individuals and 8–13 randomly selected fields were counted for each tissue section, except for lymph node sections where fewer fields were available. Liver, kidney, and lymph node sections were counted at 200 × magnification. Spleen sections were counted at both 100 × and 200 × magnification. All adhered germ tubes were counted as described previously [23]. The Student’s t-test (P < 0.05) was used to compare each tissue from control and immunocompromised animals using the log₁₀ value of each observation for either a single counter or combined counts. Results from the three counters were in agreement for the effect of immunodeficiency on adherence, although some difference in magnitude of adherent cells was noted. Results presented reflect the combined analysis.

2.3. Scanning electron microscopy (SEM)

SEM was performed as described previously [23]. Briefly, tissue sections with adhered fungi were fixed overnight in 3% glutaraldehyde in phosphate buffered saline (PBS) at 4°C. The sections were washed in PBS, dehydrated in a graded ethanol series, critical point dried with liquid CO₂ and sputter coated with gold. Tissues were examined with a Hitachi S500 scanning electron microscope (Hitachi, LTD. Tokyo, Japan).
3. Results

3.1. Immunosuppressive treatment

The immunosuppressive treatment employed has been shown to render an immunocompetent mouse colonized with \textit{C. albicans} in the gastrointestinal tract susceptible to endogenous infection \cite{27}. Infection was reported in several tissues, including spleen, liver, and kidney. In this study, during the treatment period, mice receiving drugs lost weight (Fig. 1A) and the leukocyte count was reduced and neutrophils severely depleted (Fig. 1B). Spleens removed from drug treated animals were generally observed to be smaller than those of control animals.

3.2. Ex\textit{ vivo} binding assay

Germ tubes appeared bound randomly to spleen, lymph node, kidney and liver tissue sections from both treated and control animals as observed by light microscopy (data not shown). Areas of white pulp in spleen frequently appeared smaller in sections from treated animals compared to control sections in agreement with a previous report \cite{25}. A few marginal zone profiles appeared to have enhanced binding of germ tubes. In kidney sections, although germ tubes were bound over all tissue, many glomeruli appeared to have bound organisms as previously noted \cite{23}. Quantitative analysis of binding showed that adherence to spleen and kidney sections

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![Graph A](image1.png)

**A**

![Graph B](image2.png)

**B**

Fig. 1. Effect of immunosuppressive treatment on weight, total leukocyte counts and neutrophil population. Weight of the treated and control animals during the treatment period is shown in panel A. Leukocyte (WBC) counts and total neutrophil (NEUT) for control and drug-treated animals obtained before and after treatment are shown in panel B.
from drug-treated animals was significantly reduced compared to sections from control animals while it was significantly increased to liver sections and unchanged for lymph node sections (Fig. 2). The small magnitude of these differences was approximately 16%, 26% and 20% for spleen, kidney and liver tissue sections respectively.

3.3. SEM

The distribution of splenic macrophages was determined by immunocytochemical staining and compared to distribution of adhered germ tubes. Greatest intensity was observed in marginal zone areas around white pulp. Marginal zones generally had a larger radius in control tissues and appeared to be thicker around the smaller white pulp center observed in treated tissue sections data not shown, [25]. This distribution differed from the apparently random distribution of adhered germ tubes. The attachment of germ tubes to tissue was examined in more detail with SEM. As previously described for normal tissue [23], three patterns of adhesion were observed in both control and drug treated tissue sections with germ tubes appearing (1) to be loosely attached or lie on the surface (Fig. 3A); (2) to be more tightly bound to the surface (Fig. 3B); or (3) to be embedded in the tissue (Fig. 3C, D). Some of these embedded germ tubes appeared to be lying in cavities which conformed to their contours (Fig. 3D) while some appeared to have portions of the hyphal extension of the germ tube below the tissue surface (Fig. 3C). As reported previously [23], germ tubes appeared to bind to tissue with both the parent yeast cell and the hyphal extension. In clumps containing loosely and tightly bound germ tubes, a few germ tubes were observed to lie on top or adhere to other germ tubes while others looped over germ tubes before contacting tissue (data not shown).

4. Discussion

The ex vivo assay permits an examination of both qualitative and quantitative aspects of adherence. Comparison by Brawner and Mori [25] of the qualitative aspects of adherence of hydrophilic yeast cells showed that induced immunodeficiency did not alter the apparent specificity of binding to macrophage in the splenic marginal zone or subcapsular regions and trabecular sinuses of lymph node or the lack of specificity in binding to kidney tissue [25]. In this study similar treatment did not alter the apparently random adhesion of germ tubes to all examined tissues, spleen, lymph node, kidney and liver (data not shown). In addition, the tissue interaction revealed by SEM was similar for treated and untreated tissues (Fig. 3). Thus it is unlikely that the susceptibility of the immunocompromised host to candidal infection is attributable to a qualitative change in adhesion of germ tubes. The distribution of germ tubes bound to tissues is similar to the distribution of hydrophobic yeast cells to tissues from mice with an intact host defense [22]. Germ tubes express CSH [12,13] and thus adhesion of hydrophobic yeast cells and germ tubes may be similar.

Some quantitative aspects of adhesion to tissue were altered by the treatment with cyclophosphamide and cortisone acetate. The reported reduction in adhesion of hydrophilic yeast cells to lymph node and kidney tissue (about 13% and 26% respectively) was not significant [25]. In this study, adhesion of germ tubes was reduced to kidney (26%), unchanged to lymph node and increased to liver (20%) tissue (Fig. 2). The extent to which differences of this magnitude could substantially contribute to susceptibility is unclear, particularly when
a decrease in binding would, at least superficially, not seem to have infection promoting effects in kidney. Adhesion of hydrophilic yeast cells to treated spleen tissue was reduced 10-fold compared to normal tissue [25] while in this study germ tube adhesion was minimally reduced (16%, Fig. 2). Immunocytochemical localization of macrophages did not reveal sufficient changes in the macrophage pop-

Fig. 3. SEM of germ tube adhesion. SEM shows germ tubes of *C. albicans* bound loosely to spleen tissue from a drug-treated animal (A), bound tightly to spleen tissue from a control animal (B) and embedded in liver tissue from a drug-treated animal (C), and a clump of germ tubes bound to spleen tissue from a drug-treated animal (D). Bar is 10 μm for all panels.
ulation or location [25] (data not shown) to account for this decreased adherence. Brawner and Mori [25] postulated that adhesion of yeast cells to splenic macrophages is a clearance mechanism in the normal host and that a decrease in binding to macrophages in the immunocompromised host may have infection-promoting effects by permitting uncleared organisms to infect other tissues. The lack of enhanced binding of germ tubes to tissue regions enriched in natural host defense cells may reduce accessibility of this form of the fungus to host defense. However, the small reduction in germ tube adhesion to sections of treated tissue seemed insufficient to account for the susceptibility of an immunocompromised host.

The establishment or clearance of a microbe from host tissue is the result of the outcome of the virulence attributes of the pathogen and the defense properties of the host. In the case of opportunistic pathogens, such as C. albicans, normal defense is sufficient to prevent infection and in the normal host this particular organism may establish itself as a commensal. It is probable that it is yeast cells, commonly observed in normal colonization, which hematogenously seed other tissues. A number of factors, including the ability of the organism to form germ tubes and to induce or enhance expression of various surface components, is thought to be important in pathogenesis [2,3,16]. During infection, the host may be confronted with both forms of the organism. This study showed that qualitative and quantitative aspects of adherence of the hyphal form of C. albicans to tissue were not substantially altered by immunodeficiency. While the sum of small changes in the balance between microbe and host may affect the outcome of the interaction, altered adherence of germ tubes to tissue may not be a major contributor to susceptibility, and additional interactions such as altered adherence of the hydrophilic yeast form to spleen tissue [25] and loss of leukocytes and neutrophils are likely to be more important in the susceptibility of the immunodeficient animal to candidial infection.

References


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