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How the host fights against *Candida* infections

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TABLE OF CONTENTS

1. ABSTRACT

Candida albicans is the predominant cause of both superficial and invasive forms of candidosis, although the proportion of serious infections attributed to other members of the genus is rising.. The spectrum of host defences include cell mediated immunity which is comprised of cytokine release by lymphocytes and activation of natural killer cells and lymphocytes by interleukins.. An increasing body of evidence supports a role for specific antibody in protection against invasive *Candida* infection. Clinical observations indicate that mucocutaneous *Candida* infections are commonly associated with defective cell-mediated immune responses. Innate immunity is the dominant protective mechanism against disseminated candidosis. Quantitative and qualitative abnormalities of neutrophils and monocytes are associated with systemic candidosis. In the present review virulence factors and the spectrum of immune responses are discussed in relation to the perspective for the development of appropriate vaccines against *Candida.* Here we present an overview of toll-like receptor signalling, cellular-dependent responses, the role of specific antibodies in protection against *Candida*, and the array of immune mechanisms that operate in gastrointestinal, vaginal and oral candidosis.

2. INTRODUCTION

Yeasts of the genus *Candida* are part of the normal human flora and typically grow on mucosal surfaces, such as those in the mouth and vagina. They can cause a variety of different superficial diseases, including a spectrum of mucosal infections including oropharyngeal, oesophageal, vulvovaginal, and cutaneous candidosis; as well as systemic disease (1). There is however, considerable variation in the range of organisms isolated in different hospitals and different patient groups. *C. glabrata, C. krusei, C. parasilosis* and *C. tropicalis* have become significant causes of human infection. Other species, such as *C. dubliniensis, C. guilliermondii, C. inconspicua, C. lusianiae, C. norvegenis* and *C. rugosa*, have been isolated from occasional patients. *C. albicans* is found in the mouth and gastrointestinal tract of around 30-50% of normal individuals, but much higher isolation rates have been recorded among patients receiving medical attention, including the elderly and those of fragile health. Although *C. albicans* is still the most prevalent species recovered from normal and sick individuals, it appears to be less common in the environment than many other *Candida* species.

2.1 Virulence of Candida

Properties associated with virulence and pathogenicity include adhesins and a range of secreted

hydrolases. The role of dimorphism in virulence is not clear. *Candida* mutants that are unable to form filaments are less virulent, although conversely, mutants that are unable to grow as yeast are also less virulent, suggesting that both forms are needed for pathogenicity. Most lesions contain both forms, although some infections contain only yeasts, suggesting that filaments are not necessary for virulence. Several surface-expressed and secreted proteins, such as adhesion molecules, especially mannoproteins, and secreted proteolytic enzymes, contribute to the pathogenicity of *C. albicans*. In addition, morphogenic changes during yeast to hypha transition are considered essential for the infection process. Hyphal growth can be induced by a shift of pH or change in temperature. Hyphal forms are elongated and substantially differ morphologically from the round cellular forms. Furthermore, during hyphal growth, the expression of new surface antigens causes increased adhesion of hyphae to the host cells and facilitates tissue penetration. In order to appreciate fully the pathogenesis of candidosis and host defence mechanisms it is useful to present an overview of the various aspects of *Candida* virulence and the complex immune mechanisms involved in defense against this organism. Detailed reviews can be found in the edited monograph *Candida and Candidiasis (*2).

2.2. Tissue interactions

Since *C. albicans* is the predominant cause of virtually all types of candidosis, the interactions of this organism with epithelial cells have been studied in the greatest detail. Two different mechanisms of oral epithelial cell invasion have been described. One mechanism is the production of lytic enzymes, such as secreted aspartyl proteinases (SAPs), by the organism (reviewed in reference 3). It has been proposed that these enzymes digest the surface of the epithelial cell and thereby provide an entrance into the cell. SAPs may be especially important for the invasion of keratinized epithelial cells. *C. albicans* mutants containing disruptions of various SAP genes have a reduced capacity to damage vaginal and oral epithelial cells *in vitro*. As epithelial cell invasion is likely to be a precursor of epithelial cell damage, it is possible that the reduced damage caused by the sap-null mutants is due in part to decreased epithelial cell invasion. Consistent with the *in vitro* results, many of these mutant strains have attenuated virulence in the rat model of vaginal candidosis. It is also possible that C. *albicans* SAPs play a role in tissue penetration by facilitating the passage of the organism between host cells. In this regard, the SAPs of *C. albicans* appear to be functionally similar to the cysteine proteases of *Porphyromonas gingivalis* and group A *Streptococcus*, which enable these bacteria to invade and damage epithelial cells.

At certain mucosal sites, such as the oesophageal mucosa, demonstration of fungal invasion is required for definitive diagnosis of infection, since *C. albicans* is also a commensal colonizer of mucous membranes. Moreover, at these sites the extent of fungal invasion has been shown to correlate well with the severity of infection. Fungal invasion of the superficial layers of the oral epithelium is found in human cases of advanced immunosuppression and in animal models of oropharyngeal candidosis. The tissue invasion capacity of *C. albicans* correlates with its ability to stimulate a strong inflammatory response by oral mucosal cells (4). Although the role of invasion in the virulence of *C. albicans* has been demonstrated, the mechanism by which *C. albicans* invades the mucosa is not understood fully.

Adhesion complexes, known as adherens junctions, contribute to the integrity of the epithelium. An adherens junction is a specialized region of the plasma membranes of two adjacent cells in which cadherins act as adhesion molecules, linking together the actin cytoskeletons of the cells. Proteolytic breakdown of E-cadherin, the predominant protein in epithelial adherens junctions, has been proposed to be a mechanism of invasion of the intestinal mucosa and the oral mucosa. A recent study has shown that E-cadherin is proteolytically degraded during the interaction of oral epithelial cells with *C. albicans* (5). *C. albicans*- mediated degradation of E-cadherin was completely inhibited in the presence of protease inhibitors. Using a three-dimensional model of the human oral mucosa the authors found that E-adherin was degraded in localized areas of tissue invasion by *C. albicans*. An invasion-deficient *rim101*$^{-}$/*rim101*$^{-}$ strain was deficient in its ability to degrade E-cadherin, and this finding suggested that proteases may depend on Rim101p for expression. This work supports the hypothesis that there is a mechanism by which *C. albicans* invades mucosal tissues by promoting the proteolytic degradation of E-cadherin in epithelial adherens junctions (5).

2.3. Epithelial cell endocytosis

Another mechanism of candidal invasion of epithelial cells is the induction of epithelial cell endocytosis. It has been observed that *C. albicans* induces epithelial cells to produce pseudopods that surround the organism and pull it into the cell. The formation of these pseudopods is accompanied by the accumulation of epithelial cell microfilaments around the organism. These microfilaments are required for endocytosis because disrupting them with cytochalasin D blocks the uptake of *C. albicans*.

A variety of human epithelial cell lines are able to endocytose *C. albicans*, including HeLa cells, HET1-A oesophageal cells, FaDu pharyngeal cells, and OKF6/TERT-2 oral epithelial cells. However, the formation of pseudopods by epithelial cells is difficult to observe *in vivo*. Thus, the relative contribution of endocytosis versus local proteolytic digestion to the invasion of epithelial cells by *C. albicans in vivo* remains to be determined. However, it is clear that secreted proteases are not necessary for *C. albicans* to induce its own endocytosis by epithelial cells *in vitro*, because killed organisms are endocytosed as avidly as are live organisms.

Although both yeast and hyphal phase organisms can induce endocytosis, hyphae are more efficient at stimulating this process. The greater capacity of hyphae to induce endocytosis compared with that of blastospores suggests that hyphae express specific invasin-like

molecules on their surface that bind to one or more epithelial cell receptors and induce endocytosis. However, the identities of these candidal invasins and their epithelial receptors are unknown at this time.

Two different signal transduction mutants of *C. albicans* have been discovered to have a reduced capacity to induce endocytosis by oral epithelial cells, even though they have little or no defects in hypha formation (reviewed in reference 6). These mutants lack either Tpk2, which is a catalytic subunit of protein kinase A, or Cka2, which is a catalytic subunit of protein kinase CK2. However, it is not known whether Tpk1 and Cka2 govern the expression of the same or different epithelial cell invasins. A *C. albicans* mutant that lacks the glycosylphosphatidylinositol-linked protein Ecm33 also has a reduced capacity to invade oral epithelial cells. Ecm33 is likely to be expressed on the fungal surface and is required for normal cell wall assembly. The heterologous expression of Ecm33 in *Saccharomyces cerevisiae* does not result in enhanced endocytosis of this organism, suggesting that Ecm33 itself does not mediate epithelial cell invasion. Also, the Ecm33-null mutant has aberrant expression of at least one other cell surface protein. Therefore, it is likely that the invasion defect of the Ecm33-null mutant is due to abnormal function and expression of one or more other cell surface proteins.

2.4. Endothelial cell invasion

In susceptible patients, *Candida* species can enter the bloodstream either by translocation across the gastrointestinal mucosa or via an intravascular catheter (reviewed in references 6, 7). To escape from the bloodstream and invade target organs, these organisms must cross the endothelial cell lining of the blood vessels. There are three general mechanisms by which this process can occur. The first mechanism is the phagocytosis of the organism by a leukocyte, which then diapedeses across the endothelial cell lining of the blood vessel. Indeed, *Candida* species have been observed inside leukocytes of patients with candidaemia. However, there must be other mechanisms by which these organisms can escape from the bloodstream, because disseminated candidosis can occur in patients with profound leucopenia. A second mechanism is the passage of the organism between the endothelial cells. Such a process would likely occur in vascular beds of organs such as the kidney, where the endothelial cell lining of the blood vessels is fenestrated. A third mechanism is the endocytosis of the organism by endothelial cells. This mechanism clearly occurs *in vitro*, and has been the focus of multiple investigations.

Researchers have observed that *C. albicans* is endocytosed by human umbilical vein endothelial cells, endothelial cells in porcine vascular explants, and human brain microvascular endothelial cells. In most cases, the endothelial cells produce pseudopods that engulf the organism. Endocytosis of *C. albicans* requires intact endothelial cell microfilaments and microtubules; it is also governed in part by the tyrosine phosphorylation of endothelial cell proteins . This process is passive on the part of the organism because killed organisms are able to induce endocytosis similarly to live organisms. *C. albicans* hyphae induce endocytosis by human umbilical vein endothelial cells much more efficiently than do blastospores.

The results of several studies have led to the development of a model of how *C. albicans* hyphae induce their own endocytosis by human umbilical vein endothelial cells *in vitro* (reviewed in reference 6). In this model, *C. albicans* hyphae express an invasin-like protein, Als3, which can bind to N-cadherin and other proteins on the endothelial cell surface. Binding to these surface proteins induces the phosphorylation of at least two different endothelial cell proteins, which in turn causes the rearrangement of the endothelial cell microfilaments to produce pseudopods and initiate endocytosis. The N-terminal region of Als3 contains the ligand-binding domain. This region is predicted to share structural homology with the invasin protein of *Yersinia pseudotuberculsosis*. However, this method of host cell invasion by *C. albicans* is reminiscent of that induced by internalin A of *Listeria monocytogenes*, which induces epithelial cell endocytosis by binding to E-cadherin.

It is clear that *C. albicans* interacts differently with endothelial cells from different vascular beds (reviewed in reference 6). For example, although human umbilical vein endothelial cells preferentially endocytose hyphae, blastospores have been found to be avidly endocytosed by porcine endothelial cells and human brain microvascular endothelial cells *in vitro*. In addition, endothelial cells may act differently *in vitro* than *in vivo*. Hypha formation does not appear to be necessary for *C. albicans* to escape from the bloodstream and invade target organs in immunocompetent mice (8). Furthermore, some species of *Candida*, such as *C. glabrata*, are incapable of forming hyphae, yet are able to cause hematogenously disseminated candidosis in humans. How blastospores of *Candida* species are able cross the endothelial cell lining of the vasculature is currently unknown.

2.5. Spectrum of immune responses

The risk of candidosis and particular manifestations differs, depending upon which aspect of immunity is impaired (reviewed in reference 9). Moreover, an understanding of the host response to *Candida* is important in decisions regarding use of currently available antifungal therapies and in the design of new therapeutic modalities. Moreover, it is important to appreciate the various immunomodulating effects that antifungals have on host effector cells. Cell mediated immunity is known to have an important role, particularly in mucosal infection, as patients with deficiencies in this arm of the immune system are at particularly high risk. Lymphocytes participate in antifungal host defense by releasing cytokines that enhance the activity of macrophages and neutrophils. In addition, natural killer cells and lymphocytes activated by interleukins have been shown to inhibit the growth of *C. albicans.* An increasing body of evidence also supports a role for specific antibody in protection against invasive *Candida* infection.

Clinical observations indicate that mucocutaneous *Candida* 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 infection in mouse models has demonstrated an absolute dependence on CD4 (+) T cells, although clearance also involves phagocytic cells (reviewed in reference 10). Both IL-12 and TNF-alpha 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 (reviewed in reference 10).

Innate immunity is the dominant protective mechanism against disseminated candidosis. Quantitative and qualitative abnormalities of neutrophils and monocytes are associated with systemic candidosis. Patients with lymphoma, leukaemia, chronic granulomatous disease, and recipients of intensive cancer chemotherapy with resultant neutropenia are at increased risk for disseminated infection. Neutrophils and monocytes damage and kill yeast cells, hyphae and pseudohyphae. The large size of *Candida* hyphae and pseudohyphae may preclude phagocytosis. In such cases, several phagocytes collaborate to effect extracellular killing. Neutrophils and monocytes recognize and engulf opsonised and non-opsonised yeast cells via cell-surface pattern recognition receptors, including TLRs, mannose receptors and beta-glucan receptors. Binding to individual TLRs or IL-1 receptor (IL-1R) activates specialized antifungal functions on neutrophils and other phagocytes. Killing is by oxidative mechanisms, including generation of reactive oxygen and nitrogen intermediates, and by non-oxidative mechanisms. Phagocytosis and killing are augmented by opsonisation and proinflammatory cytokines. Invasion of vascular structures facilitates dissemination of *Candida*. Endothelial cells resist vascular invasion by secretion of proinflammatory mediators and expression of leucocyte adhesion moleculaes, which recruit and bind to activated leucocytes. Mediators of inflammation at the site of damaged endothelial surfaces induce release of antimicrobial peptides from human platelets.

3. IMMUNITY IN SYSTEMIC CANDIDOSIS

3.1. Toll-like receptor signalling

Toll-like receptors (TLRs) are central mediators of the innate immune system that instruct cells of the innate and adaptive response to clear microbial infections. Recognition of pathogen-associated molecular patterns (PAMPs) by TLRs, either alone or in heterodimerization with other TLR or non-TLR receptors, induces signals responsible for the activation of innate immune response. Recent studies have demonstrated a crucial involvement of TLRs in the recognition of fungal pathogens such as *C. albicans* (reviewed in reference 11). By studying fungal infection in knock-out mice deficient in either TLRs or TLR-associated adaptor molecules, it appeared that specific TLRs such as TLR2 and TLR4 play differential roles in the activation of the various arms of the innate immune response. Recent data also suggest that TLRs offer escape mechanisms to certain pathogenic microorganisms, especially through TLR2-driven induction of anti-inflamatory cytokines.

Weindle and colleagues demonstrated that human epithelial TLR4 directly protected the oral mucosa from fungal infection via a process mediated by polymorphonuclear leukocytes (PMNs) (12). In an *in vitro* epithelial model of oral candidosis, *C. albicans* induced a chemoattractive and proinflammatory cytokine response but failed to directly modulate the expression of genes encoding TLRs. However, the addition of PMNs to the *C. albicans*-infected model strongly upregulated cytoplasmic and cell-surface epithelial TLR4 expression, which correlated directly with protection against fungal invasion and cell injury. *C. albicans* invasion and cell injury was restored by the addition of TLR4-specific neutralizing antibodies and knockdown of TLR4 using RNA interference, even in the presence of PMNs, demonstrating the direct role of epithelial TLR4 in the protective process. Furthermore, treatment with neutralizing antibodies specific for TNF-alpha resulted in strongly reduced TLR4 expression accompanied by augmented epithelial cell damage and fungal invasion. This appears to be the first description of such a PMN-dependent, TLR4-mediated protective mechanism at epithelial surfaces, which may provide significant insights into how microbial infections are managed and controlled in the oral mucosa.

Historically, the cell walls of fungi were shown to be covered by a layer of mannoproteins, which prompted much interest in mannose-based recognition systems (pathogen-associated molecular patterns, PAMPs). Subsequent evidence has suggested that this model may be too simplistic and that other PAMPs, particularly beta-glucans, are exposed on the cell surface and therefore are potentially important in immune recognition. In *C. albicans* beta-glucans can comprise up to 50% of the dry weight of the cell wall and are essential structural components that provide elasticity and mechanical strength.

Host-cell recognition of beta-glucan is mediated mainly by dectin-1, a myeloid-expressed type II transmembrane C-type lectin—like receptor that contains an immunoreceptor tyrosine-based activation motif in its cytoplasmic tail. Dectin-1 binds to many fungi, including *Saccharomyces, Candida, Coccidioides* and *Aspergillus*. *In vitro*, dectin-1 has been shown to mediate a variety of both TLR-dependent and TLR-independent antifungal cellular responses, including phagocytosis, the respiratory burst and the production of many cytokines and chemokines. In *Candida*, the ability to rapidly and reversibly switch between yeast and filamentous morphologies is crucial to pathogenicity, and it is thought that the filamentous

morphology provides some advantage during interaction with the mammalian immune system. It has been shown that the cell wall of *Candida* blastoconidia is largely shielded from dectin-1 by outer wall components (13). However, the normal mechanisms of yeast budding and cell separation create permanent scars which expose sufficient beta-glucan to trigger antimicrobial responses through dectin-1, including phagocytosis and activation of reactive oxygen production. During filamentous growth, no cell separation or subsequent beta-glucan exposure occurs, and *Candida* fails to activate dectin-1. The data demonstrate a mechanism by which *C. albicans* shape alone directly contributes to the method by which phagocytes recognise the yeast.

A recent study demonstrates that immune sensing of *C. albicans* requires cooperative recognition of cell wall mannans and glucans by lectin and toll-like receptors (14). The authors demonstrated that cytokine production by human mononuclear cells or murine macrophages was markedly reduced when stimulated by *C. albicans* mutants defective in mannosylation. Recognition of mannosyl residues was mediated by mannose receptor binding to N-linked mannosyl residues and by TLR4 binding to O-linked mannosyl residues. Residual cytokine production was mediated by recognition of beta-glucan by the dectin-1/TLR2 receptor complex. *C. albicans* mutants with a cell wall defective in mannosyl residues were less virulent in experimental disseminated candidosis and elicited reduced cytokine production *in vivo*. It was concluded that recognition of *C. albicans* by monocytes/macrophages is mediated by three recognition systems of differing importance, each of which senses specific layers of the *C. albicans* cell wall.

Another recent study has shown that deficiency of dectin-1 rendered mice susceptible to infection with *C. albicans*. Dectin-1-deficient leukocytes demonstrated significantly impaired responses to fungi even in the presence of opsonins (15). Impaired leukocyte responses were manifested *in vivo* by reduced inflammatory cell recruitment after fungal infection, resulting in substantially increased fungal burdens and enhanced fungal dissemination. The study has established a fundamental function for beta-glucan recognition by dectin-1 in antifungal immunity and has demonstrated that a signalling non-Toll-like pattern recognition receptor is required for the induction of protective immune responses. Gow and colleagues demonstrated that cytokine production by both human peripheral blood mononuclear cells and murine macrophages is dependent on the recognition of beta-glucans by dectin-1 (16). Heat killing of *C. albicans* resulted in exposure of beta-glucans on the surface of the cell wall and subsequent recognition by dectin-1, whereas live yeasts stimulated monocytes mainly via recognition of cell-surface mannans. Dectin-1 was shown to induced cytokine production through two pathways: Syk-dependent production of the T-helper (Th) 2-type anti-inflammatory cytokine interleukin-10 and Toll-like receptor-Myd88-dependent stimulation of monocyte-derived proinflammatory cytokines, such as tumor necrosis factor-alpha . In contrast, stimulation of Th1-type cytokines, such as interferon-gamma , by *C. albicans* was independent of the recognition of beta-glucans by dectin-1. The authors concluded that *C. albicans* induces production of monocyte-derived and T cell-derived cytokines through distinct pathways dependent on, or independent of dectin-1.

3.2. Cellular-dependent responses

Monocytes and macrophages are the cell types most commonly associated with the innate immune response against systemic *C. albicans* infection with many reports of experimental models demonstrating the T-cell-dependence of host responses against disseminated infection (reviewed in reference 10). Interactions between the host immune system and *Candida* organisms have been investigated for planktonic *Candida* cells (reviewed in references 9, 10). Recent studies have focused on these interactions in a biofilm environment. Various types of candidosis are associated with the formation of biofilms (reviewed in reference 17). Chandra and colleagues evaluated the ability of *C. albicans* to form biofilms in the presence or absence of adherent peripheral blood mononuclear cells (PBMCs; enriched for monocytes and macrophages by adherence) (18). Using scanning electron and confocal scanning laser microscopy it was shown that the presence of PBMCs enhanced the ability of *C. albicans* to form biofilms and that the majority of PBMCs were localized to the basal and middle layers of the biofilm. In contrast to the interactions of PBMCs with planktonic *C. albicans*, where PBMCs phagocytose yeast cells, PBMCs did not appear to phagocytose fungal cells in biofilms. Furthermore, time-lapse laser microscopy revealed dynamic interactions between *C. albicans* and PBMCs in a biofilm. Additionally, the authors found that (i) only viable PBMCs influenced *Candida* biofilm formation, (ii) cell surface components of PBMCs did not contribute to the enhancement of *C. albicans* biofilm, (iii) the biofilm-enhancing effect of PBMCs was mediated by a soluble factor released into the coculture medium of PBMCs with *C. albicans*, and (iv) supernatant collected from this coculture contained differential levels of pro- and anti-inflammatory cytokines. Studies of this nature provide new insights into the interaction between *Candida* biofilms and host immune cells and demonstrate that immunocytes may influence the ability of *C. albicans* to form biofilms.

3.3. Are specific antibodies important?

An increasing body of evidence supports a role for specific antibody protection against invasive *Candida* infections. In animal models of candidosis, both systemic and topical administration of polyclonal antibodies against *C. albicans* have been effective in preventing infection. A monoclonal antibody has also been shown to be effective in mouse models of disseminated candidosis. What has been termed the “third age of antimicrobial therapy” is the combination of Efungumab (mycograb), which is an antibody to heat shock protein 90 (hsp90) and lipid formulations of amphotericin B (19-21). Efungumab has been developed as a major recombinant antibody fragment against hsp90 that incorporates the dominant paratope found in patients who recovered from invasive candidosis. Efungumab and other hsp90 inhibitors, such as geldanamycin, have an antifungal effect *in vitro* and, in the

case of efungumab, in an animal model of fungal infection. In a randomized, double-blind study in patients with invasive candidosis, the combination of efungumab and lipid-associated amphotericin B was superior to monotherapy with lipid-associated amphotericin B, demonstrating a higher clinical response rate (86% versus 52% (P<0.001)), a higher mycological response rate (89% versus 54% (P<0.001)), a faster rate of culture-confirmed clearance (hazard ratio, 2.3; 95% confidence interval, 1.4-3.8; P=0.001) and less *Candida*-attributable mortality (18% versus 4% (P=0.025)) (21).

The mechanisms by which specific antibodies can interact with components of the cellular immune system to defend against pathogens have been the subject of intense study (reviewed in reference 22). Recent studies have shown that naturally occurring serum antibodies against *C. albicans* mannan can function as opsonins to mediate phagocytic killing (23). In a study by Bliss and colleagues serum concentrations as low as 1% resulted in 50% inhibition of *C. albicans* metabolic activity after incubation with peripheral blood mononuclear cells at an effector to target ratio of eight (24). Measurable inhibition was also achieved at lower effector to target ratios and lower serum concentrations, and at least a portion of the metabolic inhibition reflected fungal cell death. Depletion of *C. albicans*-specific antibody decreased the toxic effect while opsonization with purified human IgG restored toxicity, and cell–cell contact between peripheral blood mononuclear cells and fungus was required. Depletion of or enrichment for monocytes from the peripheral blood mononuclear cells preparation diminished the toxic effect and the monocytic cell line, THP-1, was likewise incapable of toxicity. These studies provide evidence that antibody augments antifungal host defense and underscore the complex interrelationship between humoral and cellular immunity in these infections. Furthermore, it has been observed that the protective potential of antibodies to enhanced phagocytosis and killing of the fungus is dependent upon epitope specificity, serum titre, and ability to rapidly and efficiently fix complement to the fungal surface (25).

4. THE ROLE OF DENDRITIC CELLS AT MUCOSAL SURFACES

Dendritic cells (DCs) are present in small quantities in tissues that are in contact with the external environment, mainly the skin (where a specialized dendritic cell type is called Langerhans cells) and the inner lining of the nose, lungs, stomach and intestines. Mucosal surfaces are considered to be the first line of defence to acquire protective immunity against *Candida* infection. However, little is known regarding the role of dentritic cells in candidosis. In a recent study Cambi and colleagues showed that two C-type lectins, DC-SIGN and the macrophage mannose receptor, specifically mediate *C. albicans* binding and internalization by human DCs (26). Moreover, by combining a range of *C. albicans* glycosylation mutants with receptor-specific blocking and cytokine production assays, they determined that N-linked mannan but not O-linked or phosphomannan was the fungal carbohydrate structure specifically recognized by both C-type lectins on human DCs and directly influenced the production of the proinflammatory cytokine IL-6. It is envisaged that this line of research will ultimately lead to the development of new drugs targeting specific carbohydrate antigens of *Candida*.

5. DEFENSINS AND LIPASES IN GASTROINTESTINAL *C. ALBICANS* INFECTION

Although gastric candidosis is common in debilitated cancer patients, this condition is often asymptomatic and is seldom diagnosed during life. Mucosal ulcerations are the most common lesions, but it is unclear to what extent these are chronic gastric ulcers superinfected with *Candida* species. Perforation can lead to disseminated infection. Koh and colleagues have hypothesized that in a mouse model both neutropenia and gastrointestinal tract (GI) damage are critical for allowing widespread systemic disease (27). Animal models have also been useful in exploring the immunological regulation of *Candida* in GI tract. The ability of regulatory T (Treg) cells to inhibit aspects of innate and adaptive immunity is central to their protective function in fungal infections. In murine candidosis, CD4 (+)CD25 (+) Treg cells prevent excessive inflammation but enable fungal persistence in the gastrointestinal tract, which underlies the onset of durable antifungal protection. De Luca and colleagues showed that fungal growth, inflammatory immunity, and tolerance to *Candida* were all controlled by the coordinate activation of naturally occurring Treg cells, which limited early inflammation at the sites of infection, and pathogen-induced Treg cells. (28). Naturally occurring Treg cells required the TRIF pathway for migration to inflamed sites, where the MyD88 pathway would then restrain their suppressive function. Subsequent inflammatory Th1-type immunity was modulated by induced Treg cells, which required the TRIF pathway as well, and acted through activation of IDO in dendritic cells and Th17 cell antagonism. *In vitro*, using naive CD4 (+) cells from TRIF-deficient mice, tryptophan metabolites were capable of inducing the Foxp3-encoding gene transcriptionally and suppressing the gene encoding RORgammat, Th17 lineage specification factor. This is the first study to show that the same tryptophan catabolites can foster dendritic cell-supported generation of Foxp3 (+) cells and mediate, at the same time, inhibition of RORgammat-expressing T cells.

Alpha- and beta- defensins are major constituents of the innate defence system, providing rapid antimicrobial action. To define the influence of oesophageal *Candida* infection on the expression of defensins Kiehne and colleagues studied the expression of alpha- and beta-defensins in the oesophagus and stomach by quantitative, real-time, polymerase chain reaction (PCR) in healthy individuals (29). Biopsy samples were taken from the upper gastrointestinal tract, mRNA was extracted, reverse transcribed into cDNA and real-time reverse transcription PCR (RT-PCR) analysis measuring transcript number of a-defensins and ss-defensins performed. Human beta defensin (hBD)-1, hBD-2 and hBD-3 had their highest expression levels in the oesophagus with markedly lower levels in the stomach. Candida oesophagitis resulted in

massive up-regulation of hBD-2 (800-fold), while hBD-1 and hBD-3 expression were slightly increased. In addition, expression of HNP 1-3 was detected, indicating infiltration of neutrophil granulocytes. Cytokine expression of interleukin-1beta, interleukin-6 and interleukin-8 were increased. This study showed that *Candida* colonization induced a high expression of antimicrobial peptides. Up-regulation of defensins might protect against invasive candidosis and keep candidosis limited to the mucosal surface.

Candida possesses a large family of lipase encoding genes whose extracellular activity may be important for colonization of the gastrointestinal tract. The expression of the *C. albicans* lipase gene family (LIP1-10) has been investigated using a mouse model of mucosal candidosis during alimentary tract colonization (caecum contents) and orogastric infection (30). LIPs4-8 were expressed in the caecum contents and infected mucosal tissues (stomach, hard palate, esophagus and tongue) suggesting a maintenance function for these gene products. In contrast, LIPs1, 3, and 9, which were detected consistently in infected gastric tissues, were essentially undetectable in infected oral tissues. In addition, LIP2 was expressed consistently in caecum contents but was undetectable in infected oral tissues suggesting LIP2 may be important for alimentary tract colonization, but not oral infection. Animals responded to a *Candida* infection by significantly increasing expression of the chemokines MIP-2 and KC at the site of infection. Therefore, differential LIP gene expression was observed during colonization, infection and at different infected mucosal sites.

6. EXOGENOUS AND ENDOGENOUS FACTORS IN VAGINAL CANDIDOSIS

Vulvovaginal candidosis (VVC), primarily caused by *C. albicans*, remains a significant problem in women of childbearing age (reviewed in reference 31). While cell-mediated immunity is considered the predominant host defense mechanism against mucosal candidal infections, two decades of research from animal models and clinical studies have revealed a lack of a protective role for adaptive immunity against VVC caused by putative immunoregulatory mechanisms. Moreover, natural protective mechanisms and factors associated with susceptibility to infection have remained elusive. That is until recently, when through a live challenge model in humans, it was revealed that protection against vaginitis coincides with a non-inflammatory innate presence, whereas symptomatic infection correlates with a neutrophil infiltrate in the vaginal lumen and elevated fungal burden. Thus, instead of VVC being caused by a putative deficient adaptive immune response, it is now being considered that symptomatic vaginitis is caused by an aggressive innate response.

Vulvovaginal candidiasis (VVC) is a significant problem affecting 75% of all women at least once during their lifetime (reviewed in reference 31) This high prevalence of infection correlates with millions of dollars spent on over the counter or prescription medication or physician office visits to treat the infections. As a result of this exposure, most if not all healthy individuals have developed *Candida*-specific adaptive immunity demonstrable by serum/mucosal antibodies, *in vitro* T-cell responses, and delayed skin test reactivity. It is generally accepted that these adaptive immune mechanisms are responsible for protecting against the conversion of the organism into an opportunistic pathogen of the same mucosal tissues. However, if these host defenses are modulated and/or the organism becomes modulated by environmental factors, symptomatic infections can ensue. Exogenous factors associated with acute VVC include modulations or imbalances in reproductive hormones (i.e. oral contraceptive usage, pregnancy, hormone replacement therapy (HRT)), as well as antibiotic usage, and diabetes mellitus. While infections brought on by these factors are largely treatable, they have a significant effect on the quality of life of those affected. More importantly, there is another population of women (5% and possibly up to 10%) who have recurrent VVC (RVVC) defined as three or more episodes per annum. There are two forms of RVVC. Primary RVVC is idiopathic with no known predisposing factors such as those identified for acute VVC. Secondary RVVC is simply frequent episodes of acute VVC because the women cannot avoid certain predisposing factors (i.e. diabetes mellitus, HRT). Women with RVVC generally respond to antifungal regimens adequately with no resistance, but these regimens do not prevent recurrence.

It has generally been accepted that women with primary RVVC are in effect missing important immune protective factors. Thus, following conventional antifungal therapy for a symptomatic episode, the organism that was not fully eliminated by the fungistatic agents could easily increase in population numbers and cause repeated infections by relapse. Therefore, knowledge of protective host defense mechanisms associated with resistance to infection and the factors associated with susceptibility to infection will allow for the development of immunotherapeutic strategies to prevent or treat RVVC as well as acute VVC.

As in all models of host-parasite interactions, protective cell-mediated and humoral responses need to be finely tuned and regulated to avoid overwhelming stimulation which may turn into local inflammation and host damage (reviewed in reference 31). Together with fungus attributes allowing commensalism, the immunoregulatory mechanisms may play a central role in the vaginal environment. Some immunoregulatory disorders of bacterium-harboring peripheral tissues involve excessive stimulation of Th1-type responses with the associated production of proinflammatory cytokines and chemokines and excess recruitment of inflammatory cells to the mucosal site. Many more Th1-type T-cell clones secreting proinflammatory cytokines can be generated from the vaginas of RVVC subjects than from the vaginas of non-RVVC subjects, and there is some evidence that IL-23, a cytokine whose overproduction is critically associated with some types of inflammatory intestinal disorders, is secreted during vaginal infection. IL-23 induces Th17 cells and IL-17, which potently recalls inflammatory cells at the

mucosal site. The two defensive but proinflammatory cytokines IL-12—gamma interferon and IL-23-IL-17 can both contribute to pathology when there is a great deal of stimulation or in the absence of dampening immunomodulatory mechanisms. As an attractive, although at present merely hypothetical, view, it could be suggested that the predominant forms of vaginal candidosis are inflammatory immune disorders to which overwhelming Th1-Th17 stimulation is coupled with inflammatory responses by the vaginal epithelial cells (EC) (and possibly stromal cells and resident phagocytes) stimulated or damaged by *Candida* virulence traits. This would be in accord with (i) the documented leukocyte-driven inflammation in experimental *Candida* challenge in women; (ii) the potential regulatory role of Th2 responses, plasmacytoid Il-10-secreting DC, and transforming growth factor beta; and (iii) the observation that antibodies blocking virulence traits of the fungus and inhibiting adherence to EC are protective. These antibodies may well inhibit the hyperstimulatory fungal burden in the vagina. In fact, antibodies, through their Fc binding to inhibitory Fc-gamma receptors, may constitute a rather potent means to dampen excessive inflammation.

In summary, the pathogenesis of vaginal candidosis can be assumed to comprise of a number of stages (reviewed in reference 31). Firstly, *Candida* may reside commensally in the vagina because of the equilibrium reached between the expression of virulence traits (stage 1) and the immune response, both innate and adaptive, of which an epithelial functional barrier, fungus immunoescape, immunoregulatory mechanisms, and local tolerance are important components (stage 2). Disease (stage 3) occurs when excess virulence, which may also be due simply to an overburden of fungal cells (attack numerator), causes damage to epithelial cells and overwhelms immune responses and their regulation (defense denominator). In some women, the denominator may be particularly low for unknown (possibly genetic) reasons, and these women would be prone to repeated episodes and exacerbation of vaginitis. Two noteworthy consequences of this model are that *C. albicans* adherence and damage to the epithelial cells constitute the most critical pathogenicity event and that factors inhibiting adherence and damage are likely to be protective. Antibodies (from active or passive vaccination) are direct therapeutic options; less direct options are those affecting inflammation and immunoregulation (for example, cytokines, probiotics). The model does not consider the seemingly small proportion of women in whom vaginitis may be due to allergy to *Candida* products (atopic subjects).

7. C. ALBICANS AS A CAUSE OF ORAL INFLAMMATION

Candida albicans is the major infectious agent of oral candidosis, and both innate immunity and cell-mediated immune response participate in the control of the fungal infections. Oral candidosis causes intraepithelial inflammation, and this is followed by recruitment of a heavy inflammatory cell infiltrate which includes neutrophils as the main component. Oral candidosis has many varieties of which it might be atrophic (loss of epithelium causing erythema), pseudomembranous (formation of a slough membrane), or hyperplastic (gain of more layers of epithelium). Chronic hyperplastic candidosis (CHC) is a form of oral candidosis characterized by hyphal invasion of the oral epithelium and in which appropriate antifungal therapy should lead to resolution of the condition. CHC appears clinically as firm whitish palpable lesions, which cannot be rubbed off, affecting most commonly the buccal mucosa, especially the oral commissures. One face of the immune reactions directed against candidosis is a group of small (about 8-14 KDa), mostly basic substances (cytokines) which have the ability to traffic neutrophils and other phagocytes to the site of infection.

Local defence mechanisms against mucosal infection in the oral cavity include salivary proteins, such as lactoferrin, beta-defensins, histatins, lysozyme, transferring, lactoperoxidase, mucins, and secretory immunoglobulin A. These appear to impair adhesion and growth of *Candida* in the oropharyngeal cavity. Healthy oral epithelial cells inhibit blastoconidia and/or hyphal growth of several *Candida* species (32). Local mucosal immunity appears to be at least as important as systemic cell mediated immunity. Moreover, shedding and renewal of mucosal surfaces plays an important role in local defence.

In a recent study the clinical forms of oral candidosis were correlated with the number of colony forming units (CFU) of *C. albicans* in saliva (33). An additional aim was to characterize T cell response in patients with oral candidosis. Participants included 75 subjects: 36 with lesions of candidosis and 39 without lesions of oral candidosis. Saliva was collected from all subjects for microbiological analysis. Cytokine levels were determined by ELISA in supernatants of peripheral blood mononuclear cells of 25 patients with oral candidosis, after *in vitro* stimulation with *C. albicans* antigens. In 48% of patients, no association was observed with denture use. *C. albicans* was detected in the saliva of 91.7% of patients with oral candidosis, and there was an association between the number of CFU and the presence of oral lesions. A type Th1 immune response was observed in supernatants of peripheral blood mononuclear cells stimulated with *C. albicans* antigens. In contrast, IL-5 and IL-10 levels were very low or undetectable. Together, this study shows an association between clinical forms of oral candidosis and the number of colonies of *C. albicans* in saliva, and that a systemic immune response characterized by the production of TNF-alpha and IFN-gamma is observed in patients with oral candidosis.

A specific manifestation of oral candidosis is exemplified by autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), or autoimmune polyendocrine syndrome type I (APS-I). This is an autosomal recessive disease caused by mutations of the *AIRE (*autoimmune regulator) gene (reviewed in reference 34). Recent work suggests that a regulatory T

(Treg) cell impairment is involved in the pathogenesis of APECED and emphasizes the importance of active tolerance mechanisms in preventing human autoimmunity (35). The first components appear in most cases in the first decade of life but they may be delayed until adulthood. The disease occurs all over the world, but is rare except among Finns, Iranian Jews and Sardinians. Several endocrine glands and other organs may be destroyed or damaged by the patient's immune system. The most common component is chronic mucocutaneous candidosis, specifically oral candidosis which has been suggested to be carcinogenic (36). Thus, most patients receive repeated treatment and prophylactic courses of antifungals throughout their lives. Since the late 1980's topical and systemic azole antifungals, mainly ketoconazole and fluconazole have been used (37).

Another component of oral candidosis is chronic hyperplastic candidosis (CHC) (candidal leukoplakia). This is a variant of oral candidosis that typically presents as a white patch on the commissures of the oral mucosa (37). The major etiologic agent is *C. albicans*, although other systemic co-factors, such as vitamin deficiency and generalized immune suppression, may play a contributory role. Clinically, the lesions are symptomless and regress after appropriate antifungal therapy and correction of underlying nutritional or other deficiencies. If the lesions are untreated, a minor proportion may demonstrate dysplasia and develop into carcinomas. Ali and colleagues studied the participation of interleukin-8 (IL-8) and the localization of its receptor A in the host response against chronic candidal infection (39). Biopsies from patients with CHC lesions were examined by immunohistochemistry using IL-8 and IL-8 R-A specific polyclonal antibodies. Biopsy samples from healthy individuals served as controls. In CHC lesions IL-8 and its receptor A were secreted by a variety of cells intraepithelially, interstitially in the lamina propria and around the blood vessels. It was found also that *C. albicans* secretes interleukin-8 or an analogue of it whilst the hyphae expressed IL-8 Receptor A. It appeared that *C. albicans* seemed to have manufactured IL-8 in order to attract neutrophils away from attacking the weaker, hyphal elements of *Candida*. Candidal hyphae might sense the presence of human IL-8 by means of hyphal IL-8 receptor A (or IL-8 receptor-like protein). This strategy may account for the ability of the fungus to locate its way, avoiding the chemotactic effect of this cytokine since the cells recruited i.e. neutrophils represent the first line of defence against candidal pathogenicity.

In the past two decades, *C. glabrata* has emerged as a notable pathogenic pathogenic yeast in the oral cavity, especially in immunocompromised patients. Little is know about the local immune response to *C. glabrata*. Li and Dongari-Bagtzoglou (40) studied the cytokine-inducing and cell-damaging potential of *C. glabrata* in oral epithelial cells and compared this to *C. albicans*. Oral epithelial cell lines and primary gingival epithelial cells were cocultured with strains of *C. glabrata* or *C. albicans*. Supernatants were analysed for the presence of interleukin-1alpha (IL-1alpha), IL-8 and granulocyte-macrophage colony-stimulating factor (GM-CSF) by enzyme-linked immunosorbent assay. The cytotoxity of different strains was determined using the CytoTox-96 assay. Compared to *C. albicans*, *C. glabrata* induced different proinflammatory cytokine responses in oral epithelial cells; a high level of GM-CSF induction was only detected in *C. glabrata*-infected cells and not in *C. albicans*-infected cells, regardless of the origin of these cells (cell lines or primary cells) or the strain used. Like *C. albicans, C. glabrata* induced an IL-1alpha response by oral epithelial cells, but this response was both strain-dependent and epithelial cell origin-dependent. Unlike *C. albicans, C. glabrata* failed to induce a strong IL-8 response in any of the cell systems studied. Finally, in these studies *C. glabrata* showed lower cytotoxicity than *C. albicans*. The authors concluded that *C. glabrata* is less cytotoxic than *C. albicans* and induces different proinflammatory cytokine responses in oral epithelial cells.

8. RESISTANCE *TO CANDIDA* INFECTIONS

Clinical evidence and experimental data indicate that both the innate and the adaptive immune systems regulate (reviewed in reference 41). In murine experimental models of infection, it has been demonstrated that Th cell reactivity plays a central role in regulating immune responses to the fungus, Th1 reactivity being responsible for resistance and Th2 reactivity being associated with susceptibility. The development of protective anticandidal Th1 responses requires the concerted actions of several cytokines, including IFN- and IL-12, in the relative absence of Th2 cytokines, such as IL-4 and IL-10, which inhibit development of Th1 responses.

Recent evidence indicates that dendritic cells are uniquely capable of decoding the fungus-associated information required to elicit the qualitative nature of the adaptive immune response (reviewed in reference 42). Dendritic cells finely discriminated between the two forms of *C. albicans* in terms of type of immune responses elicited. By the production of IL-12 and IL-4 in response to the nonvirulent and virulent forms of the fungus, dendritic cells were uniquely capable of Th priming and education *in vitro* and *in vivo*. This finding is particularly relevant in candidosis, because the fungus behaves as a commensal as well as a true pathogen of skin and mucosal surfaces, known to be highly enriched for dendritic cells.

9. DESTRUCTIVE IMMUNE RESPONSES IN *CANDIDA* INFECTIONS

To obtain a complete overview of the range of damage caused by *C. albicans* in a mammalian host, the histopathological consequences of an acute systemic infection can be studied in animal models. Rozell and colleagues showed that one-day post infection, animals showed clinical and behavioral changes compatible with a systemic infection (42). Consistent with the observed clinical changes, histological examination revealed extensive fungal growth and cellular changes in most organs and tissues. Fungal colonization and invasion was associated with dense neutrophilic granulocyte infiltrates

clearly indicating a rapid inflammatory response. The kidneys appeared to be the most extensively infected tissue. Initial colonization occurred in the cortex. Two-days into the infection, the kidneys were grossly enlarged. In the cortex there was clear evidence of an acute inflammatory reaction, dominated by granulocytes associated with hyphae. In addition, a centripetal fungal spread with colonisation of tubules was observed. Glomeruli did not appear to be affected. The heart was also one of the primary early targets for colonization. The extensive growth led to the destruction of myocytes, and the accumulation of dense aggregates of neutrophilic granulocytes in areas surrounding colonies of fungal cells. It appears that the bulk of the infection was cleared by neutrophilic granulocytes. The combined activity of the fungal cells and innate immune response were probably responsible for the damage in the myocardium. Interestingly, there was little growth seen in the liver and lungs. However, in the spleen there was clear evidence of fungal growth.

10. PERSPECTIVE

One of the most exciting areas of research is the development of vaccines against *Candida*. The development of a useful *Candida* vaccine is a distinct possibility despite the fact that individuals with a lifetime of commensal sensitization do not develop sterile immunity to the organism. An effective *Candida* vaccine would be invaluable in preventing hematogenously disseminated candidosis, as well as mucocutaneous disease. Mochon and Cutler have reviewed our current understanding of the interplay between commensal and pathogenic forms of *C. albicans* and approaches toward active and passive immunoprevention against candidosis (43). The evidence presented in this review suggests a clear need for the continued development of effective and useful therapeutic agents and vaccines against *C. albicans* and other *Candida* species. This suggestion is supported by the increasing incidence of morbidity and mortality associated with candidal infections and the lack of definitive diagnostic tests that distinguish between commensalism and pathogenicity.

Though the need for appropriate vaccine development against candidosis is readily apparent, the enigmatic relationship between *Candida* and the host makes this endeavor complex. Further investigations are required defining differences and similarities associated with the commensal and opportunistic forms of this fungus and determining the appropriate immunological factors necessary for the protection against the different forms of candidal disease (i.e. disseminated versus mucocutaneous candidosis) (reviewed in reference 44).

Whether an interaction between *C. albicans* and the host is a mutually reciprocal relationship must be differentiated from whether this yeast is simply a latent pathogen held in check by a competent immune system. This issue is pertinent in determining which approach, immunoprophylaxis or immunotherapy, is the best course of action in the prevention and/or treatment of candidosis. The advantage of an immunoprophylactic agent is its ability to induce an immune response that will either eliminate the fungus from the host or maintain it at sufficiently low numbers to prevent invasive disease in at-risk populations. This vaccine strategy must be approached with caution however, as others have suggested that this may lead to a harmful hyperimmune response against a mucosal-associated microbe that had primed the immune system prior to vaccine administration. Although unlikely, if this suggestion proves correct, an immunotherapeutic vaccine approach would be preferred. Presumably, this would preserve maintenance of *C. albicans* commensalisms with its host, while protecting the individual from disease. The implication of immunotherapeutic vaccines is that they must be specifically directed against fungal antigens expressed only during pathogenesis, which makes such vaccine development highly challenging.

Recent research has focused on candidal antigens that have been used as vaccine candidates (reviewed in reference 43). In some cases, interesting results have been obtained through the use of whole cell vaccine approaches, but the antigenic complexity of whole cells may lead to inconsistent protective responses. Specific antigens that certainly deserve attention are those that are involved in attachment to host sites where anti-candidal defenses are minimal, with the hope that specific antibody effects blocking of the adherence event. Hwp1 and members of the Als family show potential in this regard. Likewise, certain secreted acid proteinases (SAP) may have pathogenic activities at different anatomical sites, thus, neutralizing antibodies may well be expected to reduce the virulence during tissue invasion. The use of microarrays to determine candidal gene products required for infection will undoubtedly lead to the discovery of further vaccine candidates.

Regardless of the vaccine approach is a concern that a highly specific antigenic response may promote selection for null mutants that are resistant to the immune response. Indeed, rather than develop a vaccine that directs the immune response against a single fungal determinant, the best vaccine may be comprised of two or more unrelated antigens.

Finally, the immune status of the patient receiving a vaccine is of critical concern. The best situation would entail the use of a safe and efficacious antibody-mediated vaccine that induces long-term immunity prior to subsequent development of immunocompromising conditions. Alternatively, short-term protection afforded by passive administration of protective antibodies alone or in combination with antifungal drugs may well be useful in certain clinical situations.

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