

Candida albicans Pathogenesis: Fitting within the Host-Microbe Damage Response Framework

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Historically, the nature and extent of host damage by a microbe were considered highly dependent on virulence attributes of the microbe. However, it has become clear that disease is a complex outcome which can arise because of pathogen-mediated damage, host-mediated damage, or both, with active participation from the host microbiota. This awareness led to the formulation of the damage response framework (DRF), a revolutionary concept that defined microbial virulence as a function of host immunity. The DRF outlines six classifications of host damage outcomes based on the microbe and the strength of the immune response. In this review, we revisit this concept from the perspective of *Candida albicans*, a microbial pathogen uniquely adapted to its human host. This fungus commonly colonizes various anatomical sites without causing notable damage. However, depending on environmental conditions, a diverse array of diseases may occur, ranging from mucosal to invasive systemic infections resulting in microbe-mediated and/or host-mediated damage. Remarkably, *C. albicans* infections can fit into all six DRF classifications, depending on the anatomical site and associated host immune response. Here, we highlight some of these diverse and site-specific diseases and how they fit the DRF classifications, and we describe the animal models available to uncover pathogenic mechanisms and related host immune responses.

Historically, the nature and extent of host damage by an opportunistic microbe were considered highly dependent on virulence attributes of the microbe. However, it is now quite clear that damage to the host during infection is also reflective of the immune status of the host and often mediated by host responses. Therefore, disease itself is a complex outcome which can arise because of pathogen-mediated damage, host-mediated damage, or both. Hence, in many interactions between pathogens and normal hosts, there is a continuum between pathogen-mediated and host-mediated damage, which results in disease only when the nature of the damage impairs the normal function of the host.

In this review, we revisit the concept of the damage response framework (DRF) initially introduced by Casadevall and Pirofski in 1999, which explains microbial pathogenesis as the outcome of an interaction between the host and microbe (1). The host-relevant outcome is microbe- and/or host-mediated damage, which provides a basis for a new pathogen classification scheme based on the amount of damage as a function of the host immune response (1). Here, we utilize the DRF to explore the pathogenesis of a ubiquitous fungal pathogen, *Candida albicans*, which is uniquely adapted to its human host, existing often as a harmless commensal at various mucosal sites (2, 3). As a pathogen, however, *C. albicans* is responsible for a wide range of infections, both mucosal and systemic, in both immunocompetent and immunocompromised individuals (4, 5). With no known environmental reservoir, *C. albicans* is acquired at or shortly after birth, often being transmitted from mother to child, and can remain a commensal or cause neonatal infections. Therefore, the DRF provides an excellent means to illustrate and explain all the various forms of candidiasis in the context of host response and host damage.

Much of what we know about *C. albicans* pathogenesis and host immune responses has come from animal model systems.

The large panel of clinically relevant models available to study systemic and mucosal candidiasis highlights the diversity of niches in which this fungus can cause disease (4, 6). The rodent is ideal for studying *C. albicans* pathogenesis due to the demonstrated similarity to the human disease process and host immune responses. Experimental animal models of mucosal candidiasis, in particular, have been invaluable in elucidating the various compartmentalized host immune responses to *C. albicans* at the various mucosal sites. Similarly, rodent models have been instrumental in understanding host responses during the initiation and progression of systemic *C. albicans* infection. The main advantage of these models is that they allow for manipulations, genetically and pharmacologically, in order to mimic susceptible hosts, greatly contributing to our knowledge of factors leading to host susceptibility to *Candida* infections (7).

The versatility of *C. albicans* in its pathogenic potential and the diversity of its disease entities, depending on infection site, position it in a unique situation: based on the various clinical manifestations as a function of host immune status, this pathogen may arguably fit within each of the classes outlined by the DRF (1, 8). Accordingly, here we highlight some of the varied and site-specific

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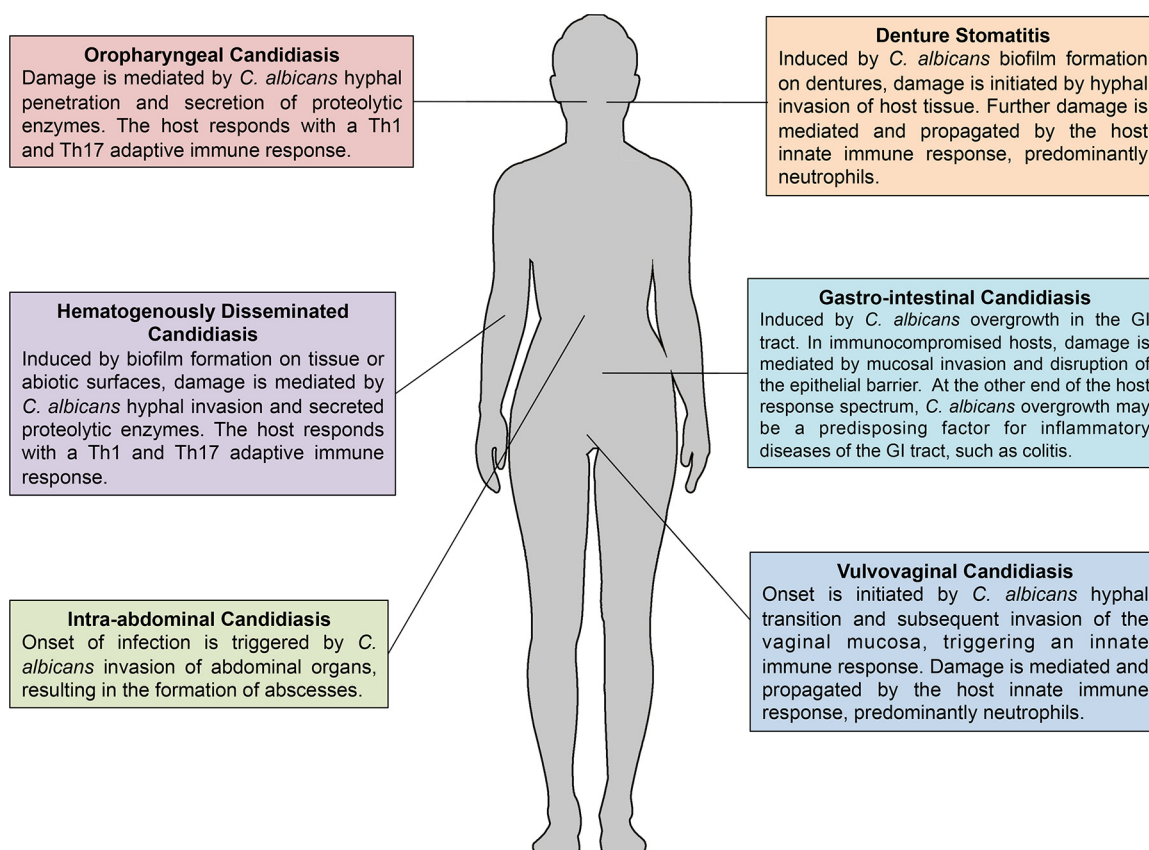


FIG 1 Anatomical defenses and host damage associated with the various manifestations of candidiasis. The illustration shows the diverse and site-specific diseases caused by *C. albicans*, highlighting the disease pathogenesis in each case and site-specific host immune responses.

diseases caused by this human pathogen (Fig. 1) in the context of the DRF. Within each class, disease pathogenesis is explored using both clinical data and data from animal models that support the DRF classification. Of note, while this perspective article focuses specifically on *C. albicans*, some of the literature cited to support the classifications of *C. albicans* within the DRF does not discriminate between individual *Candida* species. Accordingly, the classifications ultimately may be extended to other *Candida* species as well.

DAMAGE RESPONSE FRAMEWORK

The premise of the DRF is that damage to the host can be mediated by either the pathogen or the host, and therefore microbial virulence is a measure of the outcome of an interaction between a microbe and a susceptible host. This integrated view of pathogenesis in which the contributions of both the microbe and the host are incorporated became essential in light of the surge in infections caused by microbes once considered to be members of the commensal microbiota. It is well recognized that medical devices, immunosuppressive therapies, and diseases such as AIDS have created patient populations highly susceptible to infections. This awareness ultimately led to the development of the concept of microbial opportunism and opportunistic pathogens, viewed as microbes with pathogenic potential that becomes manifest in the setting of a weakened host immune system (8, 9).

The DRF is based on the core principle that there are no exclusive pathogens, commensals, or opportunists, but that microbial

pathogenesis requires a microbe and a host to interact, with the relevant outcome being damage to the host. *C. albicans* pathogenesis has often been described using phrases based on the concept that *C. albicans* is the exclusive causative agent, such as “converts from commensal to pathogen” or exists as an “opportunistic pathogen.” Under the tenets of the DRF, these phrases or terminologies are invalid and should be avoided. More importantly, the ensuing damage results from microbial and/or host factors, with the host response contributing to microbe-mediated damage. Further, the host damage can stem from either weak or strong responses to microbes. This concept is illustrated in graphs depicting six different levels of damage to the host as a function of the range of host immune responses with the potential for considerable damage occurring at either or both extremes of the host response (1, 9). In a global context, the DRF shifted the focus away from exclusively microbe-mediated damage by emphasizing the role of the host as a contributor to damage. Importantly, in addition to host and microbe, this flexible conceptual framework also focuses on outcomes that are a function of multiple factors, which include the environment and time.

DRF CLASSIFICATION OF MICROBIAL SPECIES

Class 1: pathogens that cause damage only in the setting of weak immune responses. Microorganisms placed in DRF class 1 are those usually considered opportunistic or commensal and are associated with disease only in individuals with impaired immune function.

Class 2: pathogens that cause damage either in hosts with weak immune responses or in the setting of normal immune responses. In DRF category 2 are microorganisms that cause host damage by both host- and pathogen-mediated mechanisms and are viewed as opportunists because their prevalence is higher in groups with impaired immune function. However, the capacity of class 2 microorganisms to mediate disease in individuals with apparently normal immunity is indicative of the expression of microbial characteristics that promote their ability to evade normal host defenses that would otherwise eliminate them.

Class 3: pathogens that cause damage in the setting of appropriate immune responses and produce damage at both ends of the continuum of immune responses. In DRF class 3, microorganisms can cause damage in normal hosts, which is amplified in the settings of either weak or strong immune responses.

Class 4: pathogens that cause damage primarily at the extremes of both weak and strong immune responses. In normal hosts, microbes in DRF class 4 cause relatively little damage. However, a weak immune response can promote infection and pathogen-mediated damage, while a strong immune response can produce excessive inflammation.

Class 5: pathogens that cause damage across the spectrum of immune responses, but damage can be enhanced by strong immune responses. In DRF class 5, microorganisms cause infections that result in pathogen-mediated damage but the damage is associated with protracted or chronic damage resulting from an excessive or inappropriate immune response.

Class 6: pathogens that can cause damage only as a result of strong immune responses. DRF class 6 was first thought to be a largely theoretical category to describe a growing list of diseases that may have a microbial etiology not associated with impaired immune function. These organisms may also be members of the normal microbiota and confer a benefit to the host in settings of normal or weak responses.

According to the criteria for each class, *C. albicans* was initially categorized as a class 2 pathogen. However, unlike most pathogens, *C. albicans* is a human commensal with no known environmental reservoir, making it highly host adapted at various anatomical sites. Given its adaptability and the diversity of its pathogenic potential, classifying *C. albicans* within one of the DRF classes does not account for the full complexity of its pathogenesis. In our review of the various candidal diseases, it became clear that a select spectrum of *C. albicans* infections can be categorized within each of the six classifications, depending on the site and manifestation of infection and the nature of the host immune response.

CANDIDA ALBICANS: A VERSATILE FUNGAL PATHOGEN

Among fungal species, *C. albicans* is the most common human pathogen, causing diseases ranging from superficial mucosal to life-threatening systemic infections (2, 10–12). As part of the commensal human microbiota, *C. albicans* asymptotically colonizes the gastrointestinal tract, oral cavity, and reproductive tract of healthy individuals (13, 14). At these various sites, its proliferation is controlled by the host immune system and colonization resistance is provided by other members of the microbiota. Therefore, its isolation from mucosal tissues does not necessarily indicate a diseased state (3, 4, 15, 16). However, disruptions in host immune status, barrier function, or local microenvironment (including microbiota composition) can lead to changes in *C. albi-*

cans growth and physiology (gene/protein expression, metabolism, morphology), which can cause host damage, both microbe mediated and/or host mediated. Depending on the magnitude of damage, disease ensues, which can be acute and/or recurrent in nature (4, 17–19). As a result, *C. albicans* infections are recognized as a serious public health challenge with high clinical and socioeconomic importance, representing one of the most prevalent agents identified in nosocomial infections (10, 20, 21).

As with other microbial pathogens, the ability of *C. albicans* to adhere to host surfaces is a prerequisite for both successful colonization and persistence during infection. Unsurprisingly, the majority of *C. albicans* infections are associated with its ability to form biofilms on host tissue or abiotic surfaces (6, 18, 19, 22–24). Biofilms are structured communities of surface-associated microbial populations embedded in a matrix of extracellular polysaccharides proposed to provide protection for biofilm cells (24–27). *C. albicans* biofilm-embedded cells are afforded a stable environment where they are protected from the host immune system and can tolerate extremely high concentrations of antimicrobials (28). The impact of these biofilms on public health is dramatic, as cells released from biofilms formed on implanted biomaterials, including vascular catheters, can potentiate systemic infections. Thus, *C. albicans* biofilm contamination of medical devices has been implicated as a risk factor for increased patient mortality (18, 19). The growing usage of and need for implanted medical devices and central venous catheters in managing patient care are important reasons why the incidence of *C. albicans* infections have steadily increased. In fact, *C. albicans* and other non-*albicans* *Candida* species are currently ranked by the Centers for Disease Control and Prevention as the third most commonly isolated bloodstream pathogens in hospitalized patients, with a mortality rate of up to 50% (6, 19, 24, 29). Overall, the annual cost of antifungal therapies for device-associated *Candida* species infections in the United States alone is estimated at \$2.6 billion (30, 31).

A number of properties and virulence factors attributed to *C. albicans* are known to promote its biofilm-forming potential and its persistence within the host. First and foremost is the property of morphological transition, as the distinct morphological states (yeast, pseudohyphae, hyphae) of *C. albicans* dictate phases of colonization, growth, and dissemination; the yeast form has been associated with both initial attachment and dissemination, while the filamentous hyphal form enables *C. albicans* to form a biofilm and invade host tissue (24, 32). Importantly, mutants locked in either the yeast or pseudohyphal phase are generally less virulent and more easily cleared from tissues during intravenous infections than hyphal-phase-locked mutants (33). Another important virulence property is adhesion mediated by cell wall adhesins; most notable are the agglutinin-like sequence family (Als) and a hyphal wall protein (Hwp1) that is crucial for attachment of *C. albicans* to receptors on host tissues (30, 34). In addition, *C. albicans* also produces several extracellular secreted enzymes, such as lipases, esterases, and secreted aspartyl proteinases (Saps), as well as hemolysins, that are involved in host tissue invasion and nutrient acquisition (35). Compounded by the increase in resistance to the relatively limited number of available antifungal drugs, the continued increase in the incidence of *C. albicans* (including non-*albicans* *Candida* species) infections highlights the need for elucidating the fundamental pathogenic determinants of *C. albicans* and the reciprocal host protective mechanisms against this fungus (4).

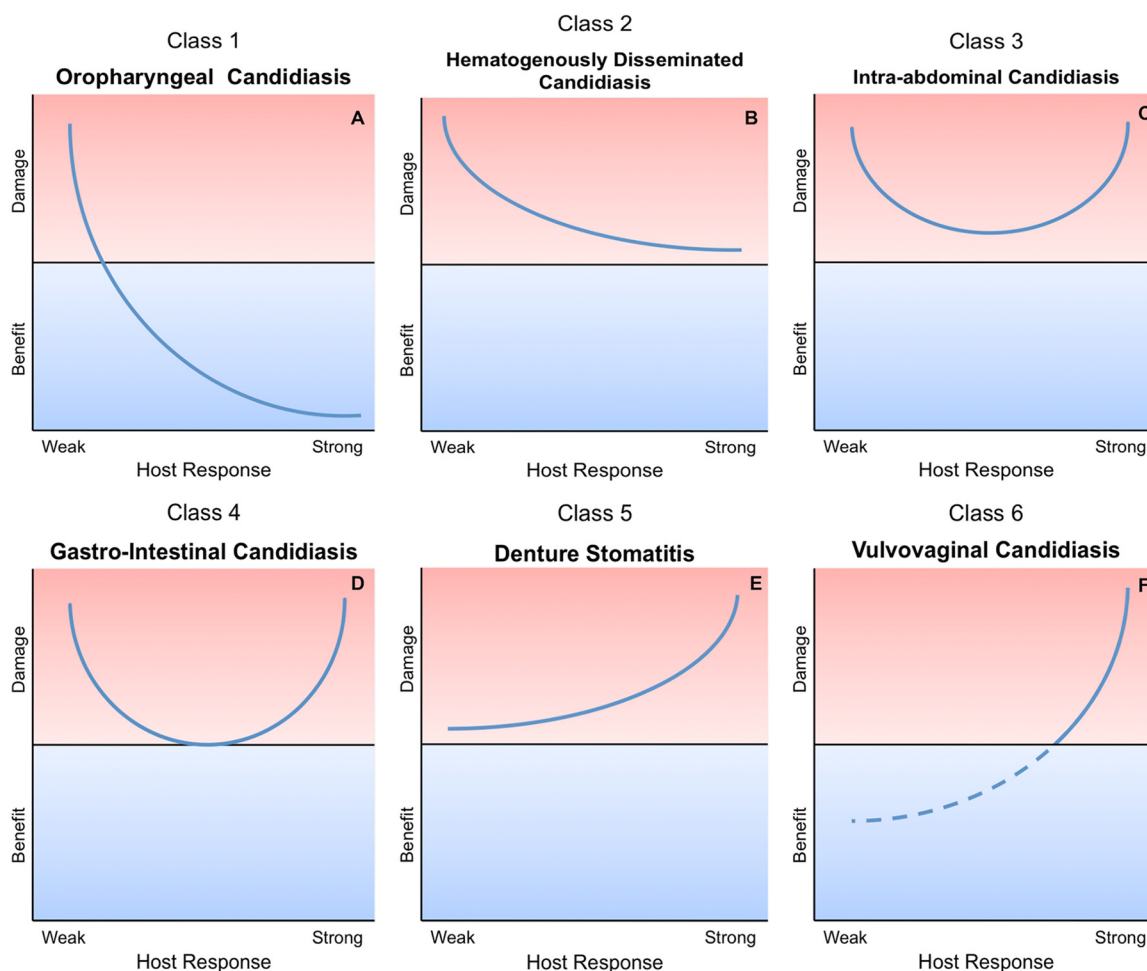


FIG 2 Damage response framework curves. Individual graphs are included for each class, with an associated *Candida* infection. (A) Class 1, pathogens that cause damage only in situations of weak immune responses (e.g., oral candidiasis). (B) Class 2, pathogens that cause damage either in hosts with weak immune responses or in the setting of normal immune responses (e.g., invasive candidiasis). (C) Class 3, pathogens that cause damage in the setting of appropriate immune responses and produce damage at both ends of the continuum of immune responses (e.g., intra-abdominal candidiasis). (D) Class 4, pathogens that cause damage primarily at the extremes of both weak and strong immune responses (e.g., gastrointestinal candidiasis). (E) Class 5, pathogens that cause damage across the spectrum of immune responses, but damage can be enhanced by strong immune responses (e.g., denture stomatitis). (F) Class 6, microorganisms that can cause damage only under conditions of strong immune responses (e.g., vaginal candidiasis). (Adapted from reference 1 with permission.)

FITTING *C. ALBICANS* WITHIN THE DAMAGE RESPONSE FRAMEWORK

The ability of *C. albicans* to adapt to various and changing host environments, such as immune status and microbiota, is key to its ability to cause such a wide range of diseases. Here, we highlight some of the most common candidal diseases and, based on infection site and nature of host immune response, we propose their classification within the DRF (Fig. 2). Furthermore, host and pathogen factors mediating damage to the host and the animal models available to study the various diseases are described in Table 1.

Class 1 (damage occurs only in situations of weakened or compromised immune system): oropharyngeal candidiasis. Oropharyngeal candidiasis (OPC), commonly known as thrush, encompasses infections of the tongue and other oral mucous membranes and may extend into the pharynx (Fig. 2A). OPC can present as white curd-like lesions (pseudomembranous) (Fig. 3A) or reddened patches (erythematous) (Fig. 3B). The diagnoses of

both manifestations of OPC are essentially clinical and are based on the recognition of the lesions by a health care provider and can be confirmed by microscopic identification or culture of *Candida* (31, 36). OPC is rare in healthy adults, occurring almost exclusively in immunocompromised patients. In fact, OPC is the most common oral infection in HIV-positive individuals, although the incidence has been reduced significantly with antiretroviral therapies (ART) (16, 31, 37, 38). In addition to HIV infection, OPC occurs in 35% of cancer patients who have recently received chemotherapy, in the elderly and infants, and under conditions of malnutrition or local immune suppression (e.g., suppression resulting from use of steroid inhalers for asthma). Further, patients with Sjogren's syndrome, diabetes, or other metabolic or hormonal disorders or those on antibiotics are also predisposed to OPC (30, 37–39).

OPC is a biofilm-associated disease that results from the adherence of yeast cells to mucosal tissue, followed by hyphal invasion, which is associated with secreted proteolytic enzyme expres-

TABLE 1 Examples of *C. albicans* infections, host predisposing factors, and animal models for each DRF class

DRF class	Candidal infection example	Primary predisposing factor(s)	Rodent model(s)
Class 1	Oropharyngeal candidiasis	Immunocompromised state (AIDS, cancer patients)	Mouse oral infection model
Class 2	Hematogenously disseminated candidiasis	GI tract mucosal disturbances; intravenous catheters; surgery; use of broad-spectrum antibiotics; neutropenia and other immunosuppressed conditions	Rodent intravenous, subcutaneous catheter and GI tract translocation models
Class 3	Intra-abdominal candidiasis	Peritoneal dialysis; bowel surgery; GI tract perforation; hepatobiliary leaks	Mouse peritonitis and abscess models
Class 4	Gastrointestinal candidiasis	Immune deficiency; cancer; antibiotic or immunosuppressive therapy	Mouse mucosal GI model
Class 5	Denture stomatitis	Denture usage; poor oral hygiene	Rat denture model
Class 6	Vulvovaginal candidiasis	Antibiotic or hormone therapy; estrogen replacement; vaginal microbiome dysbiosis	Rodent vaginal infection models

sion (40). Therefore, under conditions of weak oral immune responses, *C. albicans* invokes considerable damage upon the host, highlighting its class 1 designation as an opportunist. However, as part of the commensal microbiota in the oral cavity, *C. albicans* can exist asymptotically in individuals with normal or strong protective host defenses. Under these conditions, *C. albicans* has the potential to develop a symbiotic relationship with the host, imparting a degree of protection against potentially harmful microbes that come in contact with the oral cavity (3). This potential for benefit in the context of strong host responses and for damage in the face of weak responses defines a DRF class 1 pathogen.

Experimental and clinical evidence has provided significant advances in terms of understanding more specifically what contributes to host defense against OPC. While it is clear that innate defenses (salivary flow, antimicrobial peptides) help limit *C. albicans* overgrowth in the oral cavity, it became quite clear during the HIV epidemic that CD4⁺ T cells were the primary protective host defense mechanism against OPC (37, 38, 41–46). An established mouse model of OPC has been widely used for investigating *Candida* virulence factors, immune mechanisms against candidiasis, and the efficacy of antifungal agents (4). In this relatively simple model, mice are rendered susceptible to oral infection by injection with cortisone acetate prior to sublingual inoculation with *C. albicans* (47). This process results in a reproducible level of infection, the histopathology (Fig. 4) of which mimics that of pseudomembranous OPC in patients (47, 48). The availability of a CD4/HIV^{MutA} transgenic mouse model that develops AIDS-like

disease was instrumental in demonstrating that HIV-mediated loss of CD4⁺ T cells underlies susceptibility to mucosal candidiasis (49). Originally, it was thought that the primary protective mechanism by CD4⁺ T cells was the Th1 phagocyte-dependent response (37). However, the discovery of the Th17 axis and subsequent *in vivo* studies using the OPC model identified the CD4⁺ Th17 response as the primary protective response (17, 50). Importantly, in the absence of interleukin-17 (IL-17) and related cytokines IL-12 and gamma interferon (IFN- γ), innate cell recruitment, activation, and phagocytosis of *C. albicans* cells fail to occur. Using knockout mice, defense against OPC was shown to be more dependent on Th17-type than Th1-type immunity, with Th17-deficient mice exhibiting impaired neutrophil recruitment and high fungal burdens (51). It is also noteworthy that Th17 cells enhance the expression of antimicrobial peptides, including defensins and histatins, which are produced by oral epithelial cells and salivary glands, respectively (52). Therefore, the Th1/Th17 immune response is central to combating and preventing oral candidal infections under immunocompetent conditions, providing a large-scale benefit with little to no damage from a strong immune response. Although the incidence of OPC can be high in the absence of ART, it is postulated that under CD4⁺ Th1/Th17 immunocompromised conditions, secondary host defense mechanisms can provide some protection against infection. These include oral epithelial cells via annexin-A1 and CD8⁺ T cells, although the mechanisms are not well understood (37, 53, 54).

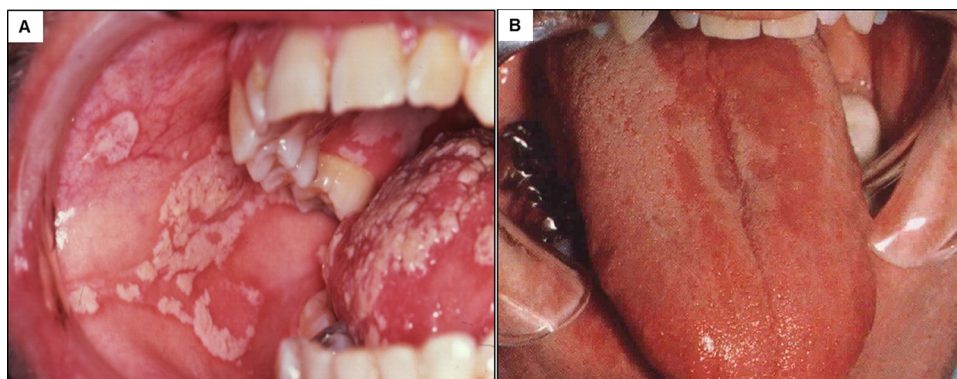


FIG 3 Clinical manifestations of oral candidiasis. (A) Pseudomembranous candidiasis is characterized by white plaques formed on the tongue and the buccal mucosa. (B) Erythematous candidiasis example, showing the subtle red lesions on the tongue, which can also occur on the palate.

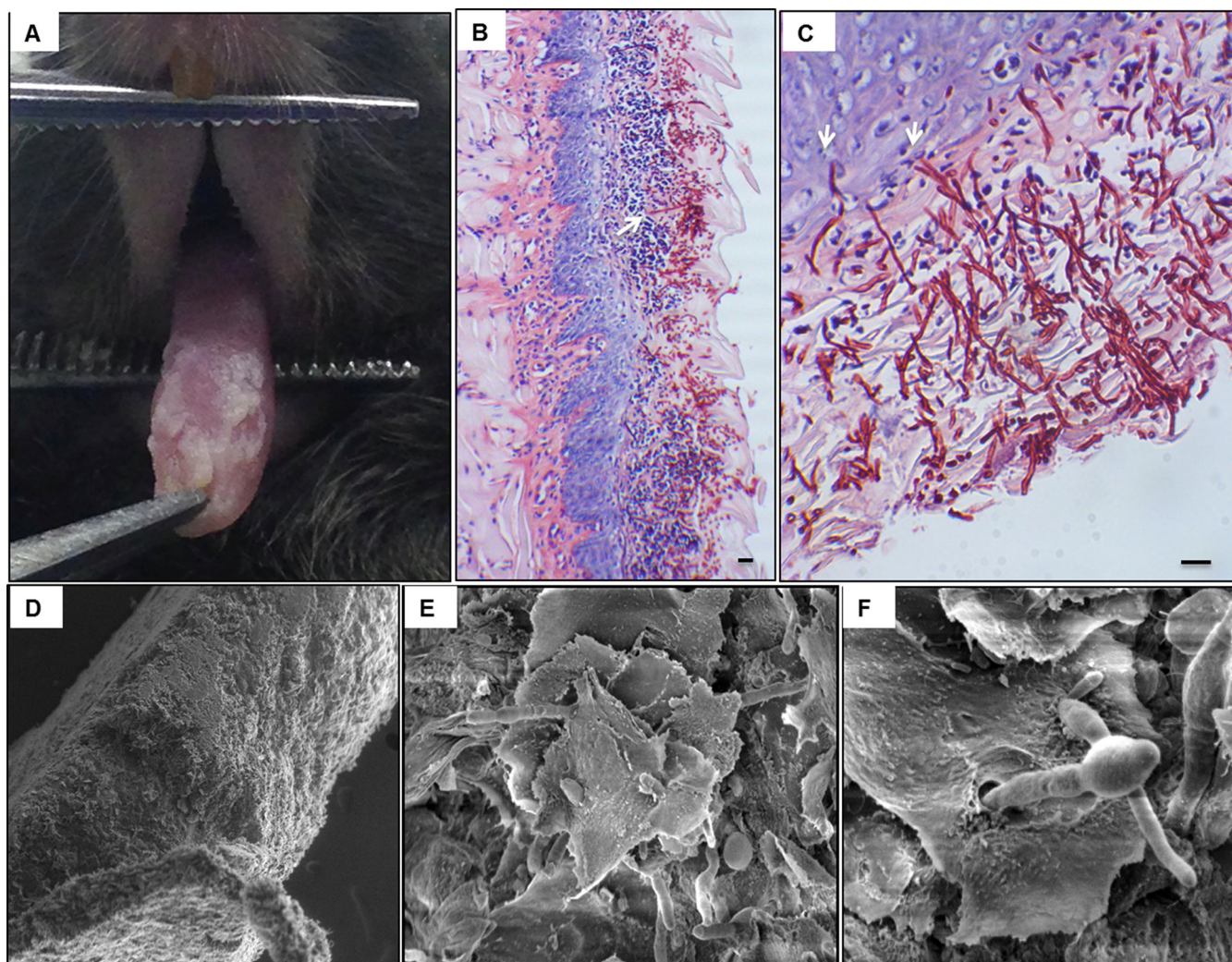


FIG 4 Mouse model of oral candidiasis. (A) An infected mouse exhibiting clinical signs of advanced candidiasis on the surface of the tongue 4 days post-sublingual infection with *C. albicans*. (B) Histopathology of an infected tongue tissue section, demonstrating the extensive presence of *C. albicans* around the periphery of the tongue. Hyphae can be seen penetrating the subepithelial tissue, along with a marked presence of host inflammatory cells. (C) Magnified image of tongue tissue, revealing the depth of hyphal invasion into the subepithelium (arrows). Bar, 20 μ m. (D) Scanning electron micrograph of excised tongue showing the thick biofilm formed on the outer epithelial surface consisting of *C. albicans* hyphae invading the subepithelium. (E) Higher-magnification image of the outer surface of the tongue showing the epithelium spiny layer with hyphae penetrating through the surface. (F) Significant gap in the tissue caused by hyphae invading from the sublingual area as it emerges through the tongue surface. (Reprinted from reference 166.)

Class 2 (damage occurs in hosts with weak or normal immune responses): hematogenously disseminated candidiasis.

Hematogenously disseminated candidiasis (HDC) arises when *C. albicans* gains access to the bloodstream (candidemia), leading to deep-seated candidiasis (DSC), which is defined as *C. albicans* infection of internal organs (Fig. 2B) (55). Overall, *C. albicans*, along with other non-*albicans* *Candida* species, is the second leading cause of invasive infection in North American intensive care units (ICUs), causing approximately 18% of infections, inclusive of all origins of infection (56). HDC is a leading cause of mycosis-associated mortality, and *C. albicans*, along with other non-*albicans* species, is currently ranked the third leading cause of bloodstream infections (BSI) in hospitalized patients in the United States (6, 19, 29, 57). Once in the bloodstream, *C. albicans* can infect a wide range of target organs, including the kidney, spleen, liver, heart, gastrointestinal (GI) tract, and lungs (58). Within tar-

get organs, invasive infections lead to significant tissue damage (59, 60). One of the major problems in managing patients with HDC is the difficulty in establishing the diagnosis, because bloodstream *Candida* infections tend to present clinically with nonspecific symptoms similar to those seen with systemic bacterial infections (7). More importantly, the sensitivity of blood cultures, the current diagnostic gold standard, is only ~50% (55). The nonspecific presentation and poor performance of blood culturing often lead to delays in the initiation of effective antifungal therapy, which contributes to the high mortality rates associated with *Candida* bloodstream infections (61).

HDC most commonly stems from translocation across a damaged GI tract mucosa into the systemic circulation or from infected vascular catheters or other access devices. GI tract translocation is often seen in neutropenic and other immunocompromised hosts, in particular cancer patients with mucositis stem-

ming from chemotherapy (62, 63). Central line catheter-associated infections typically emerge from biofilms formed on catheters, providing a niche for microorganisms where they are protected from both the host immune system and antimicrobials (24). Successful therapy of these foreign body-associated infections relies on device removal in most instances. Catheter-associated biofilms provide the opportunity for direct inoculation of *C. albicans* into the bloodstream. If the device is not removed, continual seeding of the bloodstream can overwhelm intact host defenses in nonneutropenic patients (normal host response) and cause significant mortality despite antifungal therapy and often times an intact immune system (64–66). In central line catheter-associated bloodstream infections, the weak defense is related to a breach in the barrier protecting the systemic circulation, as opposed to a more specific immune deficiency. Damage is amplified in acutely ill patients with additional immune deficiencies who acquire HDC. Thus, the fact that HDC can occur in the presence of both weak and normal defenses, albeit disproportionately, highlights the DRF class 2 designation of *C. albicans* and its behavior as an “equal opportunist” under such circumstances. The ability of *C. albicans* to cause systemic disease under conditions other than immune deficiencies highlights the fact that the term opportunistic does not always apply to this pathogen.

Animal models of HDC have been essential for our understanding of disease initiation and progression and for development of more effective diagnostics and therapies. In the standard HDC model, mice are infected intravenously (i.v.) via lateral tail vein injection, which results in rapid dissemination of *C. albicans* in a reproducible fashion (67, 68). Although fungal growth is controlled in the spleen and to a lesser extent in the liver, due to the presence of resident phagocytes, *C. albicans* causes extensive disease in the kidneys that is accompanied by increased immune infiltrates (68, 69). Whereas with lower inocula tissue burdens and host responses within the kidney are controlled, with higher inocula sepsis might occur, leading to high fatality rates (67, 70). In another model that has been useful for studying *C. albicans* dissemination to kidneys and other organs, a central venous catheter (CVC) is used to study *C. albicans* biofilm formation in catheters implanted in the central venous system of rodents. The benefit of this model is that it provides a realistic model of the central venous catheter infection site, catheter-vascular flow, and host serum proteins and other blood components, as well as humoral immunity (71–73).

With the i.v. HDC model, studies have shown that immune detection of *C. albicans* is accomplished by pattern recognition receptors (PRRs) on innate immune cells, where host Toll-like receptors (TLRs) and dectin receptors recognize cell wall components of *C. albicans*, such as mannans and glucans (74). Recognition of *C. albicans* by these PRRs activates the expression and secretion of a bevy of inflammatory cytokines, which are pivotal for optimal activation of phagocytes. In addition, the CD4⁺ T cell-independent IL-17 response is also notable and is considered essential to an effective antifungal response during systemic infection. In fact, animal studies have shown that infection of IL-17Ra knockouts results in greater mortality, higher fungal burdens, and significantly decreased neutrophil recruitment in an HDC model of infection (75, 76). In addition, IL-17 can also stimulate the production of chemokines such as IL-8, CXCL1, CXCL2, CCL20, and CCL7, which recruit polymorphonuclear leukocytes (77). Non-CD4⁺ T cell sources of IL-17 include $\gamma\delta$ T cells, which are

expanded in HIV⁺ patients and produce IL-17A in response to *C. albicans* (78). In a murine model of HDC, the major source of IL-17A was lung $\gamma\delta$ T cells, which were required for optimal neutrophil recruitment and control of infection (79). NK cells are not required for defense against intravenous infection in immunocompetent animals, but lack of NK cells in T/B-cell deficient SCID mice led to increased susceptibility to infection (80).

There are also several murine models of candidal dissemination via GI tract colonization that have been used to evaluate either host or fungal factors that promote disease (reviewed in reference 81). Most models rely upon antibiotic treatment to promote *C. albicans* GI tract colonization and upon immunosuppressive agents, such as 5-fluorouracil or cyclophosphamide. The liver is the most reliable target organ for monitoring dissemination (82, 83), presumably because translocation from the GI mucosa can occur via the portal circulation or biliary tree. To more specifically address which facets of host immunity are required to prevent dissemination, investigators sequentially disrupted specific host defense elements. Surprisingly, the findings demonstrated that depletion of lymphocytes, neutrophils, or macrophages did not predispose to candidal dissemination (83). Similarly, disruption of enteric mucosal integrity with dextran sulfate was also not sufficient to induce disseminated candidiasis. However, when agents that ablated neutrophils and also caused gut barrier disruption were administered, lethal disseminated candidiasis developed (83). Using germfree animals to facilitate *C. albicans* colonization, another study found that mice lacking phagocyte oxidase (*Phox*^{−/−}) and nitric oxide synthase 2 (*NOS2*^{−/−}) were susceptible to not only GI mucosal colonization but also dissemination and mortality (84). Overall, these studies underscore the importance of an intact GI tract barrier and oxidative pathways of granulocytes to defend against both mucosal infection and the ability to disseminate from the GI tract.

Class 3 (damage occurs throughout the continuum of immune responses but is amplified at extremes of both weak and strong immune responses): intra-abdominal candidiasis. Intra-abdominal candidiasis (IAC) results from entry of *C. albicans* into the abdominal cavity through translocation across the GI mucosal barrier or by direct inoculation, which can occur via contaminated peritoneal dialysis catheters or as a result of perforation of the GI tract (85–88) (Fig. 2C). While HDC is a DRF class 2 disease, IAC is considered a class 3 disease. Even in otherwise-immune-sufficient patients, inoculation of the abdominal cavity can cause peritonitis, an inflammatory disease of the lining of the abdominal cavity, which could be amplified with uncontrolled host response, leading to host-mediated damage. The cardinal clinical signs and symptoms of peritonitis include fevers, chills, and abdominal pain, and complications of peritonitis include invasion of adjacent organs, such as the liver and spleen, and/or abscess formation (83, 89). Treatment usually entails both source control with drainage and surgical intervention as well as antifungal therapy. In rare instances, intra-abdominal candidiasis can disseminate via the bloodstream (HDC) and cause DSC. This may be exacerbated in immunocompromised patients who lack innate defenses that could help to contain or control *C. albicans* within the peritoneal cavity. It is estimated that secondary candidemia occurs in 5 to 20% of intra-abdominal candidiasis cases (90). IAC is distinct from HDC (class 2) in that host damage is equally severe during a strong response, due to the local nature of the peritoneal immune response, characterized by neutrophil recruitment and inflamma-

tory cytokine production (90). While defects in innate defenses are associated with susceptibility to IAC, the robust response could ultimately compound the outcome of infection (91). The severity of these infections at either end of the host response continuum, with some damage occurring even under optimal host responsiveness, leads to *C. albicans* being classified in this scenario as class 3, with behavior as a “bipolar” pathogen. It is not clear, however, how pathogenesis and host responses in blood-borne *C. albicans* infections resulting from intra-abdominal candidiasis compare to those of bloodstream infections that originate through other mechanisms.

One unique feature of intra-abdominal infections involving *C. albicans* is that they are often polymicrobial, and fungal-bacterial mixed infections are associated with higher mortality rates than polymicrobial bacterial infections (92–95). Recently, several animal models were developed to study IAC in the context of bacterial contamination or coinfection (89, 96, 97). In one study, intraperitoneal inoculation of *C. albicans* along with sterile feces resulted in peritonitis within 6 h, characterized by a rise in pH and neutrophil influx into the peritoneal fluid. Organ invasion by hyphae and early abscess formation were evident 6 and 24 h after infection, respectively (89). It was postulated that the bacterial by-products acted synergistically with the fungi to cause disease. Another model using coinoculation of *C. albicans* with the bacterial pathogen *Staphylococcus aureus* showed similar synergistic effects on mortality compared with monomicrobial inoculation; the synergistic effects included dissemination of both species and sepsis (97). Intriguingly, however, mortality in the *C. albicans*-*S. aureus* coinfection model was associated with dramatic increases in inflammatory cytokines both locally and systemically very early postinoculation, but no increases in microbial burdens (96). These findings indicate that the host response is a key mediator of host damage, emphasizing the class 3 designation. Furthermore, a recent study examining the role of *C. albicans* morphogenesis in the disease process indicated that, unlike in the majority of *C. albicans* infections, hyphal formation is not a major contributor to the pathogenesis of *C. albicans*-bacterial pathogen peritonitis, although the signaling pathways governing morphogenesis are required (96, 98). Further, studies using a sublethal monomicrobial inoculum demonstrated a role for a secreted aspartyl protease (Sap6) in mediating peritoneal organ invasion and tissue damage independent of hyphal formation, supporting the concept that morphogenesis *per se* is not a virulence determinant during peritoneal infection (99). These mouse models provide powerful tools for measuring the relative virulence of infecting strains and evaluating *in vivo* gene expression. Additionally, they are also useful for understanding how *C. albicans* adapts to diverse host environments and for studying host responses within the peritoneal cavity. Importantly, the models are invaluable for identifying novel approaches for diagnosing, preventing, and treating intra-abdominal candidiasis and invasive candidiasis of intra-abdominal origin (89).

Class 4 (damage occurs primarily at the extremes of both weak and strong immune responses): gastrointestinal candidiasis. Environmental factors such as antibiotic use and diet, which can alter bacterial microbiota levels and lower colonization resistance, are believed to lead to overgrowth of *C. albicans* in the GI tract in humans (100) (Fig. 2D). While this has been demonstrated using murine models (81), monitoring human *C. albicans* intestinal colonization levels is more problematic. However, more

recent clinical studies examining fecal loads have demonstrated increased *Candida* levels with antibiotic treatment (101). Overgrowth may facilitate localized mucosal infection and/or generalized GI tract disturbances, but the validity of GI candidiasis via overgrowth as a clinical entity is controversial (102, 103). This has been difficult to verify due to the lack of specific symptoms (belching, bloating, indigestion, nausea, diarrhea, and gas) and lack of diagnostic tests. Patients considered at risk for this type of candidiasis include cancer and transplant patients receiving immunosuppressive therapy or prolonged antibiotic prophylaxis, and therefore this represents a disease associated with impaired host immunity (104, 105). At the other end of the host response spectrum, experimental and clinical evidence also suggests that GI tract *C. albicans* colonization may be a cofactor for inflammatory diseases, which could be considered host-mediated damage. Therefore, based on current, albeit limited data, GI candidiasis fits within the class 4 designation, with *C. albicans* behaving in this case as an immunoreactive opportunist, causing host damage in the context of both strong and weak host responses.

It is clear from various animal models that the major defense against *C. albicans* overgrowth in the GI tract is the presence of the bacterial microbiota and normal gut peristalsis. Antibiotic treatment or the use of germfree gnotobiotic animals enhances consistent colonization of the gut (81). In terms of innate immunity, mice with congenital granulocytic cell deficiencies are susceptible to GI candidiasis but mount *C. albicans*-specific adaptive responses and eventually clear the infection (106). Treatment with a NOS inhibitor increased the severity of GI tract infection; however, the reactive chemical species responsible is likely peroxynitrite, as nitric oxide is not directly candidicidal *in vitro* (107). Studies using congenitally athymic T cell-deficient SCID mice or CD4⁺ T cell-depleted mice showed that T cells are critical for effective protection against GI candidiasis (108–112).

There are several experimental and clinical studies that support the notion that *C. albicans* overgrowth in the GI tract exacerbates inflammatory diseases, promoting local and systemic hyperreactivity. In one such murine model, inoculation of the GI tract with *C. albicans* was accompanied by dramatic increases in pulmonary allergic responses to experimental allergens (113, 114). These studies utilized immunocompetent mice and did not involve previous systemic antigen priming, as is typically used for inducing hypersensitivity to these allergens. There was also no evidence of microbial growth in the lungs or inflammation in the GI tract in this model. Combined, the findings from these studies demonstrate experimentally that disruption of the microbiota, including fungal GI tract colonization, can reduce tolerance to aeroallergens. Mechanistically, it has been suggested that aberrant immune responses to mucosal fungal colonization in the GI tract are responsible for disrupting normal tolerance mechanisms that control hyperreactivity. For example, mice deficient in dectin-1, which recognizes β -(1,3)-glucan in the fungal cell wall, are more susceptible to induction of colitis, which was associated with increases in intestinal fungi, including *Candida* spp. (115).

In humans, polymorphisms in dectin-1 are associated with severe medically refractive ulcerative colitis more strongly than in patients with less severe disease (115). Further, GI tract inflammation was shown to promote *C. albicans* colonization in chemically induced colitis in mice, which augments inflammatory responses via the PRR galectin-3 (116). *C. albicans* colonization also primed expansion of Th17 cells with commensal specificity, driving intes-

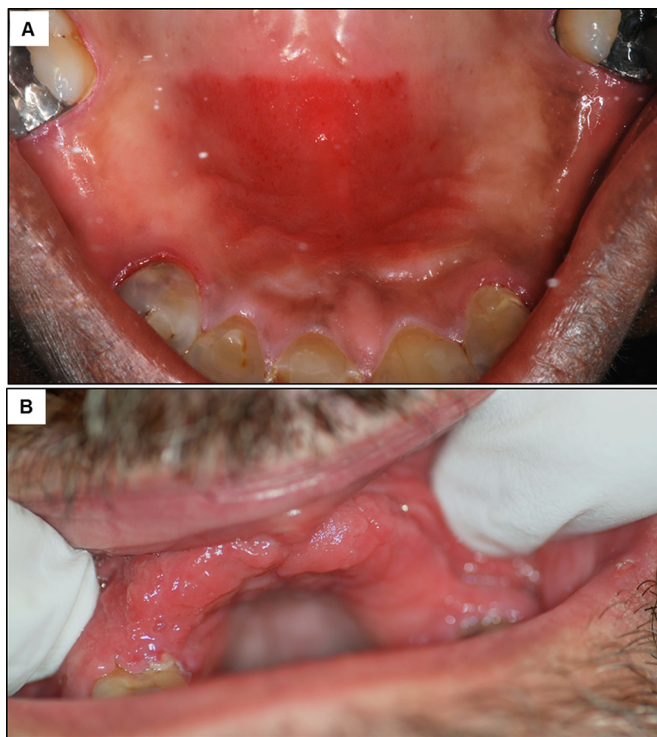


FIG 5 Clinical manifestations of denture stomatitis. (A) Red inflammatory lesions formed on the denture-associated palatal tissue in a patient with a partial denture. (B) Inflammation of the gingival tissue in a patient with a full denture.

tinal inflammation (117). Likewise, *C. albicans* colonization of the GI tract in germfree animals induced gastritis, demonstrating the inflammatory potential of this “benign” microbiota member (13). In humans, it has been noted that the fungal microbiota composition differs in patients with inflammatory bowel disease (IBD) from that in healthy individuals, with increases in fungal load and diversity (118). Therefore, PRRs that recognize fungal moieties may help promote responses to commensals in the GI tract and initiate damage. It is as yet unclear whether GI tract carriage of *C. albicans* in healthy hosts (up to 50% of individuals) imparts any benefit, but it is often asymptomatic and hence likely does not normally cause damage in the context of normal host responses (119).

Class 5 (damage occurs across the spectrum of immune responses, but damage is enhanced by strong immune responses): denture stomatitis. *Candida*-associated denture stomatitis (DS) is the most common form of oral candidal infections in otherwise-healthy individuals and is primarily caused by *C. albicans* (120, 121) (Fig. 2E). Denture stomatitis is a chronic disease characterized by localized or generalized inflammation of the denture-bearing mucosa and affects patients wearing removable partial or complete dental prostheses (Fig. 5). Symptoms of *Candida*-associated DS range from mild to severe, including palatal edema, painful inflammation, and papillary hyperplasia (small pebble-like sores) (120). This condition is prevalent in approximately 70% of denture wearers, with very high recurrence rates despite antifungal therapy (120–122). *C. albicans* readily adheres to the acrylic denture material and forms biofilms, which could result in continuous seeding of biofilm-associated organisms on the palatal

tissue. This is particularly exacerbated in situations where an ill-fitting denture and frictional irritation damage the normally protective mucosal barrier, allowing infiltration of *C. albicans* into the tissue (30). Under these conditions, a strong immune response, likely chronic in nature, will invoke considerable damage to the host, whereas a moderate response results in less damage, highlighting the DRF class 5 designation of *C. albicans* in DS as an immunoreactive pathogen. We recognize that assigning this class may be somewhat premature based on available data; clarification/confirmation of this assignment should be forthcoming, pending further research.

To study *Candida*-associated DS *in vivo*, a rat acrylic denture model that recapitulates features of DS was developed (123, 124) (Fig. 6). In this model, a custom-fitted fixed and removable denture system (U.S. patent 8,753,113 [125]) is installed in the rat palate, followed by inoculation with *C. albicans* (123). This system allows longitudinal analysis of rats, which become chronically and stably infected while the denture is in place. Microscopic analysis demonstrated biofilm formation on both the palate and denture by 4 weeks postinoculation with no obvious tissue invasion (superficial infection). Rats also exhibited gradual worsening of erythematous palatal inflammation, while histopathology of the palate tissue revealed neutrophil infiltration, indicative of a strong inflammatory response (123). Using a similar rat model of DS, a separate study indicated that *C. albicans* SAP expression correlated with histological changes, suggesting that specific fungal virulence factors promote tissue damage (126).

Clinically, even in DS patients without obvious clinical symptoms (limited inflammation/damage; normal immune response), there may be detrimental sequelae from a contaminated denture. Chronic denture infection could lead to seeding of the GI tract, which serves as a major portal for systemic infection in immunosuppressed or hospitalized patients (127). In fact, DS patients have higher rates of GI tract carriage of *C. albicans*, with similar species isolated from the oral cavity and feces (128). This could become clinically important in the elderly population, which has high rates of denture wearing and increased risk of developing immunosuppressive diseases. Clinical studies have also demonstrated that elderly denture patients have lower expression and activity levels of salivary innate defenses, which may promote oral *C. albicans* levels (129).

Class 6 (damage occurs only under conditions of strong immune responses): vulvovaginal candidiasis. Similar to the oral cavity, *C. albicans* is a resident of the normal vaginal microbiota and the leading causative agent of vulvovaginal candidiasis (VVC) (14, 130–135) (Fig. 2F). Vulvovaginal candidiasis caused by *Candida* spp. is characterized by itching, burning, pain, and redness of the vulva and vaginal mucosa and is often accompanied by vaginal discharge. It is estimated that 75% of all otherwise-healthy immunocompetent women of childbearing age will be affected by VVC at least once in their lifetime (135, 136). Although VVC is easily treated with antifungals and the infection is normally cleared, approximately 5 to 8% of afflicted women will suffer from recurring episodes characterized by having four or more episodes per year requiring continual antifungal therapy (14, 53, 131, 136, 137). Predisposing factors for primary VVC include high-estrogen oral contraceptive use, hormone replacement therapy, antibiotic usage, and underlying diabetes mellitus. Importantly, disruption of the vaginal microbiota is also considered an important contributor to this complex disease (14, 133, 138). Recurrent VVC (RVVC)

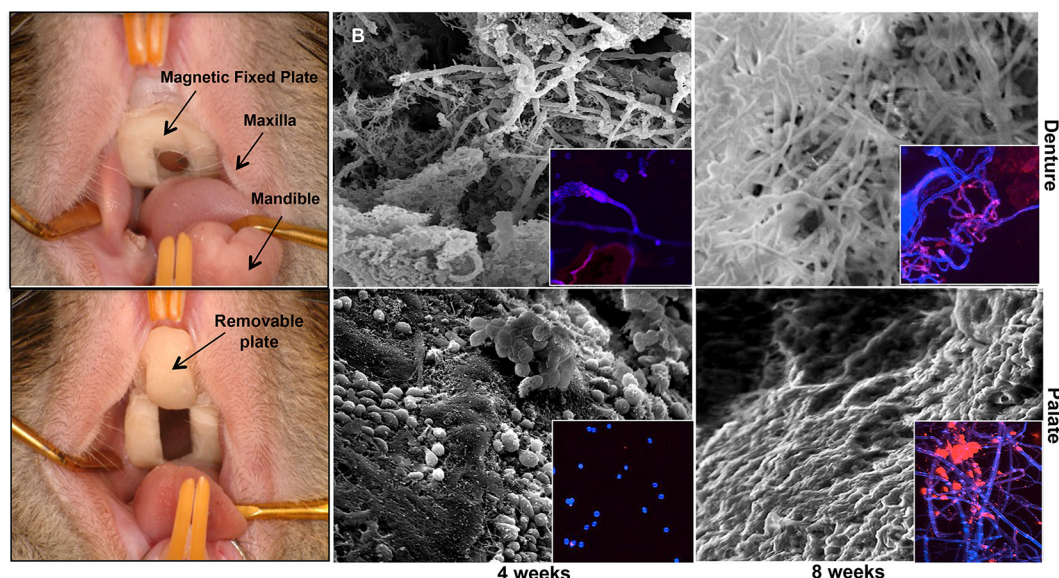


FIG 6 Rat model of *Candida*-associated denture stomatitis. Individual impressions were made for each rat, using vinyl polysiloxane impression material. Molds were made from the impressions, which were then used to construct the fixed and removable portions of the denture system. (B) Scanning electron and confocal fluorescence microscopy analysis images of a *C. albicans* biofilm formed *in vivo* on the denture and palate of rats 4 and 8 weeks postinfection with *C. albicans*. (Reprinted from reference 166.)

is considered idiopathic with no known predisposing factors, although the mechanisms of VVC and RVVC pathogenesis are likely identical. Importantly, VVC and RVVC are not associated with immunodeficiency but are instead associated with a vigorous local inflammatory response, qualifying *C. albicans* in the context of this infection as a DRF class 6 designation, as it behaves as an immunoreactive commensal.

For study of VVC, a well-established estrogen-dependent mouse model is available (139). Unlike humans, laboratory rodents do not naturally harbor *C. albicans* as a commensal; however, the experimental infection model closely parallels the human infection, and findings from the animal model are translatable to the human host (4). This includes the host immunopathological response and drug efficacy. In addition, and in contrast to humans, mice maintain a neutral vaginal pH that favors hyphal formation, thus making them a robust model system for *C. albicans* vaginitis (reviewed in reference 4). Several properties of *C. albicans* have been proposed to play major roles in causing VVC, most of which have been investigated using the animal model. Most notably, strains of *C. albicans* defective in hypha formation displayed significantly reduced vaginitis symptomatology, indicating a requirement for hyphae in the pathogenesis of VVC (130, 137). *C. albicans* tissue penetration is mediated by the invasive filamentous hyphae and is aided by candidal secreted proteolytic enzymes, which degrade epithelial cell barriers and facilitate hyphal penetration (140–143). While not a focus of this article, it should be noted that *C. glabrata*, which does not form hyphae, is the second most common cause of symptomatic VVC (144). Although one may argue that this contradicts a role for hyphae in the disease pathogenesis, a recent study employing an animal model of *C. glabrata* VVC showed no evidence of an immunopathologic response despite consistent long-term vaginal colonization (145). Hence, it is unclear how symptoms occur in clinical cases of *C. glabrata* VVC.

Although these fungal virulence factors are important for the initial onset of VVC, following initial insult from *C. albicans* the propagation of disease is largely mediated by the host immune system (130, 146). In fact, the mucosal damage in symptomatic VVC is associated with an aggressive neutrophil migration into the vagina and a subsequent acute host inflammatory response (134, 147). This neutrophil response is initiated by the interaction of *C. albicans* with vaginal epithelial cells, which have no apparent ability to clear *C. albicans* (131, 134). Therefore, neutrophils contribute more to the symptoms associated with vaginitis (i.e., damage) than they provide protection against disease (130, 135, 147).

It is postulated that the neutrophil response is triggered by the sensitivity of the vaginal epithelium to *C. albicans*, with epithelial cells of women with RVVC considered sensitive to *C. albicans* resulting in a response, whereas cells of women with no history of VVC are resistant to such responses (146). Epithelial cell triggers are considered ultimately dependent on a threshold level of *C. albicans*, such that under sensitive conditions, *C. albicans* will stimulate the epithelial cells to produce alarmins and proinflammatory cytokines that ultimately lead to neutrophil migration and the inflammatory symptomatic condition (147, 148). On the other hand, under resistant conditions, the epithelial cells fail to elicit the alarmin response and instead inhibit *C. albicans* growth in a noninflammatory manner mediated by annexin-A1 (147, 149). Interestingly, adaptive cell-mediated or humoral immunity appears to play no role in protection against infection, although certain epitope-specific antibodies characterized as protective antibodies can be used therapeutically against infection (134, 135, 150–152). Another interesting caveat is that while Th17 responses are critical to neutrophil responses at other anatomical sites (oral, bloodstream), the role for Th17 responses in the vaginal neutrophil response is controversial (139, 153). The strongest evidence to date has shown that mice deficient in several cytokines encompassing the Th17 axis showed no change in the *C. albicans*-in-

duced neutrophil response, suggesting little to no role for the Th17 response in VVC (139).

In women with asymptomatic vaginal carriage of *C. albicans*, damage is limited. *C. albicans*, as a member of the normal vaginal microbiota, colonizes approximately 70% of healthy women (154). Thus, it is tempting to speculate that there is evolutionary pressure to maintain *C. albicans* in the majority of healthy women, with colonization imparting a benefit to the host. Carriage is not associated with other common vaginal infections, such as bacterial vaginosis (BV) (154). Molecular characterization of the vaginal microbiota has revealed that lactobacilli dominate in healthy women, while BV is associated with vaginal dysbiosis and decreased diversity (155). In contrast, vaginal colonization with *C. albicans* is more common in women with a lactobacillus-dominated microbiota (healthy composition) than in women with dysbiosis (156). Therefore, *C. albicans* may support bacterial homeostasis as a benefit to the host, and *C. albicans* adherence to the vaginal epithelium may also provide colonization resistance against sexually transmitted pathogens. Such benefits to the host can be considered to be within the properties of the theoretical DRF class 6 designation.

THE HOST AND THE HOST MICROBIOTA

Scientists have estimated that there are at least as many microbial cells living in and on the human body as human cells in the body (157). The host-inhabiting microbes, defined as the microbiota, are an intricate mixture of microorganisms that have coevolved with their human host (158). Therefore, from the microbial perspective, the host is considered a complex environment. However, these host-microbe interactions influence various aspects of host physiology, and when in homeostasis, the microbiota contributes significantly to maintaining host health. Importantly, the microbiota has profound effects on host immunity and susceptibility to microbial diseases and, therefore, alterations to the microbiota could lead to host-microbe interactions that can produce host damage (8). Both extrinsic and intrinsic factors can cause perturbations to the system, which could lead to alterations in host physiology with potential adverse effects on host health (159). Consistent with the coevolved symbiotic relationship between microbes and humans, the indigenous microbiota provides many crucial functions to the host. In fact, the origin of the concept of colonization resistance dates back to 1965, when the role of the microbiota in antagonizing colonization with a potential pathogen was demonstrated (160). The introduction of broad-spectrum antimicrobial agents, in particular, provided the first evidence of the impact of loss of constituents of the microbiota (161). This was particularly applicable to *C. albicans*, for which it was first noted in the 1950s that the use of antimicrobial agents was linked to the increase in oral candidiasis in otherwise-healthy people (162).

It is well-established that the bacterial microbiome of the GI tract, including lactobacilli, plays a vital role in preventing fungal colonization, as indicated by the enhanced susceptibility of germ-free mice to *C. albicans* colonization (163). Although ample studies have focused on the ability of the GI microbiota to influence *C. albicans* levels, surprisingly little is known about the role of *C. albicans* in shaping the bacterial microbiota, particularly during antibiotic recovery (13). Similarly, VVC is a common side effect of antibiotic treatment, indicating that the vaginal microbiota might modulate colonization of *C. albicans* (14). However, the role of the vaginal microbiota in VVC is controversial in the literature; where

one study comparing the *Lactobacillus* species cultured from the vaginal secretions of women with or without VVC showed no significant differences, in another study *Lactobacillus* colonization was associated with a ≥ 4 -fold increase in symptomatic VVC (132, 133). A subsequent comprehensive study of the vaginal microbiota found no altered or unusual bacterial communities in women with VVC, suggesting that commensal vaginal bacterial species might be incapable of preventing VVC (138). Therefore, to understand the interactions of fungi and bacteria within the human GI and vaginal microbiota and their impact on health and disease states, more studies using high-throughput sequencing techniques with longitudinal samples are warranted (14).

As the microbiota is known to be critical for proper immunological function, it can be viewed as an active participant in the outcome of host-microbe interactions (164). The original formulation of the DRF did not incorporate a role for the microbiota; however, one of the utilities of the DRF is its flexibility to accommodate advances in knowledge in the field of microbial pathogenesis. Therefore, in 2015, the DRF was reformulated to incorporate the rapidly accumulating new information emerging from human microbiome studies. This reconciliation redefined the host as “the entity that houses its associated microbiome/microbiota, interacts with microbes, and responds to them in a way that results in damage, benefit, or indifference, thus resulting in the states of symbiosis, colonization, commensalism, latency, and disease” (8).

Researchers have only just begun to describe the microbial communities that are associated with humans and the extent of the interactions between a host and its microbiota. However, dissecting the host immune system responses to perturbations in microbiota remains a considerable challenge, primarily due to the enormous and diverse microbial communities that colonize various parts of the body. Understanding the mechanisms by which host homeostasis is restored is critical for future therapies aimed at manipulating the microbiota. In advancing our immunological insights into diseases, it has become clear that we also need to identify the mechanisms that allow specific members of the microbiota to modulate the host's immune health. Only with an integrated approach will it be possible to make new connections between the microbiota and the immune system. Such insights may allow for prediction of disease development and will unveil the therapeutic potential for restoration of the microbiota in order to reestablish microbial homeostasis. Therefore, improved mouse models, such as those that contain a humanized microbiota, would allow for malleable therapeutic manipulations that would assist the testing and translation of potential therapeutic interventions (162, 165).

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

C. albicans is a highly adaptable microbial species able to cause infection at various anatomical sites with equally diverse host responses. Remarkably, this diversity allows *C. albicans* to fit into all six of the DRF categories, highlighting the complexity of the pathogenesis associated with *C. albicans* infections and, importantly, emphasizing the flexibility or plasticity of the parabolas within the DRF. This obliges us to take into account the DRF as much as the virulence attributes or host responses when uncovering the mechanisms associated with each infection. Similarly, animal models that have been instrumental in identifying host response mechanisms and the requirements of the organism for pathogenesis at each anatomical site, as well as for the develop-

ment of new therapeutic approaches, should now be considered within the context of the DRF for future investigations. It is important to note, however, that we have considered only a subset of *C. albicans* infections relative to the DRF classifications. Therefore, it is likely that as additional *C. albicans* infections are considered, they will also be integrated into the diverse classifications of the DRF. Finally, as the nuances of the roles of both host and pathogen continue to be revealed through better understanding of the DRF, it has become critical to consider therapeutic strategies that target the host in an effort to modulate immune responses in order to more effectively minimize damage to the host.

In conclusion, over the past 2 decades, we have come far in our understanding of the complex host-*C. albicans* interactions. Nevertheless, there remain considerable gaps in our knowledge of *C. albicans* pathogenicity, host immune responses and, significantly, the importance of the role of *C. albicans* as a constituent of the human microbiota. Incorporation of the DRF into studies addressing those gaps should strengthen the impact of findings, provide better global perspectives on various diseases, and identify appropriate host targets for therapeutics.

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