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Review

Immunoglobulins in Defense, Pathogenesis, and Therapy of Fungal Diseases

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Only two decades ago antibodies to fungi were thought to have little or no role in protection against fungal diseases. However, subsequent research has provided convincing evidence that certain antibodies can modify the course of fungal infection to the benefit or detriment of the host. Hybridoma technology was the breakthrough that enabled the characterization of antibodies to fungi, illuminating some of the requirements for antibody efficacy. As discussed in this review, fungal-specific antibodies mediate protection through direct actions on fungal cells and through classical mechanisms such as phagocytosis and complement activation. Although mechanisms of antibody-mediated protection are often species-specific, numerous fungal antigens can be targeted to generate vaccines and therapeutic immunoglobulins. Furthermore, the study of antibody function against medically important fungi has provided fresh immunological insights into the complexity of humoral immunity that are likely to apply to other pathogens.

The past two decades have been momentous in advancing our understanding of the role of antibody-mediated immunity (AMI) in host defense against fungi, and they have brought about a paradigm shift in our thinking on this question. Prior to the 1990s, AMI was considered to be irrelevant in host defense against fungi (for review, see Casadevall, 1995), as the experimental methods that were in use at the time were not able to consistently establish a role for AMI. These methods, including passive transfer of immune sera to naive hosts and correlating the presence of serum antibody with immunity to fungal disease, often yielded negative results, and there was a lack of association between invasive fungal diseases and known antibody defects in humans. By contrast, ample evidence that cell-mediated immunity (CMI) was essential for resistance to fungal diseases resulted in CMI being viewed as the arm of the immune system responsible for host defense against fungi. In a prior essay we described the practice of characterizing microbes by whether host defense against them was dependent on AMI or CMI as the ''great immunological catastrophe of the 20th century'' because this subdivision limited research on microbial pathogenesis and immunity to a single arm of the immune system, ignoring the other(s) (Casadevall and Pirofski, 2011). However, for fungi the situation changed rapidly after Dromer and her colleagues showed that a monoclonal antibody (mAb) to *Cryptococcus neoformans* was protective against lethal cryptococcal infection in mice (Dromer et al., 1987b). At about the same, time a protective mAb was reported against the fungus *Pneumocistis carinii*, although this organism was thought to be a protozoan at the time (Gigliotti and Hughes, 1988). Subsequently, protective mAbs have been successfully generated against five medically important fungi (Table 1). The fact that certain antigens recognized by some of the aforementioned mAbs are expressed by different fungi has raised optimism that universal antifungal vaccines that protect via AMI could be generated.

The breakthrough that made the identification of protective antibodies to fungi possible was the mAb technology. In contrast to polyclonal sera, mAbs provided defined reagents that recognized a single antigenic determinant and yielded consistent and reproducible results. Furthermore, and importantly, studies with mAbs led to the discovery that depending on their specificity and isotype, mAbs to fungi can mediate three different effects in being protective, nonprotective (indifferent), or disease-enhancing. The observed disease-enhancing properties of mAbs provided an explanation for historical difficulties in establishing a role for AMI with polyclonal preparations, as these intrinsically heterogeneous reagents were likely to contain a variety of antibodies in varying proportions with each of the foregoing activities. Hence, studies with mAbs established definitively that protective immunoglobulins to fungi can be produced and that the historical inability to establish a role for AMI in protection against fungi was most likely a function of the heterogeneous preparations used, rather than a fundamental limitation of AMI.

Given the rising tide of mycotic diseases, understanding the role of AMI in host defense against fungi is particularly important. Mycotic diseases have increased significantly as a result of use of antibacterial agents, which alter the host associate microbiota and immunosuppressive therapies, which induce impaired immunity. Most invasive fungal infections occur in patients with impaired immunity due to one of the interventions noted above or acquired immunodeficiency, such as HIV/AIDS. Given that immunosuppression enhances fungal pathogenesis, it is not surprising that despite ample in vitro activity, antifungal drugs often cannot eradicate the fungal burden in patients with impaired immunity. As such, fungal diseases are difficult to treat, have high morbidity and mortality, and often result in latency that can reactivate in the setting of immune suppression. Consequently, there is a great need to develop new approaches to treat and prevent fungal diseases. Immunoglobulins offer a very attractive approach. Vaccines that elicit AMI have a long track

Table 1. Fungal Antigens that Have Been Shown to Elicit Protective Antibodies

Fungus	Antigen	Reference
Aspergillus fumigatus	Beta-1,3 glucan	(Torosantucci et al., 2009)
	Cell wall glycoprotein	(Chaturvedi et al., 2005)
Candida albicans	Secreted aspartyl proteinase2	(Sandini et al., 2011)
	Beta-1,3 glucan	(Torosantucci et al., 2009; Torosantucci et al., 2005)
	Mp58 mannoprotein	(Viudes et al., 2004)
	Beta-(1-2)-linked mannotriose	(Han et al., 1997)
	Heat-shock protein 90	(Matthews et al., 1991)
	Als3	(Brena et al., 2007)
	Phosphoglycerate kinase	(Calcedo et al., 2011)
	Fructose bisphophate aldolase	(Calcedo et al., 2011)
Cryptococcus neoformans	Beta-1,3 glucan	(Torosantucci et al., 2009)
	Glucuronoxylomannan (GXM)	(Dromer et al., 1987a; Sanford et al., 1990; Mukherjee et al., 1992; Fleuridor et al., 1998; Beenhouwer et al., 2007)
	Glucosylceramide	(Rodrigues et al., 2000)
	Melanin	(Rosas et al., 2001)
Histoplasma capsulatum	Heat shock protein 60	(Guimarães et al., 2009b)
	Histone-like protein	(Nosanchuk et al., 2003)
	70 kDa cell surface protein	(Lopes et al., 2010a)
Paracoccidioides brasiliensis	gp43	(Buissa-Filho et al., 2008)
	Melanin	(da Silva et al., 2006)
Pneumocystis species	Glycoprotein A	(Gigliotti et al., 1996)
	Major surface glycoprotein	(Smulian et al., 2000)

record of efficacy against many infectious diseases, and having served as inaugural antimicrobial agents in the preantibiotic era, immunoglobulins are increasingly being considered as potential therapies for a variety of microbes (Casadevall et al., 2004). Consequently, there is now great interest in the development of vaccines and therapeutic antibodies for fungi, but progress in this area must contend with the complexity of AMI for this group of pathogens.

In this review, we discuss the tremendous progress that has been made in the field of AMI to fungal pathogens, with emphasis on how information learned with the fungi has informed immunology on basic mechanisms of antibody action.

Challenges Posed by Fungal Pathogens

In containing fungi, the immune system faces unique challenges unlike those posed by bacteria and viruses. Many fungal pathogens exhibit dimorphism, a characteristic that enables them to exist as hyphae or yeast cells, with each form having a distinct surface and antigenic composition. Fungal cells are surrounded and protected by a cell wall, which is a structure composed of crosslinked polysaccharides including chitin and other components. An important functional effect of the fungal cell wall is to inhibit the ability of complement to damage fungal cell membranes, even though complement activation can elicit an antifungal inflammatory response. Hence, antibody-dependent complement activation does not result in direct damage to fungal cells. However, there are now several examples of mAbs that bind to cell wall components and inhibit replication (Table 2). Although the mechanism by which mAbs bind and affect the fungal cell wall is not understood, it is possible that the complex nature of the cell wall requires a tightly regulated metabolism that is disrupted by the attachment of immunoglobulin molecules. Fungal cells are notorious for producing secondary metabolites with deleterious effects. For example, mannitol production can interfere with host oxidative defenses, and mycotoxins such as gliotoxin are toxic to immune effector cells. Adding to the difficulties encountered by the immune system, fungal pathogens can exhibit antigenic variation. The phenomenon of fungal dimorphism means that many pathogenic fungi can exist in two different forms in the host, each of which is antigenically distinct. For *C. neoformans,* antigenic diversity stems from the capsular polysaccharide, and it is estimated that each cell is antigenically unique owing to combinatorial diversity of the polysaccharide capsule (McFadden et al., 2007). In addition, the *C. neoformans* capsule is antiphagocytic and capable of quenching free radicals produced by host immune cell oxidative burst (Zaragoza et al., 2008). Capsular polysaccharide released by *C. neoformans* cells in tissue interferes with the inflammatory response and inhibits recruitment and the function of effector cells that are empowered by specific antibodies. Fungi are also capable of forming very large structures in tissue. *Aspergillus* species form dense collections of hyphae in tissue that induce surrounding tissue necrosis, and *C. neoformans* and *Coccidioides immitis* can each generate enormous structures in tissue known as giant or titan cells and spherules, respectively. *C. neoformans* giant cells are far larger than macrophages, precluding phagocytosis and posing a formidable problem for host defenses (Zaragoza et al., 2010; Okagaki et al., 2010). Although fungi exert a multitude of effects that thwart host defense, these very effects provide rational targets for AMI as

Table 2. Mechanisms of Antibody-Mediated Protection against Fungi

Mode	Mechanism	Comment	References
Indirect	Phagocytosis	This mechanism may be particularly important for fungi that actively resist phagocytosis, such as *C. neoformans*. However, phagocytosis may not be sufficient as a host-protective mechanism since many fungi can survive intracellularly.	(Schlageter and Kozel, 1990)
	Complement activation	Appears to be critical for IgM but may be dispensable for IgG	(Shapiro et al., 2002; Han et al., 2001)
	Antibody-directed cellular cytotoxicity	Best example is the requirement for specific antibody in NK cell-mediated antifungal effects against *C. neoformans*	(Nabavi and Murphy, 1986)
	Phagosome activation	mAbs to *H. capsulatum* promote phagosome maturation and thus interfere with fungal mechanisms of intracellular survival	(Shi et al., 2008)
	Modulation of the inflammatory response	mAbs to *C. neoformans* mediate changes in inflammatory responses that are associated with better control of infection in tissue	(Feldmesser et al., 2002; Feldmesser and Casadevall, 1997)
Direct	Inhibition of biofilm formation	An in vitro observation for *C. neoformans* that could reflect the ability of specific antibody to the capsule to interfere with polysaccharide release	(Martinez and Casadevall, 2005)
	Inhibition of polysaccharide release	Given the many deleterious effects associated with polysaccharide in tissue, the ability of antibody to reduce capsule shedding from *C. neoformans* could translate into more effective immune responses.	(Martinez et al., 2004)
	Inhibition of replication and cytotoxicity	The mechanism is unknown, but antibodies that bind cell wall components can inhibit replication and/or trigger cell death.	(Brena et al., 2007; Moragues et al., 2003; Magliani et al., 2005; Rosas et al., 2001)
	Interference with dimorphic changes	Some mAbs to *C. albicans* interfere with germ tube formation.	(Moragues et al., 2003)
	Alterations in gene expression	Antibody binding to the *C. neoformans* capsule triggers changes in gene expression that can make the fungus more vulnerable to antifungal agents.	(McClelland et al., 2010)
	Iron starvation	Iron starvation appears to be the mechanism by which a mAb is cytotoxic and interferes with morphogenesis.	(Brena et al., 2011)
	Mimicking killer toxin	Anti-idiotypic antibodies to yeast killer toxin mimic the structure of killer toxin and are active against many fungal species.	(Magliani et al., 2005)

antibodies to fungal cell wall determinants, metabolites, and capsular polysaccharides can serve to inhibit their deleterious effects.

Mechanisms of AMI against Fungi

Most current knowledge about mechanisms of AMI against fungi comes from studies with *C. neoformans* and *C. albicans*, which are among the most common and most studied human pathogenic fungi. Historically, mechanisms of AMI, or classical mechanisms, were held to include opsonization, complement activation, viral and toxin neutralization, and antibody-dependent cellular cytotoxicity (ADCC) (Casadevall and Pirofski, 2011). Among these mechanisms, viral neutralization is not immediately relevant, although some of the mechanisms by which viral neutralization is mediated could have parallels in fungal-antibody interactions. Each of the other classical functions could have a role in host defense against fungi. For example, opsonization is very important for host defense against *C. neoformans*, as the polysaccharide capsule abrogates phagocytosis and complement is not an effective opsonin for some strains (Zaragoza et al., 2003). Whether ingestion is an effective mechanism for controlling *C. neoformans* depends on the activation state of the cell and the immunological state of the host, as this fungus has the capacity for intracellular replication and can even escape the phagocytic cell by nonlytic exocytosis. In fact, intracellular replication can be faster than extracellular growth. In animal

models of cryptococcosis, complement activation appears to be essential for the function of specific IgM, but may be dispensable for IgG (Shapiro et al., 2002). In theory, toxin neutralization could contribute to host defense against fungi that produce mycotoxins, but this has not been demonstrated to date. Fc receptor-dependent ADCC can mediate protection against *C. albicans* (van Spriel et al., 2001), and antibody-dependent NK cell-mediated activity against *C. neoformans* has been demonstrated (Miller et al., 1990; Nabavi and Murphy, 1986). Another antibody-mediated antifungal mechanism is exemplified by the ability of anti-idiotypic antibodies mimicking killer toxin to directly damage fungal cells by interacting with the killer toxin receptor (Magliani et al., 2005).

In addition to the aforementioned classical mechanisms of AMI, new mechanisms of antibody action against fungi have been identified (Table 2). One such mechanism is that antibodies to certain fungal determinants are able to mediate direct antifungal effects without the need for host cells. For example, a mAb to *Candida albicans* mannoprotein has been shown to mediate three different activities: inhibition of adherence, inhibition of germination, and direct candidacidal activity (Moragues et al., 2003), with the direct effects involving antibody-mediated iron starvation (Brena et al., 2011). In addition, antibody-mediated inhibition of an aspartyl proteinase has been associated with protection against vaginal candidiasis (De Bernardis et al., 1997), and antibodies to 1,2 β mannotriose (Xin et al., 2008) and cell wall protein-derived peptides protect against disseminated candidiasis in mice (Xin and Cutler, 2011). Antibodies to cell wall β-glucans have been shown to interfere with adherence and cell wall remodeling (Torosantucci et al., 2005, 2009) and have demonstrated the capacity for direct antifungal activity via growth inhibition of *C. albicans* as well as *Cryptococcus neoformans* (Torosantucci et al., 2009). As exemplified by the direct antifungal and growth-inhibitory capacity of β-glucan antibodies, and the ability of AMI elicited by a cell wall β-glucan-based vaccine to protect against multiple fungi (Torosantucci et al., 2005), studies with mAbs have provided proof of principle that the development of universal fungal therapies and vaccines that provide AMI upon binding conserved fungal β-glucans could be possible.

For *Cryptococcus neoformans*, studies with mAbs have demonstrated several direct antibody-mediated effects, including interference with capsular polysaccharide release and biofilm formation. However, there is also considerable evidence that specific antibody to the *C. neoformans* capsule can mediate protection indirectly. These indirect mechanisms of protection alter the inflammatory response and depend on collaboration with effector cells and/or CMI. In fact, the primary mechanism of antibody-mediated protection against *C. neoformans* is most likely via modification of the inflammatory response in a manner that enhances fungal clearance and minimizes inflammatory damage to tissue (reviewed in Casadevall and Pirofski, 2003). Specific antibody can also alter the nature of *C. neoformans*-macrophage interaction, as exemplified by data showing that antibody-opsonized *C. neoformans* exits macrophages as biofilm-like microcolonies, whereas planktonic cells exit after complement opsonization (Alvarez et al., 2008). A similar mechanism may underlie antibody-mediated protection against murine histoplasmosis, as antibody administration was associated with reduced inflammatory damage (Guimarães et al., 2009a). The mechanism for these effects may be a result of antibody-mediated changes in the *H. capsulatum*-macrophage interaction, as antibody-dependent opsonization enhanced phagosome activation, acidification, and maturation, which can result in more efficient T cell activation and more effective CMI (Shi et al., 2008).

AMI in Natural Resistance to Fungi

Cryptococcus neoformans

Despite overwhelming evidence that individual mAbs can modify the course of fungal infection to the benefit of the host (see Table 1), a definitive role for natural AMI in host defense against fungi has been difficult to establish. Historically, the lack of (1) a clear association between known B cell defects and a predisposition to fungal disease and (2) a clear relationship between serum antibody to fungal antigens and resistance to fungi led most to conclude that AMI has no role in host defense against fungi in patients. However, this conclusion was flawed, as it was based on negative (a lack of) association. Part of the difficulty in establishing a relationship between antibody and resistance to fungal disease is that most immunologically intact individuals produce many antibodies that bind fungal antigens, entities to which they are constantly exposed. In fact, immunologically intact individuals are at very low risk of ever developing disease. Since antibodies to major fungal pathogens, such as *Cryptococcus neoformans* and *C. albicans*, are commonly present in individuals who have no history of disease as well as many who do, the presence of serum antibody does not discriminate those who are immune from those who are susceptible (Casadevall and Pirofski, 2007). However, the same information could be interpreted differently. For example, one could argue that the high prevalence of serum antibodies to fungal determinants in normal individuals together with the very low incidence of fungal disease in this group is consistent with a protective role for AMI against fungi. Further, some of these observations might be reconciled if AMI is beneficial when present but not necessarily required. Nonetheless, serum antibody can identify individuals who are susceptible to reactivation-associated disease. For example, as serological evidence against *C. neoformans* is detectable early in childhood (Goldman et al., 2001; Abadi and Pirofski, 1999), the presence of specific antibody has been cited to suggest that solid organ transplantation-associated cryptococcosis stems from reactivation of latent infection in patients with prior infection (Saha et al., 2007). However, such data also imply that pre-existing antibody did not prevent reactivation. This is not surprising in view of the critical importance of CMI in resistance to *C. neoformans* and ample evidence that natural resistance to disease requires both AMI and CMI (Rivera et al., 2005; Yuan et al., 1997; Beenhouwer et al., 2001). Nonetheless, cryptococcal disease has been reported in patients with B cell defects, including X-linked hyperIgM (Winkelstein et al., 2003; de Górgolas et al., 2005), a disorder with B, in addition to T, cell defects (Agematsu et al., 1998), and hypogammaglobulinemia (Gupta et al., 1987; Wahab et al., 1995; Neto et al., 2000). In addition, IgG2 deficiency was identified in an otherwise normal individual who developed meningitis with *C. gattii* (Marr et al., 2012), and compared to those without HIV/AIDS, adults and children with HIV/AIDS have lower titers of specific IgG2, the cryptococcal

capsular polysaccharide glucuronoxylomannan (GXM) (Abadi and Pirofski, 1999; Deshaw and Pirofski, 1995). IgG2 is the predominant IgG subclass to polysaccharide antigens in humans (Pirofski, 2001).

The most common immune deficiency among patients with cryptococcal disease is HIV, and HIV-associated B cell defects have been linked to cryptococcal disease susceptibility. HIV-infected participants in the Multicenter AIDS Cohort Study (MACS) who developed cryptococcosis had lower levels of serum antibody-expressing VH3 genes and IgM to GXM than those who did not. Similar observations have been reported with HIV-infected individuals in other cohorts (Deshaw and Pirofski, 1995; Fleuridor et al., 1999; Subramaniam et al., 2009). The loss of B cells and serum antibody-expressing VH3 depletion is a well-documented HIV-associated defect (Berberian et al., 1994, 1993). Hence, as human antibodies to GXM use VH3 genes (Fleuridor et al., 1998, 1999; Pirofski et al., 1995), HIV-associated depletion of VH3-expressing B cells could translate into a hole in the GXM-reactive repertoire. Another HIV-associated deficit that has been linked to cryptococcal disease susceptibility is IgM memory B cell depletion. Identified as one of the original HIV-associated B cell deficiencies (Lane et al., 1983), IgM memory B cell depletion remains a complication of HIV/AIDS even in the HAART era (Moir and Fauci, 2009) and has been linked to HIV-associated cryptococcal disease susceptibility (Subramaniam et al., 2009). IgM memory B cells are the source of natural IgM that has the capacity to bind conserved microbial carbohydrates (Baumgarth et al., 2005; Griffin et al., 2011). Hence, the lower levels of IgM to GXM that have been reported in the sera of patients with HIV/AIDS (Deshaw and Pirofski, 1995; Fleuridor et al., 1999; Subramaniam et al., 2009), as well as pretransplant sera of patients who developed cryptococcal disease after solid organ transplantation (Jalali et al., 2006), could contribute to the development of cryptococcal disease in addition to being a possible sentinel for IgM memory B cell deficiency.

In contrast to the uncertain relationship between AMI and resistance to fungi that has arisen from serological studies in humans, investigations in experimental animals provide strong evidence for a contribution of natural AMI to host defense against fungi. An early study demonstrated increased susceptibility to *C. neoformans* in mice with X-linked immunodeficiency (Marquis et al., 1985). More recently, B cell-deficient (Rivera et al., 2005) and IgM-deficient (sIgM$^{-/-}$) (Subramaniam et al., 2010b) mice have each been shown to be more susceptible to pulmonary infection with *C. neoformans*. Interestingly, when sIgM$^{-/-}$ mice were infected intraperitoneally, they were protected against *C. neoformans* (Subramaniam et al., 2010a). Seemingly paradoxically, this finding underscores the complexity of AMI to fungi and highlights that it is often tissue specific. Hence, while secreted IgM appears to be crucial for granuloma formation and preventing dissemination of *C. neoformans* from the lungs (Subramaniam et al., 2010b), an excess of B-1 B cells and IgG2 (which is characteristic of sIgM$^{-/-}$ mice) appears to mediate protection in the peritoneal cavity despite the absence of IgM, most likely by enhancing the fungicidal activity of macrophages (Subramaniam et al., 2010a). Hence, IgM and B cells exhibit different, tissue-specific roles in resistance to cryptococccal dissemination and death in mice.

Candida albicans

Observational studies in patients with candidiasis led to the identification of antibodies to an antigen, subsequently recognized as hsp90, that were associated with survival (Matthews et al., 1991). This observation spawned work that led to the development of an antibody-based fragment for treatment of fungal disease, Mycograb (Pachl et al., 2006). Although Mycograb enhanced the activity of antifungal agents against *C. albicans* in mice (Matthews et al., 2003) and improved outcome in invasive candidiasis in combination with liposomal amphotericin in a randomized, multicenter clinical trial (Pachl et al., 2006), its clinical development has been discontinued (see below). The complex states of human *C. albicans* infection, from commensalism to disease, present a unique challenge in efforts to characterize the role of AMI in resistance to disease and harness the potential of antibody-based therapy in patients. Studies in mice have been more straightforward, but have yielded different results depending on the fungal strain, route of infection, and animal model. B cell-deficient mice succumbed more rapidly than wild-type mice when infected with filamentous forms of *C. albicans* (Saville et al., 2008), and mice depleted of B cells were more susceptible (Sinha et al., 1987). In another model, B cell-deficient mice survived primary infection with *C. albicans* and *Aspergillus fumigatus* (Montagnoli et al., 2003), but *C. albicans*-infected mice did not survive a secondary challenge due to a failure of immunoregulation, rather than fungal clearance. Normal mouse serum reversed the effects and reconstituted a survival phenotype (Montagnoli et al., 2003). This study reiterated findings from the aforementioned *C. neoformans* models in which AMI conferred protection via immunoregulation and control of inflammation.

Other Fungal Pathogens

In contrast to *C. albicans* and *C. neoformans*, B cell depletion did not alter mortality in mice with primary *H. capsulatum* infection (Allendörfer et al., 1999), however, B cells protected against fungal reactivation in immune mice (Allen and Deepe, 2006).

B cells have also been shown to have an immunoregulatory role in immunity to *Pneumocystis* in mice (Lund et al., 2006). B-1 B cells and natural IgM to conserved β-glucan determinants secreted by B-1 B cells were shown to enhance innate immunity to *Pneumocystis* via dendritic cell priming (Rapaka et al., 2010), providing another instance of IgM-mediated antifungal immunity as demonstrated for *C. neoformans* (Subramaniam et al., 2010b). Further evidence for AMI-mediated resistance to *Pneumocystis* is found in a report that simian immunodeficiency virus-infected macaques with IgG to a *Pneumocystis* protein (KEX-1) were more resistant to *Pneumocystis* colonization and lung disease than macaques without IgG (Rapaka et al., 2010). In contrast to their protective role in *Pneumocystis* and intraperitoneal infection with *C. neoformans* (as IgM-deficient mice have elevated levels of B-1 B cells; Subramaniam et al., 2010a), B-1 B cells had a detrimental effect in pulmonary *P. brasilienses* infection in mice, albeit in a different mouse model in which disease enhancement was attributed to B-1 B cell production of IL-10 and impairment of phagocytosis (Popi et al., 2008).

AMI has been shown to be important for the generation of long lasting immunity to *C. albicans* and *A. fumigatus* (Montagnoli et al., 2003) and, as noted above, for maintenance of immunity to *H. capsulatum* (Allen and Deepe, 2006). In addition to IgM,

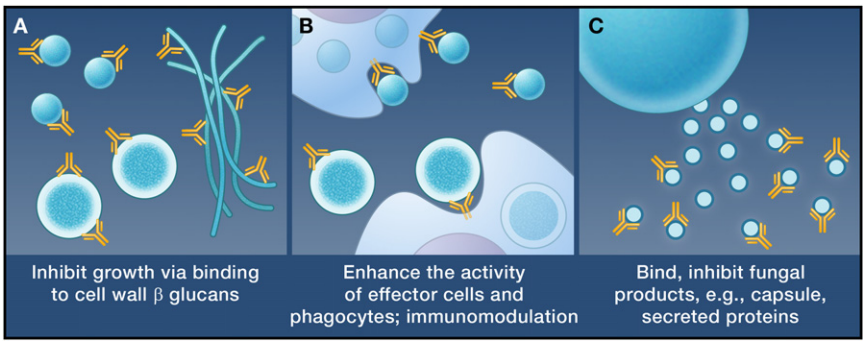

Figure 1. Proposed Mechanisms of Antibody Action against Fungi
(A–C) The proposed mechanisms of antibody action against fungi can be considered under three general categories involving (A) direct inhibition of growth, (B) immunomodulation and potentiation of innate immune mechanisms, and (C) neutralization of the untoward effects of fungal products on host tissues.

IgA could also play a role in natural resistance to *C. albicans* as tetraspanin protein (CD37)-deficient mice, which have 15-fold higher levels of IgA, were less susceptible than wild-type mice, and sera from CD37-deficient mice enhanced resistance of wild-type mice (van Spriel et al., 2009). The major mechanisms by which AMI contributes to host defense against fungi are shown in Figure 1.

Contribution of AMI in Pathogenesis and Susceptibility to Fungi

Given that AMI is a critical arm of the immune response, and fungi cause disease in the setting of immunodeficiency and excessive immune responses, i.e., at both ends of the damage-response spectrum (Casadevall and Pirofski, 2011), it is likely that some antibody responses can be deleterious. For example, IgE to fungal antigens has been implicated in the development of allergic pulmonary aspergillosis, fungal sinusitis, and forms of asthma that are caused by *C. neoformans* (Goldman et al., 2006). However, evidence that AMI contributes to the pathogenesis of and susceptibility to invasive fungal disease is indirect at best, with most data coming from experimental animal models. The capacity of AMI to enhance fungal disease was discovered with the identification of disease-enhancing mAbs that shortened survival when they were administered to *C. neoformans*-infected mice (Nussbaum et al., 1996). However, enhancement was limited to certain mouse strains and the same mAb that was enhancing in one strain was protective in another (Rivera and Casadevall, 2005). Recently, disease-enhancing monoclonal antibodies have also been reported for *Scedosporium apiospermum*, a soil fungus that causes invasive and noninvasive infection in susceptible individuals (Lopes et al., 2010b). A comparison of Balb/c and C.B-17 mice, which differ in their capacity to clear pulmonary *C. neoformans* infection revealed that human immunoglobulin locus genes were associated with slower clearance in Balb/c mice (Lovchik et al., 1999). The enhanced susceptibility of Balb/c mice to cryptococcosis has been associated with significantly higher levels of *C. neoformans*-specific IgM, IgG1, IgG2a, and IgG3 and Th2 polarization of the pulmonary inflammatory response (Lovchik et al., 1999). In contrast, susceptible C57BL/6 mice had higher levels of IgG2b after intraperitoneal infection with *C. neoformans* (Subramaniam et al., 2010a). IgG2b has been associated with inferior opsonic activity in vitro (Sanford et al., 1990) for some mAbs, but not others (Yuan et al., 1998). Methamphetamine administration increased susceptibility to *H. capsulatum* in mice, a phenomenon associated with pleitropic effects on the immune system including alterations in antibody responses (Martinez et al., 2009). For *C. albicans*, a comparison of genetically resistant and susceptible mouse strains revealed qualitative and quantitative differences in the antibody response, but such results do not infer causality (Costantino et al., 1995). Similarly, differences in the susceptibility of mice to *Paracoccidioides brasiliensis* correlated with differences in the isotype composition such that resistant and susceptible strains produced IgG2a and IgG2b isotypes, respectively (Kashino et al., 2000). However, we note that these differences occurred in a background of many immunological differences, making the degree to which AMI contributed to disease pathogenesis unclear. On the other hand, specific IgG was associated with resistance to colonization and lung disease after exposure to *Pneumocystis* in macaques (Kling et al., 2010), and IgM-deficient mice that exhibited resistance to intraperitoneal infection with *C. neoformans* had higher levels of IgG2a than susceptible mice (Subramaniam et al., 2010a). However, it should be noted that a given antibody type can be the byproduct of a Th2-polarized response, thus being only a small part of a complex B and T cell response. Thus, links between the presence of antibody and an outcome of fungal infection are associations that do not imply or establish a causal relationship between AMI and an outcome of fungal infection. Nonetheless, immune responses to fungi are considered notorious for eliciting antibodies that are not considered to be protective as exemplified for antibodies to *Candida* (Farah and Ashman, 2005) and *Coccidiodes* species (Cole et al., 2004), and the phenomenon may be more widespread.

Efficacy of Induced AMI against Fungi in Prophylaxis and Therapy

Given the high mortality, chronicity, and refractoriness of fungal diseases to therapy, there is currently considerable interest in the use of antibodies to prevent disease and as adjuncts to standard antifungal therapy. AMI can be induced actively and passively. The efficacy of passive antibody administration is convincingly established against various fungi (Table 1), and several vaccine formulations have also been shown to mediate protection by eliciting protective antibody responses in mice (Table 3). One concern in inducing AMI is that prozone-like effects have been demonstrated against fungi, in which loss of protective efficacy is apparent at high relative to lower antibody concentrations (Taborda et al., 2003). Hence, it is conceivable that highly immunogenic vaccines could fail if they elicit exuberant antibody responses with high antibody titers. Nevertheless, the experience with many effective vaccines against bacterial and viral pathogens provides strong encouragement that effective antifungal vaccines can be developed. A vaccine against recurrent

Table 3. Experimental Vaccines that Protect through AMI

Vaccine	Target	Mechanism	Reference
Glucuronoxylomannan-tetanus toxoid conjugate	*C. neoformans*	Vaccine elicits antibody responses to the polysaccharide capsule	(Devi, 1996)
Peptide mimotope	*C. neoformans*	Peptide elicits antibody responses that react with the capsule	(Fleuridor et al., 2001)
Synthetic glycopeptide	*C. albicans*	Antibodies to cell wall beta mannans	(Xin et al., 2008)
Mannan-protein conjugate	*C. albicans*	Antibodies to cell wall beta mannans	(Han et al., 1999)
Beta-glucan conjugate	*C. albicans, A. fumigatus, C. neoformans*	Antibodies to cell wall beta glucans	(Torosantucci et al., 2005; Torosantucci et al., 2009)
Recombinant and truncated aspartic protease	*C. albicans*	Protease neutralization	(Sandini et al., 2011)
Glycan-peptide conjugates	*C. albicans*	Antibodies to cell wall	(Xin and Cutler, 2011)

vulvovaginal candidiasis known as PEV7 which expresses recombinant aspartyl protease on virosomes produced by Pevion Biotech AG (Bern, Switzerland) is currently in clinical evaluation. A murine IgG1 mAb for *C. neoformans* that was studied in a clinical trial was found to be safe and to reduce serum GXM when used at high doses (Larsen et al., 2005). Further clinical development of this mAb has been hampered by economic issues resulting from the high costs of drug development for a disease of relatively low prevalence. A recombinant antibody fragment to *C. albicans* HSP90 (efungumab, Mycograb) showed promising efficacy in clinical trials (Pachl et al., 2006), but subsequent development of this immunoglobulin derivative has been hampered by production difficulties and was abandoned by Novartis (http://www.novartis.com/newsroom/media-releases/en/2010/1449020.shtml). Despite the absence of licensed products that prevent or treat fungal diseases based on AMI, the fact that antibodies and vaccines have been tested clinically is a measure of the interest in and acceptance of the concept that the pursuit of AMI for fungal diseases warrants consideration in clinical medicine. Furthermore, newer types of antibody preparations are on the horizon. For example, the efficacy of antibody can be enhanced by linking it to a radioactive isotope, since this converts the antibody in a fungicidal molecule (Dadachova et al., 2003) irrespective of its natural biological function. In fact, by targeting antigens shared across fungal species, it may be possible to develop broad spectrum radioimmunotherapy for fungal diseases (Bryan et al., 2011). This could be an attractive option for developing new antifungal strategies against the devastating mold infections that occur in severely immunocomprised individuals.

Summary

In a little more than two decades the field of AMI to medically important fungi has gone from being a remote backwater of scientific study to a vibrant field that is now generating promising vaccines and therapeutic immunoglobulins while making major contributions to basic immunology and microbial pathogenesis. In fact, we have recently proposed a new synthesis for AMI based largely on discoveries made with antibodies to fungi (Casadevall and Pirofski, 2011). Regardless of this progress and despite the work of many investigators, it is clear that we have barely scratched the surface in this complex topic. We anticipate that in the coming years many new targets of AMI will be identified, and hopefully the promising antibody-based reagents and vaccines that are currently in various stages of preclinical and clinical development will lead to licensed products that will transform our approach to fungal diseases. However, in our view, the greatest challenge facing this field, and all of immunology, is how to integrate AMI with the other components of the immune system to produce a coherent picture of effective and noneffective immune responses that would be predictive and useful in guiding the design of new vaccines and immune therapies.

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REFERENCES

Abadi, J., and Pirofski, L. (1999). Antibodies reactive with the cryptococcal capsular polysaccharide glucuronoxylomannan are present in sera from children with and without human immunodeficiency virus infection. J. Infect. Dis. *180*, 915–919.

Agematsu, K., Nagumo, H., Shinozaki, K., Hokibara, S., Yasui, K., Terada, K., Kawamura, N., Toba, T., Nonoyama, S., Ochs, H.D., and Komiyama, A. (1998). Absence of IgD-CD27(+) memory B cell population in X-linked hyper-IgM syndrome. J. Clin. Invest. *102*, 853–860.

Allen, H.L., and Deepe, G.S., Jr. (2006). B cells and CD4-CD8- T cells are key regulators of the severity of reactivation histoplasmosis. J. Immunol. *177*, 1763–1771.

Allendörfer, R., Brunner, G.D., and Deepe, G.S., Jr. (1999). Complex requirements for nascent and memory immunity in pulmonary histoplasmosis. J. Immunol. *162*, 7389–7396.

Alvarez, M., Saylor, C., and Casadevall, A. (2008). Antibody action after phagocytosis promotes Cryptococcus neoformans and Cryptococcus gattii macrophage exocytosis with biofilm-like microcolony formation. Cell. Microbiol. *10*, 1622–1633.

Baumgarth, N., Tung, J.W., and Herzenberg, L.A. (2005). Inherent specificities in natural antibodies: a key to immune defense against pathogen invasion. Springer Semin. Immunopathol. *26*, 347–362.

Beenhouwer, D.O., Shapiro, S., Feldmesser, M., Casadevall, A., and Scharff, M.D. (2001). Both Th1 and Th2 cytokines affect the ability of monoclonal antibodies to protect mice against *Cryptococcus neoformans*. Infect. Immun. *69*, 6445–6455.

Beenhouwer, D.O., Yoo, E.M., Lai, C.W., Rocha, M.A., and Morrison, S.L. (2007). Human immunoglobulin G2 (IgG2) and IgG4, but not IgG1 or IgG3,

protect mice against *Cryptococcus neoformans* infection. Infect. Immun. *75*, 1424–1435.

Berberian, L., Goodglick, L., Kipps, T.J., and Braun, J. (1993). Immunoglobulin VH3 gene products: natural ligands for HIV gp120. Science *261*, 1588–1591.

Berberian, L., Shukla, J., Jefferis, R., and Braun, J. (1994). Effects of HIV infection on VH3 (D12 idiotope) B cells in vivo. J. Acquir. Immune Defic. Syndr. *7*, 641–646.

Brena, S., Omaetxebarría, M.J., Elguezabal, N., Cabezas, J., Moragues, M.D., and Pontón, J. (2007). Fungicidal monoclonal antibody C7 binds to Candida albicans Als3. Infect. Immun. *75*, 3680–3682.

Brena, S., Cabezas-Olcoz, J., Moragues, M.D., Fernández de Larrinoa, I., Domínguez, A., Quindós, G., and Pontón, J. (2011). Fungicidal monoclonal antibody C7 interferes with iron acquisition in *Candida albicans*. Antimicrob. Agents Chemother. *55*, 3156–3163.

Bryan, R.A., Guimaraes, A.J., Hopcraft, S., Jiang, Z., Bonilla, K., Morgenstern, A., Bruchertseifer, F., Del Poeta, M., Torosantucci, A., Cassone, A., et al. (2011). Toward Developing a Universal Treatment for Fungal Disease Using Radioimmunotherapy Targeting Common Fungal Antigens. Mycopathologia, in press.

Buissa-Filho, R., Puccia, R., Marques, A.F., Pinto, F.A., Muñoz, J.E., Nosanchuk, J.D., Travassos, L.R., and Taborda, C.P. (2008). The monoclonal antibody against the major diagnostic antigen of *Paracoccidioides brasiliensis* mediates immune protection in infected BALB/c mice challenged intratracheally with the fungus. Infect. Immun. *76*, 3321–3328.

Calcedo, R., Ramirez-Garcia, A., Abad, A., Rementeria, A., Pontón, J., and Hernando, F.L. (2011). Phosphoglycerate kinase and fructose bisphosphate aldolase of *Candida albicans* as new antigens recognized by human salivary IgA. Rev. Iberoam. Micol., in press.

Casadevall, A. (1995). Antibody immunity and invasive fungal infections. Infect. Immun. *63*, 4211–4218.

Casadevall, A., and Pirofski, L.A. (2003). Antibody-mediated regulation of cellular immunity and the inflammatory response. Trends Immunol. *24*, 474–478.

Casadevall, A., and Pirofski, L.A. (2007). Accidental virulence, cryptic pathogenesis, martians, lost hosts, and the pathogenicity of environmental microbes. Eukaryot. Cell *6*, 2169–2174.

Casadevall, A., and Pirofski, L.A. (2011). A new synthesis for antibody-mediated immunity. Nat. Immunol. *13*, 21–28.

Casadevall, A., Dadachova, E., and Pirofski, L.A. (2004). Passive antibody therapy for infectious diseases. Nat. Rev. Microbiol. *2*, 695–703.

Chaturvedi, A.K., Kavishwar, A., Shiva Keshava, G.B., and Shukla, P.K. (2005). Monoclonal immunoglobulin G1 directed against *Aspergillus fumigatus* cell wall glycoprotein protects against experimental murine aspergillosis. Clin. Diagn. Lab. Immunol. *12*, 1063–1068.

Cole, G.T., Xue, J.M., Okeke, C.N., Tarcha, E.J., Basrur, V., Schaller, R.A., Herr, R.A., Yu, J.J., and Hung, C.Y. (2004). A vaccine against coccidioidomycosis is justified and attainable. Med. Mycol. *42*, 189–216.

Costantino, P.J., Gare, N.F., and Warmington, J.R. (1995). Humoral immune responses to systemic Candida albicans infection in inbred mouse strains. Immunol. Cell Biol. *73*, 125–133.

da Silva, M.B., Marques, A.F., Nosanchuk, J.D., Casadevall, A., Travassos, L.R., and Taborda, C.P. (2006). Melanin in the dimorphic fungal pathogen *Paracoccidioides brasiliensis*: effects on phagocytosis, intracellular resistance and drug susceptibility. Microbes Infect. *8*, 197–205.

Dadachova, E., Nakouzi, A., Bryan, R.A., and Casadevall, A. (2003). Ionizing radiation delivered by specific antibody is therapeutic against a fungal infection. Proc. Natl. Acad. Sci. USA *100*, 10942–10947.

De Bernardis, F., Boccanera, M., Adriani, D., Spreghini, E., Santoni, G., and Cassone, A. (1997). Protective role of antimannan and anti-aspartyl proteinase antibodies in an experimental model of *Candida albicans* vaginitis in rats. Infect. Immun. *65*, 3399–3405.

de Górgolas, M., Erice, A., Gil, A., Gutiérrez, J., Rivas, P., Hernando, C., and Rodríguez, M.C. (2005). Cryptococcal meningitis in a patient with X-linked hyper-IgM1 syndrome. Scand. J. Infect. Dis. *37*, 526–528.

Deshaw, M., and Pirofski, L.A. (1995). Antibodies to the *Cryptococcus neoformans* capsular glucuronoxylomannan are ubiquitous in serum from HIV+ and HIV- individuals. Clin. Exp. Immunol. *99*, 425–432.

Devi, S.J.N. (1996). Preclinical efficacy of a glucuronoxylomannan-tetanus toxoid conjugate vaccine of *Cryptococcus neoformans* in a murine model. Vaccine *14*, 841–844.

Dromer, F., Charreire, J., Contrepois, A., Carbon, C., and Yeni, P. (1987a). Protection of mice against experimental cryptococcosis by anti-*Cryptococcus neoformans* monoclonal antibody. Infect. Immun. *55*, 749–752.

Dromer, F., Salamero, J., Contrepois, A., Carbon, C., and Yeni, P. (1987b). Production, characterization, and antibody specificity of a mouse monoclonal antibody reactive with *Cryptococcus neoformans* capsular polysaccharide. Infect. Immun. *55*, 742–748.

Farah, C.S., and Ashman, R.B. (2005). Active and passive immunization against oral Candida albicans infection in a murine model. Oral Microbiol. Immunol. *20*, 376–381.

Feldmesser, M., and Casadevall, A. (1997). Effect of serum IgG1 to Cryptococcus neoformans glucuronoxylomannan on murine pulmonary infection. J. Immunol. *158*, 790–799.

Feldmesser, M., Mednick, A., and Casadevall, A. (2002). Antibody-mediated protection in murine Cryptococcus neoformans infection is associated with pleotrophic effects on cytokine and leukocyte responses. Infect. Immun. *70*, 1571–1580.

Fleuridor, R., Zhong, Z., and Pirofski, L. (1998). A human IgM monoclonal antibody prolongs survival of mice with lethal cryptococcosis. J. Infect. Dis. *178*, 1213–1216.

Fleuridor, R., Lyles, R.H., and Pirofski, L. (1999). Quantitative and qualitative differences in the serum antibody profiles of human immunodeficiency virus-infected persons with and without *Cryptococcus neoformans* meningitis. J. Infect. Dis. *180*, 1526–1535.

Fleuridor, R., Lees, A., and Pirofski, L. (2001). A cryptococcal capsular polysaccharide mimotope prolongs the survival of mice with *Cryptococcus neoformans* infection. J. Immunol. *166*, 1087–1096.

Gigliotti, F., and Hughes, W.T. (1988). Passive immunoprophylaxis with specific monoclonal antibody confers partial protection against Pneumocystis carinii pneumonitis in animal models. J. Clin. Invest. *81*, 1666–1668.

Gigliotti, F., Garvy, B.A., and Harmsen, A.G. (1996). Antibody-mediated shift in the profile of glycoprotein A phenotypes observed in a mouse model of *Pneumocystis carinii* pneumonia. Infect. Immun. *64*, 1892–1899.

Goldman, D.L., Khine, H., Abadi, J., Lindenberg, D.J., Pirofski La, Niang, R., and Casadevall, A. (2001). Serologic evidence for Cryptococcus neoformans infection in early childhood. Pediatrics *107*, E66.

Goldman, D.L., Davis, J., Bommarito, F., Shao, X., and Casadevall, A. (2006). Enhanced allergic inflammation and airway responsiveness in rats with chronic Cryptococcus neoformans infection: potential role for fungal pulmonary infection in the pathogenesis of asthma. J. Infect. Dis. *193*, 1178–1186.

Griffin, D.O., Holodick, N.E., and Rothstein, T.L. (2011). Human B1 cells in umbilical cord and adult peripheral blood express the novel phenotype CD20+ CD27+ CD43+ CD70-. J. Exp. Med. *208*, 67–80.

Guimarães, A.J., Frases, S., Gomez, F.J., Zancopé-Oliveira, R.M., and Nosanchuk, J.D. (2009a). Monoclonal antibodies to heat shock protein 60 alter the pathogenesis of Histoplasma capsulatum. Infect. Immun. *77*, 1357–1367.

Guimarães, A.J., Frases, S., Gomez, F.J., Zancopé-Oliveira, R.M., and Nosanchuk, J.D. (2009b). Monoclonal antibodies to heat shock protein 60 alter the pathogenesis of *Histoplasma capsulatum*. Infect. Immun. *77*, 1357–1367.

Gupta, S., Ellis, M., Cesario, T., Ruhling, M., and Vayuvegula, B. (1987). Disseminated cryptococcal infection in a patient with hypogammaglobulinemia and normal T cell functions. Am. J. Med. *82*, 129–131.

Han, Y., Kanbe, T., Cherniak, R., and Cutler, J.E. (1997). Biochemical characterization of *Candida albicans* epitopes that can elicit protective and nonprotective antibodies. Infect. Immun. *65*, 4100–4107.

Han, Y., Ulrich, M.A., and Cutler, J.E. (1999). *Candida albicans* mannan extract-protein conjugates induce a protective immune response against experimental candidiasis. J. Infect. Dis. *179*, 1477–1484.

Han, Y., Kozel, T.R., Zhang, M.X., MacGill, R.S., Carroll, M.C., and Cutler, J.E. (2001). Complement is essential for protection by an IgM and an IgG3 monoclonal antibody against experimental, hematogenously disseminated candidiasis. J. Immunol. *167*, 1550–1557.

Jalali, Z., Ng, L., Singh, N., and Pirofski, L.A. (2006). Antibody response to Cryptococcus neoformans capsular polysaccharide glucuronoxylomannan in patients after solid-organ transplantation. Clin. Vaccine Immunol. *13*, 740–746.

Kashino, S.S., Fazioli, R.A., Cafalli-Favati, C., Meloni-Bruneri, L.H., Vaz, C.A., Burger, E., Singer, L.M., and Calich, V.L. (2000). Resistance to *Paracoccidioides brasiliensis* infection is linked to a preferential Th1 immune response, whereas susceptibility is associated with absence of IFN-gamma production. J. Interferon Cytokine Res. *20*, 89–97.

Kling, H.M., Shipley, T.W., Patil, S.P., Kristoff, J., Bryan, M., Montelaro, R.C., Morris, A., and Norris, K.A. (2010). Relationship of *Pneumocystis jiroveci* humoral immunity to prevention of colonization and chronic obstructive pulmonary disease in a primate model of HIV infection. Infect. Immun. *78*, 4320–4330.

Lane, H.C., Masur, H., Edgar, L.C., Whalen, G., Rook, A.H., and Fauci, A.S. (1983). Abnormalities of B-cell activation and immunoregulation in patients with the acquired immunodeficiency syndrome. N. Engl. J. Med. *309*, 453–458.

Larsen, R.A., Pappas, P.G., Perfect, J.R., Aberg, J.A., Casadevall, A., Cloud, G.A., James, R., Filler, S., and Dismukes, W.E. (2005). Phase I evaluation of the safety and pharmacokinetics of murine-derived anticryptococcal antibody 18B7 in subjects with treated cryptococcal meningitis. Antimicrob. Agents Chemother. *49*, 952–958.

Lopes, L.C., Guimarães, A.J., de Cerqueira, M.D., Gómez, B.L., and Nosanchuk, J.D. (2010a). A *histoplasma capsulatum*-specific IgG1 isotype monoclonal antibody, H1C, to a 70-kilodalton cell surface protein is not protective in murine histoplasmosis. Clin. Vaccine Immunol. *17*, 1155–1158.

Lopes, L.C., Rollin-Pinheiro, R., Guimarães, A.J., Bittencourt, V.C., Martinez, L.R., Koba, W., Farias, S.E., Nosanchuk, J.D., and Barreto-Bergter, E. (2010b). Monoclonal antibodies against peptidorhamnomannans of *Scedosporium apiospermum* enhance the pathogenicity of the fungus. PLoS Negl. Trop. Dis. *4*, e853.

Lovchik, J.A., Wilder, J.A., Huffnagle, G.B., Riblet, R., Lyons, C.R., and Lipscomb, M.F. (1999). Ig heavy chain complex-linked genes influence the immune response in a murine cryptococcal infection. J. Immunol. *163*, 3907–3913.

Lund, F.E., Hollifield, M., Schuer, K., Lines, J.L., Randall, T.D., and Garvy, B.A. (2006). B cells are required for generation of protective effector and memory CD4 cells in response to Pneumocystis lung infection. J. Immunol. *176*, 6147–6154.

Magliani, W., Conti, S., Frazzi, R., Ravanetti, L., Maffei, D.L., and Polonelli, L. (2005). Protective antifungal yeast killer toxin-like antibodies. Curr. Mol. Med. *5*, 443–452.

Marquis, G., Montplaisir, S., Pelletier, M., Mousseau, S., and Auger, P. (1985). Genetic resistance to murine cryptococcosis: increased susceptibility in the CBA/N XID mutant strain of mice. Infect. Immun. *47*, 282–287.

Marr, K.A., Datta, K., Pirofski, L.A., and Barnes, R. (2012). Cryptococcus gattii infection in healthy hosts: a sentinel for subclinical immunodeficiency? Clin. Infect. Dis. *54*, 153–154.

Martinez, L.R., and Casadevall, A. (2005). Specific antibody can prevent fungal biofilm formation and this effect correlates with protective efficacy. Infect. Immun. *73*, 6350–6362.

Martinez, L.R., Moussai, D., and Casadevall, A. (2004). Antibody to *Cryptococcus neoformans* glucuronoxylomannan inhibits the release of capsular antigen. Infect. Immun. *72*, 3674–3679.

Martinez, L.R., Mihu, M.R., Gácser, A., Santambrogio, L., and Nosanchuk, J.D. (2009). Methamphetamine enhances histoplasmosis by immunosuppression of the host. J. Infect. Dis. *200*, 131–141.

Matthews, R.C., Burnie, J.P., Howat, D., Rowland, T., and Walton, F. (1991). Autoantibody to heat-shock protein 90 can mediate protection against systemic candidosis. Immunology *74*, 20–24.

Matthews, R.C., Rigg, G., Hodgetts, S., Carter, T., Chapman, C., Gregory, C., Illidge, C., and Burnie, J. (2003). Preclinical assessment of the efficacy of mycograb, a human recombinant antibody against fungal HSP90. Antimicrob. Agents Chemother. *47*, 2208–2216.

McClelland, E.E., Nicola, A.M., Prados-Rosales, R., and Casadevall, A. (2010). Ab binding alters gene expression in Cryptococcus neoformans and directly modulates fungal metabolism. J. Clin. Invest. *120*, 1355–1361.

McFadden, D.C., Fries, B.C., Wang, F., and Casadevall, A. (2007). Capsule structural heterogeneity and antigenic variation in *Cryptococcus neoformans*. Eukaryot. Cell *6*, 1464–1473.

Miller, M.F., Mitchell, T.G., Storkus, W.J., and Dawson, J.R. (1990). Human natural killer cells do not inhibit growth of *Cryptococcus neoformans* in the absence of antibody. Infect. Immun. *58*, 639–645.

Moir, S., and Fauci, A.S. (2009). B cells in HIV infection and disease. Nat. Rev. Immunol. *9*, 235–245.

Montagnoli, C., Bozza, S., Bacci, A., Gaziano, R., Mosci, P., Morschhäuser, J., Pitzurra, L., Kopf, M., Cutler, J., and Romani, L. (2003). A role for antibodies in the generation of memory antifungal immunity. Eur. J. Immunol. *33*, 1193–1204.

Moragues, M.D., Omaetxebarria, M.J., Elguezabal, N., Sevilla, M.J., Conti, S., Polonelli, L., and Pontón, J. (2003). A monoclonal antibody directed against a Candida albicans cell wall mannoprotein exerts three anti-*C. albicans* activities. Infect. Immun. *71*, 5273–5279.

Mukherjee, J., Scharff, M.D., and Casadevall, A. (1992). Protective murine monoclonal antibodies to *Cryptococcus neoformans*. Infect. Immun. *60*, 4534–4541.

Nabavi, N., and Murphy, J.W. (1986). Antibody-dependent natural killer cell-mediated growth inhibition of *Cryptococcus neoformans*. Infect. Immun. *51*, 556–562.

Neto, Rda.J., Guimarães, M.C., Moya, M.J., Oliveira, F.R., Louzada, P.L., Jr., and Martinez, R. (2000). Hypogammaglobulinemia as risk factor for Cryptococcus neoformans infection: report of 2 cases. Rev. Soc. Bras. Med. Trop. *33*, 603–608.

Nosanchuk, J.D., Steenbergen, J.N., Shi, L., Deepe, G.S., Jr., and Casadevall, A. (2003). Antibodies to a cell surface histone-like protein protect against *Histoplasma capsulatum*. J. Clin. Invest. *112*, 1164–1175.

Nussbaum, G., Yuan, R., Casadevall, A., and Scharff, M.D. (1996). Immunoglobulin G3 blocking antibodies to the fungal pathogen Cryptococcus neoformans. J. Exp. Med. *183*, 1905–1909.

Okagaki, L.H., Strain, A.K., Nielsen, J.N., Charlier, C., Baltes, N.J., Chrétien, F., Heitman, J., Dromer, F., and Nielsen, K. (2010). Cryptococcal cell morphology affects host cell interactions and pathogenicity. PLoS Pathog. *6*, e1000953.

Pachl, J., Svoboda, P., Jacobs, F., Vandewoude, K., van der Hoven, B., Spronk, P., Masterson, G., Malbrain, M., Aoun, M., Garbino, J., Takala, J., et al. (2006). A randomized, blinded, multicenter trial of lipid-associated amphotericin B alone versus in combination with an antibody-based inhibitor of heat shock protein 90 in patients with invasive candidiasis. Clin. Infect. Dis. *42*, 1404–1413.

Pirofski, L.A. (2001). Polysaccharides, mimotopes and vaccines for fungal and encapsulated pathogens. Trends Microbiol. *9*, 445–451.

Pirofski, L., Lui, R., DeShaw, M., Kressel, A.B., and Zhong, Z. (1995). Analysis of human monoclonal antibodies elicited by vaccination with a *Cryptococcus neoformans* glucuronoxylomannan capsular polysaccharide vaccine. Infect. Immun. *63*, 3005–3014.

Popi, A.F., Godoy, L.C., Xander, P., Lopes, J.D., and Mariano, M. (2008). B-1 cells facilitate *Paracoccidioides brasiliensis* infection in mice via IL-10 secretion. Microbes Infect. *10*, 817–824.

Rapaka, R.R., Ricks, D.M., Alcorn, J.F., Chen, K., Khader, S.A., Zheng, M., Plevy, S., Bengtén, E., and Kolls, J.K. (2010). Conserved natural IgM antibodies mediate innate and adaptive immunity against the opportunistic fungus Pneumocystis murina. J. Exp. Med. *207*, 2907–2919.

Rivera, J., and Casadevall, A. (2005). Mouse genetic background is a major determinant of isotype-related differences for antibody-mediated protective efficacy against Cryptococcus neoformans. J. Immunol. *174*, 8017–8026.

Rivera, J., Zaragoza, O., and Casadevall, A. (2005). Antibody-mediated protection against Cryptococcus neoformans is dependent on B cells. Infect. Immun. *73*, 1141–1150.

Rodrigues, M.L., Travassos, L.R., Miranda, K.R., Franzen, A.J., Rozental, S., de Souza, W., Alviano, C.S., and Barreto-Bergter, E. (2000). Human antibodies against a purified glucosylceramide from *Cryptococcus neoformans* inhibit cell budding and fungal growth. Infect. Immun. *68*, 7049–7060.

Rosas, A.L., Nosanchuk, J.D., and Casadevall, A. (2001). Passive immunization with melanin-binding monoclonal antibodies prolongs survival of mice with lethal Cryptococcus neoformans infection. Infect. Immun. *69*, 3410–3412.

Saha, D.C., Goldman, D.L., Shao, X., Casadevall, A., Husain, S., Limaye, A.P., Lyon, M., Somani, J., Pursell, K., Pruett, T.L., and Singh, N. (2007). Serologic evidence for reactivation of cryptococcosis in solid-organ transplant recipients. Clin. Vaccine Immunol. *14*, 1550–1554.

Sandini, S., La Valle, R., Deaglio, S., Malavasi, F., Cassone, A., and De Bernardis, F. (2011). A highly immunogenic recombinant and truncated protein of the secreted aspartic proteases family (rSap2t) of Candida albicans as a mucosal anticandidal vaccine. FEMS Immunol. Med. Microbiol. *62*, 215–224.

Sanford, J.E., Lupan, D.M., Schlageter, A.M., and Kozel, T.R. (1990). Passive immunization against *Cryptococcus neoformans* with an isotype-switch family of monoclonal antibodies reactive with cryptococcal polysaccharide. Infect. Immun. *58*, 1919–1923.

Saville, S.P., Lazzell, A.L., Chaturvedi, A.K., Monteagudo, C., and Lopez-Ribot, J.L. (2008). Use of a genetically engineered strain to evaluate the pathogenic potential of yeast cell and filamentous forms during Candida albicans systemic infection in immunodeficient mice. Infect. Immun. *76*, 97–102.

Schlageter, A.M., and Kozel, T.R. (1990). Opsonization of *Cryptococcus neoformans* by a family of isotype-switch variant antibodies specific for the capsular polysaccharide. Infect. Immun. *58*, 1914–1918.

Shapiro, S., Beenhouwer, D.O., Feldmesser, M., Taborda, C., Carroll, M.C., Casadevall, A., and Scharff, M.D. (2002). Immunoglobulin G monoclonal antibodies to *Cryptococcus neoformans* protect mice deficient in complement component C3. Infect. Immun. *70*, 2598–2604.

Shi, L., Albuquerque, P.C., Lazar-Molnar, E., Wang, X., Santambrogio, L., Gácser, A., and Nosanchuk, J.D. (2008). A monoclonal antibody to Histoplasma capsulatum alters the intracellular fate of the fungus in murine macrophages. Eukaryot. Cell *7*, 1109–1117.

Sinha, B.K., Prasad, S., and Monga, D.P. (1987). Studies of the role of B-cells in the resistance of mice to experimental candidiasis. Zentralbl. Bakteriol. Mikrobiol. Hyg. [A] *266*, 316–322.

Smulian, A.G., Sullivan, D.W., and Theus, S.A. (2000). Immunization with recombinant Pneumocystis carinii p55 antigen provides partial protection against infection: characterization of epitope recognition associated with immunization. Microbes Infect. *2*, 127–136.

Subramaniam, K., Metzger, B., Hanau, L.H., Guh, A., Rucker, L., Badri, S., and Pirofski, L.A. (2009). IgM(+) memory B cell expression predicts HIV-associated cryptococcosis status. J. Infect. Dis. *200*, 244–251.

Subramaniam, K.S., Datta, K., Marks, M.S., and Pirofski, L.A. (2010a). Improved survival of mice deficient in secretory immunoglobulin M following systemic infection with Cryptococcus neoformans. Infect. Immun. *78*, 441–452.

Subramaniam, K.S., Datta, K., Quintero, E., Manix, C., Marks, M.S., and Pirofski, L.A. (2010b). The absence of serum IgM enhances the susceptibility of mice to pulmonary challenge with Cryptococcus neoformans. J. Immunol. *184*, 5755–5767.

Taborda, C.P., Rivera, J., Zaragoza, O., and Casadevall, A. (2003). More is not necessarily better: prozone-like effects in passive immunization with IgG. J. Immunol. *140*, 3621–3630.

Torosantucci, A., Bromuro, C., Chiani, P., De Bernardis, F., Berti, F., Galli, C., Norelli, F., Bellucci, C., Polonelli, L., Costantino, P., et al. (2005). A novel glycoconjugate vaccine against fungal pathogens. J. Exp. Med. *202*, 597–606.

Torosantucci, A., Chiani, P., Bromuro, C., De Bernardis, F., Palma, A.S., Liu, Y., Mignogna, G., Maras, B., Colone, M., Stringaro, A., et al. (2009). Protection by anti-beta-glucan antibodies is associated with restricted beta-1,3 glucan binding specificity and inhibition of fungal growth and adherence. PLoS ONE *4*, e5392.

van Spriel, A.B., Leusen, J.H., van Egmond, M., Dijkman, H.B., Assmann, K.J., Mayadas, T.N., and van de Winkel, J.G. (2001). Mac-1 (CD11b/CD18) is essential for Fc receptor-mediated neutrophil cytotoxicity and immunologic synapse formation. Blood *97*, 2478–2486.

van Spriel, A.B., Sofi, M., Gartlan, K.H., van der Schaaf, A., Verschueren, I., Torensma, R., Raymakers, R.A., Loveland, B.E., Netea, M.G., Adema, G.J., et al. (2009). The tetraspanin protein CD37 regulates IgA responses and antifungal immunity. PLoS Pathog. *5*, e1000338.

Viudes, A., Lazzell, A., Perea, S., Kirkpatrick, W.R., Peman, J., Patterson, T.F., Martinez, J.P., and López-Ribot, J.L. (2004). The C-terminal antibody binding domain of *Candida albicans* mp58 represents a protective epitope during candidiasis. FEMS Microbiol. Lett. *232*, 133–138.

Wahab, J.A., Hanifah, M.J., and Choo, K.E. (1995). Bruton's agammaglobulinaemia in a child presenting with cryptococcal empyema thoracis and periauricular pyogenic abscess. Singapore Med. J. *36*, 686–689.

Winkelstein, J.A., Marino, M.C., Ochs, H., Fuleihan, R., Scholl, P.R., Geha, R., Stiehm, E.R., and Conley, M.E. (2003). The X-linked hyper-IgM syndrome: clinical and immunologic features of 79 patients. Medicine (Baltimore) *82*, 373–384.

Xin, H., and Cutler, J.E. (2011). Vaccine and monoclonal antibody that enhance mouse resistance to candidiasis. Clin. Vaccine Immunol. *18*, 1656–1667.

Xin, H., Dziadek, S., Bundle, D.R., and Cutler, J.E. (2008). Synthetic glycopeptide vaccines combining beta-mannan and peptide epitopes induce protection against candidiasis. Proc. Natl. Acad. Sci. USA *105*, 13526–13531.

Yuan, R.R., Casadevall, A., Oh, J., and Scharff, M.D. (1997). T cells cooperate with passive antibody to modify *Cryptococcus neoformans* infection in mice. Proc. Natl. Acad. Sci. USA *94*, 2483–2488.

Yuan, R., Spira, G., Oh, J., Paizi, M., Casadevall, A., and Scharff, M.D. (1998). Isotype switching increases efficacy of antibody protection against Cryptococcus neoformans infection in mice. Infect. Immun. *66*, 1057–1062.

Zaragoza, O., Taborda, C.P., and Casadevall, A. (2003). The efficacy of complement-mediated phagocytosis of *Cryptococcus neoformans* is dependent on the location of C3 in the polysaccharide capsule and involves both direct and indirect C3-mediated interactions. Eur. J. Immunol. *33*, 1957–1967.

Zaragoza, O., Chrisman, C.J., Castelli, M.V., Frases, S., Cuenca-Estrella, M., Rodríguez-Tudela, J.L., and Casadevall, A. (2008). Capsule enlargement in *Cryptococcus neoformans* confers resistance to oxidative stress suggesting a mechanism for intracellular survival. Cell. Microbiol. *10*, 2043–2057.

Zaragoza, O., García-Rodas, R., Nosanchuk, J.D., Cuenca-Estrella, M., Rodríguez-Tudela, J.L., and Casadevall, A. (2010). Fungal cell gigantism during mammalian infection. PLoS Pathog. *6*, e1000945.