Antibodies and fungi: an evolving paradigm with opportunities for the development of new antifungal therapies and vaccines

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The role of antibody immunity in protection against fungal infections has been uncertain for decades. This is in sharp contrast to bacteria, viruses, and protozoa where antibody immunity is widely acknowledged to contribute to protection. This raises the questions: Do protective antibodies against fungi exist?; Why has it been so difficult to demonstrate conclusively that antibody immunity protects against fungi?; Does antibody immunity influence the course of fungal infections?

The issue of whether antibody immunity contributes to host defense against fungi is no longer academic. In recent years there has been a marked increase in fungal infections associated with the HIV epidemic and immunosuppressive therapies. Systemic mycoses in immunocompromised patients are difficult to treat effectively because antifungal therapy frequently fails to eradicate the infection in the setting of defective immunity. For example, *C. neoformans* infections are usually incurable in AIDS patients. In the 1960s Morris Gordon and collaborators established that antibody administration could enhance the efficacy of antifungal chemotherapy [1]. Hence a better understanding of the role of antibody immunity may help in the design of effective vaccines and/or antibody-based therapies.

Numerous investigators have studied the function of antibody immunity in host defence against fungi [2]. A role for antibody immunity in protection against individual pathogens is usually established by correlating the presence of serum antibody with protection and/or demonstrating protection after passive antibody administration. Among the medically important fungi, the role of antibody immunity has been most extensively studied for *C. albicans* and *C. neoformans* [2]. For both fungi studies with polyclonal antibody reagents (i.e. immune sera) have produced conflicting evidence for and against the importance of antibody immunity [2]. Until the mid-1980s all studies of antibody protection against fungi utilized polyclonal reagents. In 1987 Dromer et al. [3] demonstrated that a monoclonal antibody (mAb) was protective against *C. neoformans* and this observation marks the beginning of new era in the study of antibody function against fungi. In the past decade, experiments with mAbs have established the existence of protective and non-protective antibodies against *C. albicans* [4,5] and *C. neoformans* [3,6,7]. Furthermore, some mAbs against the *C. neoformans* capsular polysaccharide have been shown to function as blocking antibodies that can enhance infection and interfere with the function of protective antibodies [8]. Hence there is now evidence for "good" and "bad" antibodies against fungi.

Protection studies with mAbs suggest an explanation for the inconclusive and often contradictory results obtained from experiments with polyclonal sera [2]. Polyclonal antibody preparations contain antibodies of multiple specificities and isotypes. MAb preparations differ from polyclonal antibodv preparations in that they contain one antibody type of a defined specificity and isotype. The discovery that protective, non-protective and deleterious mAbs exist suggests that the efficacy of polyclonal antibody preparations reflects their relative proportion of these types of antibodies. In fact, experiments with mAbs in mice have shown that mixing protective and non-protective mAbs reduces the efficacy of the protective mAbs [8]. Immunization strategies which elicit a predominance of protective antibodies may result in useful antibody immunity. The experiments with mAbs suggest a new working paradigm for the fungi: the efficacy of antibody immunity depends on the type of antibody response made [2].

For both *C. albicans* and *C. neoformans* epitope specificity has been demonstrated to be an important determinant of antibody efficacy in protection [4,9]. For *C. neoformans*, antibody isotype has been shown to be a critical determinant of antibody efficacy: murine IgG3 mAbs are not protective but switching from IgG3 to IgGl converted a non-protective mAb into a protective mAb [8,10]. This observations suggest that, for some fungi, the generation of protective antibody responses is dependent on the ability of the host to make antibodies to specific epitopes and these antibodies must be of a particular isotype.

Specific antibodies may contribute to host defence through a variety of mechanisms. For *C. neoformans*, capsule binding antibodies have been shown to be opsonic [11], to enhance killing of yeast cells by macrophages and microglia [11,12], and to reduce capsular polysaccharide antigen [2]. Some antibodies to *C. albicans* may be directly fungicidal [13]. Antibodies also activate the complement system which is important in host defence against many fungi [14]. Antibodies may also neutralize fungal products that contribute to virulence.

The observations that some mAbs protect against fungi suggest a potential role for antibody immunity in host defence against mycotic infections. The discovery that some mAbs to fungal antigens are protective does not challenge the existing view that cell mediated immunity is the main line of defence against fungal infections. Protective antibodies may not be made in sufficient quantities to affect the course of infection or their effects may be counterbalanced by non-protective or blocking antibodies [8]. Nevertheless, the identification of protective antibodies against fungal pathogens is a major development

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because it raises the hope that vaccines which elicit protective antibody immunity can be developed. Such vaccines could function by eliciting antibody opsonins that enhance the function of non-specific (i.e. macrophage, NK cell, neutrophil) and specific (lymphocyte) cell mediated immunity and prevent or help eradicate fungal infections. A polysaccharide-tetanus toxoid conjugate vaccine is being developed [15] which can elicit protective antibodies in mice.

Protective antibodies could be potentially useful for the therapy of fungal infections. Antibody administration was widely used in the pre-antibiotic era for the treatment of a variety of infectious diseases and antibody therapy and continues to be used today for some medical conditions. Antibody-based therapies, if developed, are likely to be used as adjuncts of standard antifungal chemotherapy. For *C. neoformans*, antibodies can enhance the efficacy of amphotericin B [16,17], fluconazole [18], and 5-flucytosine [13]. Antibody administration can rapidly clear serum polysaccharide antigen in mice and humans with cryptococcal infection. Since cryptococcal antigen has been associated with a variety of deleterious effects on host immunity [20] the ability of antibody to reduce serum antigen could conceivably translate into a therapeutic benefit.

Given that protective, non-protective and deleterious (disease-enhancing or blocking) antibodies exist it is possible that the type of antibody response made in response to fungal infection will affect the course of infection. It is striking that only a minority of patients at risk for invasive fungal infection actually become ill with fungal infections. For example, only 6-8% of HIV-infected patients with CD4 + lymphocytes counts less than 200 cells/mm^3 develop cryptococcosis in New York City [21] despite the fact that *C. neoformans* is ubiquitous in the environment. Quantitative and qualitative differences in the serum antibody responses to *C. neoformans* have been described between patients with and without HIV infection [22,23]. Thus it is conceivable that derangements in antibody immunity contribute to the marked susceptibility of some populations to fungal infections.

In summary, the field of antibody immunity against fungi is in a renaissance. The application of monoclonal antibody technology to re-examine the role of antibody immunity has established that antibodies can be protective against fungi. The task ahead is to understand the mechanisms by which antibodies mediate protective effects against fungi and design vaccines which elicit protective antibody immunity.

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