Review

Receptor-mediated Recognition of Cryptococcus neoformans

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Abstract

Cryptococcus neoformans, a facultative intracellular pathogen of macrophages, is unique among medically important fungi in its possession of a polysaccharide capsule. Capsule represents the organism’s major virulence factor. In the absence of opsonins, binding of encapsulated C. neoformans to macrophages is minimal. Following incubation in serum, C. neoformans potently activates complement, resulting in surface deposition of the third component of complement. Macrophages bind and phagocytose opsonized C. neoformans via three major complement receptors (CR) for C3 fragments, designated CD35 (CR1), CD11b/CD18 (CR3), and CD11c/CD18 (CR4). Antibody in normal human serum generally lacks opsonic activity, although vaccination can elicit anticapsular antibodies that are opsonic. The major component of cryptococcal capsule, glucuronoxylomannan (GXM), is shed from the fungus and circulates in the blood and cerebrospinal fluid of patients with cryptococcosis. Cellular receptors defined for GXM include CD14, toll-like receptor-2, toll-like receptor-4, and CD18. GXM binding to macrophage receptors triggers activation of nuclear factor-κB, but not mitogen-activated protein kinases. This results in no proinflammatory gene expression or release. C. neoformans also secretes mannoproteins, which are recognized by mannose receptors as well as by mannose-binding lectin, perhaps in conjunction with CD14. Strategies directed at modulating how intact C. neoformans and its released components are recognized by phagocytes could lead to novel approaches to treating cryptococcosis.

Key words: Cryptococcus neoformans, complement receptor, toll-like receptor, mannoprotein, phagocytosis, capsule, mitogen-activated protein kinase

Cryptococcus neoformans and cryptococcosis

The encapsulated yeast, C. neoformans, has emerged as a common pathogen causing infections in patients with impaired cell-mediated immunity. Hardest hit are those with the acquired immune deficiency syndrome (AIDS). In much of the developing world, cryptococcosis is one of the two or three most common life-threatening opportunistic infections in persons with AIDS. In areas of the world where HIV is endemic, C. neoformans is the most common cause of culture-positive meningitis. Most infections are thought to originate following inhalation of airborne fungi. The size of environmental isolates favors deposition in the alveoli of the lungs. Thus, broncoalveolar macrophages presumably play an important role in first line defenses against this pathogen.

Although virtually any organ system can be affected, meningitis is the most common clinical manifestation. Morbidity and mortality remain high despite advances in therapy. Of the medically important fungi, C. neoformans is the only one that possesses a capsule. Capsule is composed primarily of a high molecular weight polysaccharide that has a backbone of α-1,3-D-mannopyranose with attached β-D-glucuronopyranosyl and β-D-xylopyranosyl residues. In the environment, C. neoformans has a very thin capsule. In vivo, capsule production is stimulated by physiological levels of bicarbonate and iron, resulting in a capsule thickness which ranges from 1 µm to greater than 30 µm, depending upon the strain. During cryptococcal infections, capsule is continuously shed from the fungus and circulates with a long half life in the cerebrospinal fluid and blood. Shed capsule, known as cryptococcal antigen, forms the basis for rapid sensitive and specific tests for cryptococcosis available in most clinical microbiology laboratories.
Deletional mutagenesis studies have established conclusively that cryptococcal capsule is a virulence factor. Thus, disruption of genes essential for capsule formation results in acapsular strains that lack virulence in animal models of cryptococcosis. Moreover, reinsertion of the genes results in restoration of virulence. In broad terms, capsule can affect virulence in one of two ways. First, by coating the surface of the fungus, it masks cell wall components and presents a new surface that, as discussed below, is antiphagocytic. Second, shed capsular polysaccharide may have deleterious immunological effects by inducing antibody unresponsiveness, promoting immunological tolerance, and inhibiting leukocyte migration.

The macrophage plays a central role in both innate and acquired immune responses. C. neoformans is capable of intracellular parasitism, but it is not an obligate intracellular parasite. It is likely that the ability to survive both intracellularly and extracellularly is critical for the virulence of C. neoformans. Early in the course of infection, C. neoformans tends to be found intracellularly in macrophages and multinucleated giant cells. Unactivated macrophages have limited antifungal activity against C. neoformans. It is postulated that macrophage-activating cytokines produced during the course of a cell-mediated immune response are necessary to arm macrophages to kill C. neoformans. As with other organisms controlled by cell-mediated immunity, such as Mycobacterium tuberculosis, intracellular organisms may also survive long periods of time and serve as latent foci should T cell function decline. In patients with disseminated disease, large numbers of extracellular organisms can often be found, sometimes as part of discrete masses known as cryptococcomas.

**Phagocyte recognition of intact C. neoformans**

In the absence of opsonins, macrophages generally do not bind C. neoformans to any appreciable extent. This is a direct consequence of the capsule, which masks potential ligands on the cell wall of the fungus. Thus, acapsular mutants of C. neoformans are readily taken up by macrophages whereas unopsonized, encapsulated organisms defy recognition. The major opsonin which promotes recognition of C. neoformans by macrophages is complement. Following incubation of normal human serum with encapsulated C. neoformans, large amounts of the third component of complement (C3) get deposited on the capsule. Roughly ten times greater numbers of complement molecules get deposited on encapsulated C. neoformans than on acapsular C. neoformans or other fungi of comparative size. A clinical consequence of the ability of C. neoformans to potently activate and consume complement is that complement depletion may occur. This may be especially relevant in anatomic sites where complement levels normally are low, particularly in the central nervous system. In such situations, phagocyte recognition of C. neoformans may not occur.

Complement activation proceeds mostly through the alternative pathway. Moreover, almost all of the C3 is in the form of iC3b. Macrophages possess three receptors for C3 fragments, designated CD35 (CR1), CD11b/CD18 (CR3), and CD11c/CD18 (CR4). Two independent lines of evidence suggest that all three receptors participate in binding of serum-opsonized C. neoformans. First, monoclonal antibodies directed against any of the three receptors inhibit cryptococcal binding. Second, C. neoformans will bind to Chinese hamster ovary (CHO) cells transfected with human CR1, CR3 or CR4, but not to untransfected CHO cells.

Normal human serum contains antibodies reactive with C. neoformans but such antibodies tend not to be opsonic. Thus, removal of antibody from serum has little effect on C3 deposition on the fungus or on cryptococcal binding to macrophages. Consistent with this finding, pooled human IgG also is not opsonic. Capsule is poorly immunogenic and a strong antibody response is rarely seen in cryptococcosis. Moreover, the little antibody that is produced is likely to be complex with circulating capsular polysaccharide rather than binding to intact organisms where it can be opsonic. Nevertheless, specific anticapsular antibody generally is opsonic and greatly facilitates phagocyte recognition of C. neoformans. This has led to strategies to elicit anticapsular antibodies by active or passive vaccination. Conjugation of capsule to a protein such as tetanus toxoid makes the carbohydrate highly immunogenic. Some, but not all, anticapsular monoclonal antibodies are protective in mouse models of cryptococcosis. A clinical trial of an anticapsular monoclonal antibody is underway in humans with cryptococcosis.

Following binding of C. neoformans, phagocytosis (defined as internalization of the fungus into the cell) proceeds. Phagocytosis of complement-opsonized C. neoformans proceeds at a slower rate compared with acapsular yeast. Phagocytosis of cryptococcal strains with very thick capsules is particularly inefficient, or may not occur at all. C. neoformans opsonized with anticapsular antibody enters the macrophage quickly following binding to immunoglobulin (Fc) receptors.
Receptor recognition of shed glucuronoxylomannan and secreted mannoproteins

As discussed above, the major component of capsule, GXM, is shed from the fungus where it mediates a variety of immunological effects. Immunohistochemical studies have demonstrated the GXM localizes to macrophages in vivo. Moreover, depletion of macrophages from mice significantly prolongs the serum half life of intravenously administered GXM. These studies suggested to us that macrophages have functional receptors for GXM. We studied the role of Toll-Like Receptors (TLR), a family of cell surface receptors that enable phagocytic inflammatory responses to a variety of microbial products. TLR often function in association with CD14. Activation via these receptors generally triggers signaling cascades, resulting in nuclear translocation of NF-kB and a proinflammatory response including tumor necrosis factor-alpha (TNFα) production. We investigated whether TLRs participate in the host response to C. neoformans GXM. CHO cells transfected with human TLR2, TLR4 and/or CD14 bound fluorescently labeled GXM. The transfected CHO cells were challenged with GXM and activation of an NF-κB-dependent reporter construct was evaluated. Activation of the construct was observed in cells transfected with both CD14 and TLR4. GXM also stimulated nuclear NF-κB translocation in human peripheral blood mononuclear cells and the murine macrophage cell line, RAW264.7. However, stimulation of these cells with GXM resulted in no TNFα gene expression or secretion. Further investigation revealed that GXM failed to trigger mitogen-activated protein (MAP) kinase pathways that are necessary for TNFα release. These findings suggest that TLRs, in conjunction with CD14, function as pattern recognition receptors for GXM. Furthermore, whereas GXM stimulates cells to translocate NF-κB to the nucleus it does not induce activation of MAP kinase pathways or release of TNFα. Neutrophils too bind GXM, at least in part via CD18, the beta chain of the beta 2 integrin family of adhesion molecules. It is likely that macrophage CD18 could also bind GXM.

Mannoproteins are a heterogeneous group of glycoproteins characterized by a protein core decorated with carbohydrate groups terminating in exposed mannose residues. Mannoproteins account for approximately half of the proteins secreted by C. neoformans. Antigen-presenting cells, likely macrophages and dendritic cells, have mannose receptors which bind mannoproteins. Moreover, CHO cells transfected with the human macrophage mannose receptor efficiently internalize mannoproteins. Other receptors that may be critical for binding mannoproteins include CD14, perhaps acting in concert with mannose-binding lectin present in serum.

References

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