Immunity in cryptococcosis: An overview

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Abstract

Cryptococcosis is an often fatal opportunistic fungal infection. Despite efforts to elucidate the role of immunity in host defense against the disease, much remains to be learned. The purpose of this brief review is to provide the reader with an overview of the history of research concerned with host immunity in cryptococcosis. Both humoral and cell-mediated studies are included. An effort has been made to present the reader with a comprehensive list of references in the hope of encouraging additional reading and research in this important area.

Cryptococcosis is a chronic, subacute, or acute pulmonary, meningeal or systemic mycosis. The etiologic agent is the encapsulated yeast, *Cryptococcus neoformans,* a saprophyte which has been isolated most frequently from soils enriched with pigeon excreta. The infectious particle is believed to be a small yeast cell that enters the body by inhalation (77, 80). A primary pulmonary infection ensues with subsequent localization or, frequently, fatal dissemination; the organism has a predilection for the central nervous system. The true incidence of cryptococcosis is unknown, but there is increasing evidence that the disease may be a major mycosis in the world, both in incidence and prevalence (49, 52).

Patients with cryptococcosis characteristically demonstrate minimal and variable immune responses to the invading fungus. The polysaccharide capsule of *C. neoformans* is a key factor in the development of host humoral immune responses. Unfortunately, the pattern of immune responses to cryptococcal polysaccharide is erratic. Several investigators have failed to demonstrate any type of antibody production in diseased humans (53, 91) or immunized mice (9). Others, however, have demonstrated antibody in patient's sera using agglutination and indirect fluorescent antibody techniques (81, 94). Bloomfield *et al.* (10) and Gordon and Veddar (35) detected antigen and/or antibody in the sera of patients suffering from cryptococcosis by using antibody-sensitized latex particles in a yeast cell agglutination test. Kozel and Cazin (56, 57) detected anticryptococcal antibody in sensitized rabbits and mice by using a passive hemagglutination assay. These discrepant results can be explained best by the wide variation in the sensitivity of the various assays employed, as well as the fact that soluble cryptococcal capsular polysaccharide can be detected in the serum of some patients which may alter or mask humoral responses. If free antigen is in circulation, one might not detect free antibody when antigen is in excess (25, 56).

In addition to its action as an antigen 'sponge', excess quantities of cryptococcal polysaccharide

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may be able to induce immune tolerance. This concept of 'immunological paralysis' was first proposed by Gadebusch in 1958 (31). Abrahams and Gilleran (2) determined that a narrow range existed between the quantity of antigen that elicited effective resistance and a larger quantity that resulted in a significant reduction in protection. Bennett and Hasencleaver (7) showed in experimental animals that serum levels of cryptococcal polysaccharide persisted for weeks following a single injection of capsular material. Goren (36) suggested that the immunologic paralysis proposed in cryptococcosis was relevant only to the polysaccharide antigen. Murphy and Cozad (73) demonstrated that immunological paralysis can occur, and that the unresponsiveness appeared to be the result of a decline in the number of cells capable of producing antibody rather than neutralization of antibody by antigen. Kozel *et al.* (59) confirmed immunological unresponsiveness in mice injected with varying amounts of cryptococcal polysaccharide. Additionally, they determined that the capsular material was sequestered in the tubular epithelial cells of the kidney. Muchmore *et al.* (72) recently expanded the work of Kozel *et al.* (59) and reported that cryptococcal polysaccharide could be detected in the liver and spleen of mice 70 days after an intravenous injection of purified capsule.

Besides a possible role for serum antibody to opsonize for clearance and killing of cryptococcal organisms, nonantibody serum factors also may play an important role in the primary host defense against *C. neoformans*. Normal sera from humans and from other mammals (33) have been shown to exhibit growth inhibitory factors against *C. neoformans*. Reiss *et al.* (85) reported anticryptococcal activity in the alpha 2 and gamma zone of normal human serum. A nondialyzable heat stable protein-like substance that inhibited the growth of *C. neoformans in vitro* was isolated by Igel and Bolande (47). Baum and Artis (5) also have characterized a growth inhibition factor for *C. neoformans* in human serum.

Complement components are necessary for effective phagocytosis (19, 20, 62) of *C. neoformans*. The fact that capsular material can react with complement components (38) and that complement is depleted during infection (68), may contribute to the pathogenesis of this disease by inhibiting the phagocytic and cell killing action of late complement components. Additionally, a role of specific anticapsular antibody (immunoglobulin G) in opsonization of *C. neoformans* for phagocytosis has been proposed (58, 60, 67). Immunoglubulin G (IgG) apparently plays a primary role in attachment of macrophages to cells of *C. neoformans*; however, cryptococcal polysaccharide can mask the opsonizing IgG, thus preventing it from participating in Fc-mediated phagocytosis.

The capsular polysaccharide of *C. neoformans* is a virulence factor which can prevent phagocytosis of the organism. Bulmer and Sans (11, 12) reported that only 24 percent of human leukocytes phagocytized an encapsulated strain of *C. neoformans,* whereas 74 to 84 percent of nonencapsulated mutants were phagocytized. Additionally, they reported that soluble capsular polysaccharide could inhibit phagocytosis of nonencapsulated strains. Tacker, Farhi and Bulmer (93) extended these observations and reported that once cells of *C. neoformans* were engulfed they were killed by human peripheral leukocytes. Diamond *et al.* (21) also noted effective killing of *C. neoformans* by human peripheral neutrophils within 2 hours. The killing mechanisms appeared to be dependent on the generation of hydrogen peroxide. In contrast, Bulmer and Tacker (14) demonstrated that guinea pig alveolar macrophages could ingest nonencapsulated cells of *C. neoformans,* but were unable to kill the engulfed yeast cells during the first few hours following exposure. This observation was supported by Karaoui *et al.* (50, 51) using alveolar and peritoneal macrophages from sensitized mice, by Diamond (16) using guinea pig macrophages, and by Diamond and Bennett (18) who demonstrated growth of *C. neoformans* within *C. neoformans* activated human macrophages. These observations led to the speculation that the macrophage may serve as a vehicle in the dissemination of *C. neoformans*.

This concept was not supported by Mitchell and Friedman (70) who examined the intracellular fate of *C. neoformans* in rat peritoneal exudate cells. These authors found that while many ingested yeast cells were resistant to intracellular killing within the macrophage, many did not multiply and were presumably dead. Two important differences between their work and that of Bulmer and Tacker (14) should be noted. First, Mitchell and Friedman used peritoneal macrophages, and second, their

incubation period was much longer than the 3 hours incubation utilized by Bulmer and Tacker. Additionally, Mitchell and Friedman reported on the ability of yeast cells to replicate within individ ual macrophages, but did not culture for viable intracellular yeast cells. They observed that the ability of macrophages to engulf some strains of *C. neoformans* was inversely proportional to the size of the capsule, but that once cryptococci were ingested, intracellular fate was not correlated with the size of the capsule. This observation was in contrast to that of Sethi and Pelster (90) who reported that peritoneal macrophages could actively inhibit the intracellular multiplication of thinly encapsulated yeast cells, but cells of *C. neoformans* having medium to large capsules multiplied intracellularly.

The importance of cell-mediated immunity in cryptococcosis involving interaction between macrophages and lymphocytes has been suggested by Gentry and Remington (34). They noted that persistent infection with intracellular bacteria and protozoa conferred resistance against *Cryptococcus* infection. The activated macrophages in their model resisted destruction following phagocytosis of *C. neoformans*. Thus, they proposed that the activated macrophage may play a major role in the observed resistance. Additionally, Regelson *et al.* (82, 83) used pyran copolymer to activate macrophages and demonstrated that pre-treatment of mice resulted in the conversion of *C. neoformans* infected mice to a healthy carrier state for prolonged periods. Both observations suggest that activated macrophages may play a role in regulation of cryptococcosis.

Several investigators have reported that mononuclear phagocytes surround cryptococci, but do not ingest them (48, 89). Papadimitrious *et al.* (79) reported that immune serum increased the number of monocytes which would surround cryptococcal cells. This interaction was followed by thinning of the fungal capsule and degradation of the organism. This observation implies that the macrophage may be capable of killing cryptococcal organisms extracellularly. Kozel and Mastroianni (61) reported that the capsular polysaccharide could inhibit the attachment of macrophages to yeast cells, again pointing to the role of capsule in protection of the yeast cell.

Numerous studies have been done in the attempt to elucidate the role of humoral and/or cellular immunity in host response to cryptococcosis. A futile attempt at immunization was made first in 1925 by Shapiro and Neil (91), who injected a patient intraspinally with specific hyperimmune rabbit serum. In 1942, Hoff (46) vaccinated mice with heat-killed cells of *C. neoformans* and found that the survival time following infectious challenge was not significantly increased. Kligman (54) used a crude capsular polysaccharide from an unknown strain of *Cryptococcus* in attempts to protect mice and rabbits. No increased survival of immunized animals was observed and he concluded that the polysaccharide was nonantigenic. Neill *et al.* (76) reported that vaccines made from weakly encapsulated cells of *C. neoformans* had greater immunogenic capacity in rabbits than did vaccines prepared from heavily encapsulated cells. They did not study animal survival following virulent challenge. Gadebusch (32) and Marcus and Rambo (69), using formalin-killed vaccines, failed to observe any significant difference in survival time between vaccinated and control animals. However, Abrahams and Gilleran (2) and Abrahams (1) reported that under properly selected conditions immunization with formalin-killed cells of *C. neoformans* protected 60 to 70 percent of mice challenged with lethal doses of *C. neoformans*. Abrahams (1) was able to increase the number of survivors to 95 percent by incorporating *Bordetella pertussis* organisms into the vaccine. In an attempt to define the mechanism of the resistance induced, he transferred antibody from immune animals to normal, syngeneic recipients and challenged the recipient mice with virulent cells of *C. neoformans*. Although he observed a slight prolongation of survival, no lasting protection occurred. Later, Abrahams (3) reported that protection could be transferred with peritoneal exudate cells and was presumably cell mediated. However, he did not define the cell type which was responsible for this protection. Beemer *et al.* (6) reported that intradermally administered phenol-killed cells of *C. neoformans* proved effective in treating patients with cryptococcosis when the vaccine was administered in combination with mycostatin therapy. Reiss and Alture-Werber (84) reported protection in 69 percent of subdermally and 50 percent of subcutaneously immunized mice using an avirulent, gama-irradiated mutant of *C. neoformans* as the

immunizing agent. Dykstra and Friedman (22) noted that subcutaneously vaccinated mice lived longer after lethal intravenous challenge than did nonvaccinated animals. Protection was seen only with low dose inocula 3 weeks or longer after vaccination. Staib and Mishra (92) showed 91 percent survival of mice previously inoculated intramuscularly with a low virulence strain of *C. neoformans,* in comparison to 23 percent survival in control mice. Fromtling (26) and Fromtling *et al.* (27–29) immunized mice with repeated intraperitoneal (i.p.) inoculations of an avirulent pseudohyphal form of *C. neoformans* (78). They reported significantly longer survival times in mice receiving 6 or 8 weekly i.p. inoculations of immunizing cells in comparison to control animals. Additionally, passive transfer of immunity was accomplished with sensitized spleen cells, but not with sera (27). The authors suggested that humoral factors played a lesser role than cell-mediated immunity in protecting the host against infection with *C. neoformans.* Graybill and Mitchell (41) and Lim and Murphy (63) also have demonstrated passive transfer of immunity (41) and immunity associated with delayed-type hypersensitivity (63). In spite of noting passive transfer of protection, none of the investigators examined which specific subsets in the unfractionated spleen preparations were responsible for the observed immunity.

In 1967, Goren and Middlebrook (37) described a procedure to elicit an antibody response to purified cryptococcal capsular polysaccharide in which polysaccharide-bovine gamma globulin conjugates were used as the immunogens. Although the antibody response to this challenge was significant, animals challenged with viable yeast cells were not protected and, in fact, the immunized animals died faster than those in the control group. These results are in disagreement with those of Louria (64) and Louria *et al.* (65, 66) who reported that enhanced resistance was correlated with the presence of antibody and not to an enhanced cellular response.

Delayed-type hypersensitivity (DTH) in cryptococcosis has been examined by several investigators, but the role of DTH in the disease is still undetermined. Delayed-type hypersensitivity to extracts of *C. neoformans* in laboratory personnel who work with the yeast (39, 88) as well as patients in environments from which *C. neoformans* was cultured (71) have been observed. Salvin and Smith (87) and Atkinson and Bennett (4) developed cryptococcal skin test antigens and showed the development of DTH in guinea pigs, although cross-reactivity with other fungi was noted. Murphy *et al.* (74) developed a culture filtrate skin test antigen that proved to be specific for *Cryptococcus* and showed a high degree of sensitivity (74, 75). Graybill and Taylor (43) and Hay and Reiss (45) have shown development of DTH in mice sensitized with disrupted cell extracts of *C. neoformans.* Development of DTH was associated with protection against lethal challenge by *C. neoformans.* Although delayed skin test reactivity (DTH) to soluble cryptococcal antigens has been useful in diagnosis of disease (4, 8, 74) this reaction does not necessarily imply that protective immunity has been established, since DTH can be seen during active infection (42) in animals that eventually succumb to the disease.

An increased susceptibility to cryptococcal disease has been associated with immunological defects, e.g., about half of patients have some underlying illness such as malignancy or have had or are undergoing immunosuppressive treatment. This has led to speculation that even when apparent underlying disease is not readily evident, subtle immune deficiencies may exist. In a study of cell mediated responses of patients who had recovered from cryptococcal disease, Graybill and Alford (39) observed diminished DTH reactions to two commonly encountered fungi, *Histoplasma capsulatum* and *Candida albicans.* They interpreted this finding as evidence that most cryptococcal patients have a deficiency in cell mediated responses to fungal antigens even when an underlying disease process can not be identified. In a similar study Diamond and Bennett (17) reported that in vitro lymphocyte transformation to cryptococcal antigen was significantly lower in successfully treated patients than that of normal individuals who had positive skin tests. They suggested that patients with cryptococcosis had a defect in the ability of their lymphocytes to recognize fungal antigens.

Studies of cryptococcosis in congenitally athymic (nude) mice suggest a strong role of cell-mediated immunity in host defense against the disease. Graybill and Drutz (40) showed that nude Balb/c and Swiss mice could not develop DTH to cryptococcal extracts and were highly susceptible to challenge by high and low virulence strains of

C. neoformans. Cauley and Murphy (15) confirmed these observations and noted that nude mice were able to produce antibodies against cryptococcal cells, indicating that some immunogens of *C. neoformans* may be T-independent, and that antibody alone is not protective.

Several branches of immunity in cryptococcosis have been examined during the past few decades; however, a complete understanding of host protection against *C. neoformans* has not been attained. The weak immunogenicity and antiphagocytic characteristics of the polysaccharide capsule have frustrated immunologists. Capsule-deficient forms of *C. neoformans* (13, 23, 24, 30, 44, 55) may be effective immunizing agents in the study of cryptococcosis. Some of these strains have been shown to produce marked granulomatous responses in human disease (24, 44) unlike encapsulated cells of *C. neoformans*. Additional strains have been shown to be avirulent (13, 23, 30) or of low virulence (55) for experimental animals.

Based on the evidence presented above, the following experimental hypotheses may be suggested. First, although delayed type hypersensitivity (DTH) is associated with positive immunity and a favorable prognosis in cryptococcosis, some humoral components play a role as well. The absence of complement component C5a has been associated with decreased resistance to *C. neoformans* infection (86); thus, it may be hypothesized that late acting complement components could be important in opsonization and clearance of the yeast. Secondly, macrophages have been shown to inhibit, kill or permit dissemination of *C. neoformans*. Macrophages must be activated to become effective in killing *C. neoformans* and this activation may be mediated by delayed-type hypersensitivity. Thus, immunity to *C. neoformans* may depend upon a complex interaction of humoral and cellular immune factors, i.e., stimulation of a sensitized host by *C. neoformans* may trigger a DTH reaction which primarily serves to initiate lymphokine production and subsequently enhance complement production and activate macrophages which kill phagocytized organisms. New lines of research in this area may elucidate the host factors responsible for defense against not only cryptococcosis, but other infectious diseases as well.

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