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Macrophages in Resistance to Candidiasis

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INTRODUCTION

Candida albicans is part of the normal microbial flora that colonizes mucocutaneous surfaces of the oral cavity, gastrointestinal tract, and vagina of many mammals and birds (205). For the most part, *C. albicans* colonizes and is infectious for the host, since both antibody- and cell-mediated immune responses to *Candida* antigens are evoked in healthy individuals; however, *C. albicans* does not normally cause disease in immunocompetent colonized hosts. It is in the setting of congenital, induced, or disease-related immune dysfunction(s) that *C. albicans* can cause cutaneous, mucocutaneous, and life-threatening systemic disease. An understanding of the host-parasite interactions that allow *C. albicans* to switch from a commensal to a pathogen capable of infecting a variety of tissues would benefit the design of innovative approaches for the prophylaxis and therapy of this widespread and common infection.

C. albicans is able to compete with other microbes as well as to adhere to and survive on mucosal surfaces of hosts with *Candida*-specific antibody- and cell-mediated immunity (AMI and CMI). Several publications have described the existence of numerous putative *C. albicans* virulence factors (4, 119, 179, 193, 233, 235, 239, 242) that might enable this opportunistic fungus to survive and thrive in the adverse conditions of host tissues. Among these putative virulence factors, the cell wall of *C. albicans* is one of the most important. The cell wall provides rigidity as well as protection against osmotic lysis, and it promotes infection by supporting the interaction of *C. albicans* adhesins and host-cell receptors (4, 119; reviewed in references 31 and 193). Also, the *C. albicans* cell wall contains mannoproteins, which have immunosuppressive properties that can enhance the persistence of the fungus in lesions (reviewed in reference 173). *C. albicans* not only can adhere to but also can

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penetrate into mucosal surfaces. Extracellular aspartyl proteinases may assist *C. albicans* in the initial stages of tissue invasion on keratinized mucocutaneous surfaces (126, 143, 180, 271). Another important virulence factor of *C. albicans* is related to its dimorphic nature. Blastoconidia are usually found as members of the normal flora in healthy individuals; however, during infection, blastoconidia, pseudohyphae, and hyphae are frequently present in lesions. Strains of *C. albicans* that produce abundant hyphae have been shown to be more pathogenic than those that grow predominantly as yeasts (219).

Although all the virulence factors of *C. albicans* are likely to contribute to its capacity to adhere to, penetrate, and survive in tissues, the immunocompetent host is colonized and infected but rarely suffers from clinical candidiasis (178). *Candida* usually causes disease in immunocompromised hosts such as leukemic, organ-transplanted, diabetic, diabetic and myeloperoxidase (MPO)-deficient, and human immunodeficiency virus (HIV)-infected patients (43, 78, 98, 122, 145, 178, 269). Innate and acquired humoral and cellular immune mechanisms are involved in resistance to candidiasis. Elucidation of the role that different immune cells and their cytokines play in resistance to *C. albicans* is critical if we are going to understand how this fungus can cause such a variety of diseases and how we can prevent or control them.

Neutrophils

The multiple clinical presentations (cutaneous, mucosal, mucocutaneous, and systemic) that *C. albicans* can produce in a host have been associated with specific immune defects. In general, neutropenia or congenital defects that affect neutrophil function (e.g., MPO deficiency and any of the mutations of the NADPH oxidase in chronic granulomatous disease) have been associated with enhanced susceptibility to systemic candidiasis (108, 164, 186). The occurrence of systemic candidiasis in hosts with normal neutrophil function (268) suggests a protective role for other cells, including mononuclear phagocytic cells, in resistance to this increasingly frequent and life-threatening form of the disease. Furthermore, the paradigm defining a predominant role for neutrophils in mediating resistance to systemic candidiasis does not hold for certain neutrophil dysfunctions. The fact that patients with chronic, congenital, or autoimmune neutropenia are not overtly susceptible to systemic *Candida* infections (57, 83, 142) argues against an exclusive role for neutrophils in resistance to the systemic form of the disease and points out that alternative mechanisms (e.g., monocytes) can control systemic candidiasis. Quoting Dale et al., "patients with this disorder (i.e., chronic neutropenia) are often remarkably free of morbidity from infection and capable of delivering a nearly normal number of phagocytes (usually monocytes) into induced inflammatory lesions" (57). The expression of MPO and NADPH oxidase in other professional phagocytes (52, 94, 130, 133) as well as in neutrophils may explain why defects in either of these two enzymes can predispose patients to candidiasis, and emphasizes the redundancy and diversity of the immune response to *C. albicans*. Although neutrophils are important components of the innate immune response to *C. albicans*, this review will not concentrate on these interesting cells.

Antibody-Mediated Immunity

AMI has also been implicated in resistance to systemic candidiasis (35, 154, 155, 264). Indirect evidence in support of a protective role for *Candida*-specific antibodies in resistance to systemic candidiasis was provided by the observation that transfer of *Candida*-immune serum bestowed considerable protection to naive animals against an otherwise lethal *C. albicans* challenge given intravenously (i.v.) (2, 155, 189). Protection against the *C. albicans* challenge by passively transferred hyperimmune serum in mice has been demonstrated with very large i.v. doses (1×10^8 to 5×10^8 cells) of *C. albicans* (155, 189). Although *Candida*-specific antibodies may have increased the resistance of mice to systemic candidiasis in the latter studies, their contribution cannot be separated from other serum factors that could have been transferred at the same time. More direct proof in favor of the hypothesis that AMI is protective against systemic candidiasis comes from the observation that genetically engineered B-cell-deficient mice, although resistant to mucosal and disseminated candidiasis of endogenous (gastrointestinal tract) origin, were susceptible to acute systemic candidiasis induced by i.v. inoculation (264).

However, not all experimental and clinical evidence supports a role for antibodies in resistance to candidiasis. Arguments against a protective contribution for AMI in candidiasis emanate from observations that rabbits treated with *C. albicans*-hyperimmune serum have not shown enhanced resistance to an i.v. challenge with *C. albicans* (2). Furthermore, other murine models such as T- and B-cell-deficient SCID mice are as resistant to systemic candidiasis as are immunocompetent mice (90, 103, 104), thus indicating that antibodies are not critical for defense against the disseminated form of the disease.

In analogy to cryptococcosis (reviewed in reference 35), the disparity in the reports on the role of AMI in resistance to systemic candidiasis might be explained by the existence of protective and nonprotective antibodies. In support of the latter hypothesis, some immunoglobulin M (IgM) antibodies to a *C. albicans* adhesin were protective; however, other IgM antibodies were not (91). The role of antibodies in resistance to candidiasis has been recently reviewed (35). In this report, we will discuss AMI primarily in the context of its potential as an opsonin for enhancing *C. albicans* phagocytosis by macrophages.

Cell-Mediated Immunity and Macrophages

Involvement of CMI in the control of infections, where macrophages serve as the effector cells of an immune response orchestrated by T cells, has been demonstrated for several pathogens. CMI, probably by T-cell-mediated activation of macrophages, is also considered to be important for resistance to mucocutaneous and systemic candidiasis (17, 106, 212). However, several reports on the in vitro candidacidal activity of macrophages have downplayed the role of these cells in resistance to *C. albicans* (5, 58, 240). Whereas some of the latter studies showed that macrophages were less efficient than neutrophils in killing *C. albicans* in vitro (5), others stressed the poor candidacidal capacity of mononuclear phagocytic cells from different species (e.g., humans, mice, and rabbits) and different tissues (e.g., peripheral blood monocytes, peritoneal macrophages, and alveolar macrophages) (5, 58, 240). These studies noted that mononuclear phagocytic cells not only were unable to kill *C. albicans* in vitro but also were destroyed by the fungus (5, 58, 240). More recently, it has been suggested that the increased susceptibility of HIV-infected individuals to orogastric candidiasis was due to a possible impairment of macrophages (49); however, others have noted that human monocytes from HIV-infected individuals and monocyte-derived macrophages infected in vitro with HIV phagocytized *C. albicans* and produced as much superoxide anion, a molecule that has been correlated with macrophage candidacidal capacity

TABLE 1. Effect of source, differentiation state, and activation on the phagocytosis and killing of *C. albicans* by mononuclear phagocytic cells

Source	Differentiation state of mononuclear phagocytes	Treatment	Phagocytosis	Killing	Reference(s)
Human	Multinucleated giant cells	IFN-γ plus IL-3	Normal[a]	Increased	72
Human	Macrophages	Aerobic culture	Normal	Increased	248
Human	Blood monocytes	Aged in vitro	Normal	Decreased	222
Human	Alveolar macrophages	Aged in vitro	Normal	Decreased	263
Murine	GG2EE	None	Normal	Decreased	19
Murine	Peritoneal exudate macrophages	Conditioned media[b]	Increased	Increased	144
Murine	Peritoneal exudate macrophages	BCG-PPD vaccination[c]	Increased	Increased	144, 252
Murine	Resident peritoneal macrophages	IFN-γ	Normal	Increased	27, 258, 259
Murine	Hepatic macrophages (not parenchimal)	Cyclophosphamide[c]	None	Increased	60
Rabbit	Peritoneal exudate macrophages	Freund's adjuvant	Normal	Increased	132

[a] Compared to untreated control.
[b] Conditioned media from phytohemagglutinin-stimulated splenocytes.
[c] Macrophages were obtained from BCG-purified protein derivative (BCG-PPD)-vaccinated mice or from cyclophosphamide-treated mice.

(reviewed in this paper), as did control mononuclear phagocytic cells (174, 176).

In contrast to the negative reports on the importance of macrophages in immunity to candidiasis (5, 58, 174, 176, 240, 251), other studies suggest that macrophages do participate in resistance to this frequent infection (17, 39, 73, 103, 114, 130). Macrophages from a variety of tissues and from several different hosts have been shown to phagocytize (5, 9, 44, 123, 133, 136, 150) and kill (10, 27, 38, 60, 136, 221, 225, 258, 259) *C. albicans*. In addition, human monocytes can ingest and kill *C. albicans* blastoconidia in vitro better than or similar to the killing by human peripheral blood neutrophils (133, 229). The importance of the participation of mononuclear phagocytic cells in resistance to either systemic or mucosal candidiasis has been suggested in several other studies (17, 38, 103, 197, 225, 275).

Because the contribution of macrophages to resistance to candidiasis is still controversial and because multiple reviews on immunity to candidiasis have not treated macrophages in detail (7, 8, 47, 78, 81, 89, 166, 196, 205, 207, 217, 253), the aims of the present review are to describe the interactions of macrophages with *C. albicans* at the molecular and cellular levels and to focus on their role in resistance to the spectrum of diseases caused by this prominent pathogenic fungus.

PHAGOCYTOSIS OF *C. ALBICANS* BY MACROPHAGES

Methods

The majority of studies on phagocytosis of *C. albicans* by mononuclear phagocytes were performed with an excess of the fungus and used different stains (e.g., trypan blue, methylene blue, and Giemsa) to estimate the number of *C. albicans* organisms phagocytized (19, 27, 72, 263). By using a combination of trypan blue and eosin, some investigators (132) have differentiated between attached and interiorized *C. albicans*, since intracellular and extracellular fungi are unstained and purple, respectively, under these conditions. Other methods to differentiate between interiorized and noninteriorized *C. albicans* have taken advantage of the fact that the green fluorescence of fluorescein isothiocyanate-labelled *C. albicans* organisms which have been interiorized is protected from quenching by crystal violet or ethidium bromide (222, 258).

Phagocytosis is an important step in the intracellular killing of *C. albicans* blastoconidia by mononuclear phagocytic cells. However, phagocytosis and macrophage candidacidal activity are not always correlated (Table 1). Differences in the capacity of macrophages to phagocytize do not appear to correlate with differences in the pathogenicity of several *Candida* spp. Although mononuclear phagocytic cells ingest *C. albicans* and less pathogenic *Candida* spp. such as *C. tropicalis*, *C. parapsilosis*, *C. pseudotropicalis* (now *C. kefyr*), *C. krusei*, and *C. guilliermondi* at a similar rate, phagocytized *Candida* spp. are not always killed by macrophages (150, 221, 240). For instance, *C. albicans* and *C. tropicalis* can destroy the macrophages by forming hyphal filaments within a few hours after phagocytosis; conversely, *C. guilliermondi*, a less pathogenic species than *C. albicans*, was phagocytized, did not form hyphae and caused very little damage to the macrophages after long-term culture (240, 260).

In addition to the fact that phagocytized *C. albicans* organisms are not always killed by the macrophages, macrophage activation does not always increase the capacity of mononuclear phagocytic cells to ingest *C. albicans* (Table 1) (reviewed in reference 78). The heterogeneity of macrophages used in studies on phagocytosis and killing of *C. albicans*, as well as the variety of mechanisms used to activate macrophages, may explain the conflicting results that have been reported on the state of macrophage activation and the capacity of these cells to phagocytize and kill this pathogenic fungus. A case that exemplifies the latter result is a report that GG2EE macrophages incubated with either tumor necrosis factor (TNF), interleukin-1 (IL-1), IL-3, or IL-4 did not phagocytize more zymosan particles than did control macrophages (19); however, the phagocytic activity of the same line of macrophages for zymosan was enhanced after the macrophages were treated with gamma interferon (IFN-γ) or macrophage colony-stimulating factor (M-CSF) (19).

One conclusion that can be drawn from the previous studies, which address macrophage phagocytosis and killing of *C. albicans*, is that phagocytosis of *C. albicans* is a well-conserved characteristic of mononuclear phagocytic cells from different mammalian species, anatomical sites, and states of differentiation and activation. Because *C. albicans* can be ingested by so many different populations of mononuclear phagocytic cells, a diverse array of opsonic and nonopsonic interactions between *C. albicans* and these phagocytes must exist.

Phagocytosis of Opsonized *C. albicans*

Phagocytosis of *C. albicans* by human monocytes and by human or murine macrophages appears to be optimal when the yeasts are opsonized with "normal" serum (114, 248). Of the opsonins present in normal serum, specific anti-*Candida* antibodies and complement components have been studied

most extensively in relation to phagocytosis of *C. albicans* by mononuclear phagocytic cells.

Antibodies. Several reports suggest that *C. albicans*-specific antibodies mediate the ingestion of *C. albicans* by mononuclear phagocytic cells (44, 144). Human monocytes phagocytized *C. albicans* better when fresh human serum was present in the medium (44, 214); the opsonic properties of the serum were attributed to IgG but not to complement factors (44). Similarly, compared to nonimmune serum, *Candida*-immune serum supported enhanced phagocytosis of *C. albicans* by rabbit macrophages (214). Conversely, Morrison and Cutler (163), using a murine model, reported that elimination of antibodies specific for *C. albicans* did not influence the opsonic characteristics of the serum. Also, addition of anti-*C. albicans*-specific antibodies did not increase the phagocytic activity of murine macrophages for *C. albicans* in saline buffer (114).

The diverse positive and negative results of studies designed to clarify the need for antibodies in the phagocytosis of *C. albicans* by macrophages could be due to the variety of mononuclear phagocytic cells used in such experiments. A clear case that exemplifies the latter hypothesis is the fact that addition of *Candida*-immune mouse serum enhanced the capacity of nonactivated murine macrophages (but not that of activated macrophages obtained either from bacillus Calmette-Guérin [BCG]-vaccinated mice or by in vitro exposure of normal macrophages to phytohemagglutinin-induced lymphokines) to phagocytize *C. albicans* blastoconidia (144). The increased phagocytosis of immune serum-opsonized *C. albicans* by nonactivated macrophages suggests that AMI plays a role in resistance to *C. albicans* infections in hosts that lack the CMI necessary for macrophage activation. In favor of the notion that antibodies might be needed in situations where CMI has not been established, B-cell-deficient mice did not show splenocyte proliferation in response to *C. albicans* antigens in vitro and were indeed susceptible to acute systemic candidiasis induced by i.v. inoculation (264). Nevertheless, these B-cell-deficient mice showed resistance to acute systemic candidiasis after they were orally colonized with *C. albicans* and developed *Candida*-specific T-cell-mediated immunity (110, 264).

Differences in the capacity of opsonic antibodies to increase the phagocytosis of *C. albicans* by mononuclear phagocytes from different species might not reflect species differences but could instead be associated with the differentiation and/or activation state of the macrophages. Undifferentiated monocytes utilize Fc receptors for optimal phagocytosis of *C. albicans*, whereas some differentiated macrophages express other surface molecules that can recognize *C. albicans* independently of antibody. In addition to the state of activation and differentiation, the isotype and specificity of the antibody may influence the outcome of the interaction of antibody-opsonized *C. albicans* and mononuclear phagocytes. Another factor that should be considered when the opsonic properties of *Candida*-specific antibodies are studied is their capacity to enhance macrophage candidacidal activity. Identification of opsonic antibodies that enhance the phagocytosis and candidacidal activity of macrophages may ultimately lead to the production of specific antibodies for the therapy of candidiasis or for its prevention in individuals at high risk.

Complement. Complement components can also promote the phagocytosis of *C. albicans* by macrophages. Heat-inactivated serum was found to be less efficient than normal serum in enhancing *C. albicans* phagocytosis by human monocytes (133). The latter observation suggests that heat-labile complement components are important in the ingestion of *C. albicans* by human monocytes. In fact, phagocytosis of *C. albicans* and *C. parapsilosis* by human monocytes requires calcium and opsonization by components from both the classic and alternative complement pathways (150). As in the human system, murine macrophages appear to phagocytize *C. albicans* optimally if the complement system is intact (114, 163).

Several investigators (114, 163, 199) have shown that components of the alternative complement pathway enhance the phagocytosis of *C. albicans* by murine macrophages. Murine serum preincubated with zymosan at 17°C, a treatment that removes properdin and thus prevents the activation of the C3 component, diminished the uptake of *C. albicans* by murine macrophages (163). Similarly, murine serum heated at 50°C, a temperature that inactivates factor B (a heat-labile serum protein of the alternative complement pathway), decreased the phagocytic activity of murine macrophages (163, 199). Chelation of magnesium ion, which is required for activation of the alternative complement pathway, but not calcium, which is important for the activation of the classical complement pathway, inhibited the phagocytic-promoting characteristics of fresh mouse serum.

More direct proof that the alternative complement pathway promotes the phagocytosis of *C. albicans* by macrophages has been demonstrated by reports of decreased phagocytosis of *C. albicans* when murine serum is treated with anti-C3 (163). The relevance of the C3 component in the phagocytosis of *C. albicans* by macrophages is emphasized by the fact that *C. albicans* blastoconidia are able to activate the alternative complement pathway (198, 199). Specifically, *C. albicans* mannans, and glucans with β-D-(1,3)- and β-D-(1,6)-cross-links, can activate the alternative complement pathway (56, 198). The activation of C3 complement component occurs in vitro as well as in vivo. Sohnle et al. (236) noted that C3 complement component and properdin, and in some instances *Candida* antigen together with C3 complement component and properdin, were present in basement membranes of patients with chronic mucocutaneous candidiasis.

Most of our knowledge about opsonins that enhance the phagocytosis of *C. albicans* by macrophages has been generated by studies performed in vitro. In vivo, however, opsonization of *C. albicans* also seems to take place. *C. albicans* organisms placed in chambers that were inserted into the peritoneal cavity of mice were phagocytized by macrophages in vitro (54). Although complement components and antibodies probably contributed to the opsonization of *C. albicans* in vivo, participation of other opsonic components cannot be excluded from the latter study. The observation that vitronectin enhanced the attachment of *C. albicans* to macrophages (139) suggests the existence of serum factors (other than complement and *C. albicans*-specific antibodies) that can enhance the strength of the binding of the fungus to phagocytes.

Mannose binding protein. Mannose binding protein (MBP), a C-type lectin secreted by the liver, is an important component of innate immunity (243) that may participate in the innate response to *C. albicans* infections. In the first hour after mice were inoculated with *C. albicans*, the concentration of MBP in serum dropped, but it increased above normal levels by 72 h after the *C. albicans* challenge (245). Kitz et al. (121) demonstrated that MBP, isolated from rabbit serum, inhibited the phagocytosis of *C. albicans* by murine macrophages and suggested that soluble MBP could interfere with the *Candida*-macrophage interaction. Soluble MBP could also interfere with the adherence of *C. albicans* to host tissue, since tissue adherence also seems to partially rely on mannan-mannose (receptor) interactions. In fact, the adherence of *C. albicans* to buccal epithelial cells can be blocked by mannose (121). The anti-adhesive properties of MBP make this peptide an interesting target for treatment of mucosal and mucocutaneous

candidiasis; however, whether MBP reaches superficial sites infected with *C. albicans* or whether it remains anti-adhesive in the environment normally found in anatomical sites populated by *Candida* are questions that have to be resolved before it is used for prophylaxis or therapy.

The initial decrease of the MBP concentration in serum coincides with a decrease in the concentration of C3 at early times after i.v. *C. albicans* inoculation (245). The decrease in C3 concentration is probably due to the excellent capacity of MBP to activate the classical complement cascade (82, 107). MBP has an associated serine protease subunit that is similar to the serine proteases of the first complement component cascade, C1r-C1s (153, 223). The ability of MBP to activate the complement cascade may promote the release of both chemotaxins and opsonins. Apparent contradictions about the role of MBP in the phagocytosis of *C. albicans* by macrophages (i.e., it has been shown to abrogate phagocytosis by itself but has the capacity to enhance it by catalyzing the generation of opsonins from the complement cascade) illustrate our vague understanding of this lectin in resistance to candidiasis.

Phagocytosis of Nonopsonized *C. albicans*

Because mononuclear phagocytic cells recognize a diverse number of antigens expressed by microorganisms, phagocytosis can occur in the absence of opsonins (183). Similar to other microbes, *C. albicans* can be phagocytized by human macrophages equally well in the presence and absence of fresh human serum (150), indicating that macrophages have several mechanisms of recognizing this dimorphic fungus. Direct recognition of *C. albicans* by macrophages could be important in tissues that are poor in opsonins (e.g., lungs, renal medulla and cerebrospinal fluid) and at the beginning of the microbial infection, when the concentration of specific antibodies is low.

Two nonopsonic mechanisms used by mononuclear phagocytic cells to recognize *C. albicans* have been described: recognition of *Candida* mannan and glucan by mannose and glucan receptors, respectively, on mononuclear phagocytic cells.

Mannose receptors. Mannose receptors on the surface of macrophages participate in the phagocytosis of microbes with a mannan-rich cell surface (183, 238). Mannose receptor-mediated phagocytosis may be especially important for *C. albicans*, since mannan is a main constituent of the outer layer of the yeast cell wall (31, 74). Direct proof that mannose receptors can mediate the phagocytosis of *C. albicans* comes from the observation that COS cells, which do not usually express mannose receptors on their surfaces, phagocytized *C. albicans* when transfected with a mannose receptor cDNA (74). The expression of mannose receptors is considered to be an early surface marker in the differentiation of macrophages from monocytes (117). Because monocytes lack mannose receptors, mannan does not interfere with the uptake of *C. albicans* by human monocytes (102). Conversely, the phagocytosis of *C. albicans* by human and murine macrophages can be blocked by mannan, mannose, β-1,2-oligomannosides or β-1,2-mannotetraose (75, 80, 123, 149, 150). Mannose receptors are also important in mediating phagocytosis of other *Candida* spp. by macrophages (266). Murine alveolar macrophages ingested *C. krusei* in the absence of opsonins, and phagocytosis of *C. krusei* was inhibited by D-mannose (266).

Based on the observation that phagocytosis of *C. albicans* by murine peritoneal exudate macrophages incubated with M-CSF can be inhibited by soluble mannan, Karbassi et al. (116) suggested that mannose receptors on macrophages can be upregulated by M-CSF to enhance the phagocytosis of *C. albicans*. Upregulation of mannose receptor expression on activated macrophages could influence the host resistance to *C. albicans* because the increased phagocytic activity of M-CSF-treated macrophages is paralleled by enhanced candidacidal activity (116). However, M-CSF does not seem to be protective in experimental systemic candidiasis (100).

Mannose receptors are also involved in the increased phagocytosis and candidacidal activity of IFN-γ-activated human macrophages (148). The increased phagocytosis and candidacidal activity induced by IFN-γ are not associated with an upregulation in the expression of mannose receptors (151). It is possible that IFN-γ activates macrophage candidacidal activity by coupling mannose receptors to microbicidal mechanisms, such as the generation of superoxide anion (151).

Soluble mannan can inhibit the phagocytosis of C3-opsonized *C. albicans* by murine macrophages (123). The fact that mannan can inhibit the complement-mediated phagocytosis of *C. albicans* suggests either that stoichiometric or spatial competition between complement (e.g., CR1) and mannan receptors may occur or that a synergy between the two receptors is needed for optimal phagocytosis of *C. albicans.*

Glucan receptors. Although soluble β-1,3-D-glucans are found in the serum of patients with candidiasis more frequently than are mannans (160), and although glucans, in the form of β-1,3-D-glucans and β-1,6-D-glucans, are more abundant than mannans in the cell wall of *C. albicans* (31), little is known about possible interactions of *C. albicans* glucans and host cells. Glucans are located mainly in inner layers of the cell wall of *C. albicans*, where they provide rigidity (31). Nevertheless, glucans are also expressed on the surface of *C. albicans* (258) and may also interact with mononuclear phagocytic cells. Accordingly, zymosan, which is a glucan-enriched preparation from *Saccharomyces cerevisiae* and glucan particles, can be ingested by monocytes (239).

The ingestion of glucan particles by human monocytes suggests the existence of a glucan receptor. The glucan receptor has been cloned from human monocytes, and, similar to the mannose receptor, expression of glucan receptors depends on the differentiation state of the phagocytic cells. Soluble glucan inhibited the ingestion of *C. albicans* by human monocytes in a dose-dependent manner, but glucan did not interfere with the phagocytosis of *C. albicans* by human macrophages (102, 150). It is still unknown whether murine mononuclear phagocytic cells express glucan receptors. The observation that soluble glucan diminished the production of peroxynitrite by macrophages suggests the existence of a glucan-like receptor in murine macrophages (258). If the expression of the glucan receptor is restricted to monocytes, macrophages must recognize *C. albicans* glucans by other means, such as complement receptors (88).

Activation of Macrophages by Phagocytosis of *C. albicans*

In addition to being essential for intracellular killing, phagocytosis may deliver signals that dictate the subsequent regulatory and microbicidal functions of the phagocytes. In neutrophils, the receptors involved in the ingestion of *C. albicans* can influence the outcome of the host cell-yeast interaction. Ingestion of *C. albicans* by neutrophils in the presence of C3 components promoted yeast killing, whereas in the absence of C3 components, phagocytosis was sustained but candidacidal activity was not enhanced (278). Mixed results have been obtained from studies of whether the *C. albicans* coupling of different receptors on mononuclear phagocytic cells affects their candidacidal activity. For example, killing of *C. albicans* and *C. parapsilosis* by murine peritoneal macrophages did not differ in the presence or absence of *C. albicans*-specific anti-

TABLE 2. Activation of mononuclear phagocytic cells by glucan and mannan moieties

Source	Differentiation state of mononuclear phagocytes	Fungal cell wall component	Response of mononuclear phagocytes	Reference(s)
Human	Monocytes	Particulate β-glucan	IL-1 receptor agonist, TNF-α, IL-1[a] Platelet-activating factor[b] Leukotrienes	1, 55, 71, 194, 273
Murine	Peritoneal macrophages	Particulate β-glucan	Superoxide anion, peroxynitrite	114, 258
Murine	Bone marrow-derived macrophages	β-1,3-Glucan	Acid hydrolase, β-glucuronidase, platelet-derived factor	127
Murine	Peritoneal macrophages	β-1,3-Glucan	Hexosaminidase, TNF-α, IL-1	124, 137
Murine	Bone marrow-derived macrophages	β-1,3-Glucan	TGF-β	175
Murine	Alveolar macrophages	β-1,3-Glucan	NO, IL-1, IL-6, TNF-α	218
Murine	Alveolar macrophages	Mannan	TNF-α[c]	87
Rabbit	Alveolar macrophages	Particulate β-glucan	Arachidonic acid	37, 59, 118
Rabbit	Alveolar macrophages	α-Mannan	Arachidonic acid	37
Rat	Alveolar macrophages	β-Glucan	TNF-α	96

[a] In reference 190, IL-1 was not produced by human monocytes in response to β-glucan.
[b] Platelet-activating factor was produced by ligating simultaneously the glucan and complement receptors.
[c] Stimulation of TNF-α production by mannan could be inhibited by either D-mannose, α-methyl-D-mannoside, or concanavalin A.

bodies or complement (114, 221). In contrast, after phagocytosis of preopsonized *C. albicans* or *C. parapsilosis*, human monocytes and monocyte-derived macrophages produced more superoxide anion and killed more yeasts than when the fungi were not preopsonized with serum (147). The killing of opsonized *C. albicans* by human macrophages cannot be explained by increased phagocytic activity, because the macrophages also ingested *C. albicans* in the absence of serum (147). One of the problems in analyzing the contribution of opsonins to the candidacidal activity of macrophages is the difficulty in separating opsonic from nonopsonic processes. The latter problem may be clarified by using *C. albicans* mutants deficient in one or more of their cell surface antigens and/or macrophages that are free of one or more of the receptors used to phagocytize the fungus.

Regardless of whether the receptor that macrophages use to ingest *C. albicans* modifies their candidacidal activity, engaging different receptors on the mononuclear phagocytic cells with ligands that are normally present in the *C. albicans* cell wall results in the activation of the mononuclear phagocytic cells (Table 2). In general, mannans have been reported to trigger TNF-α and arachidonic acid production by mononuclear phagocytic cells (Table 2). Glucans can activate the production of TNF-α, IL-1, several lysosomal enzymes such as acid hydrolase and β-glucuronidase, and the 5-lipooxygenase pathway with the consequent production of leukotrienes (Table 2).

Some investigators have studied the activation of macrophages by components isolated from *C. albicans*. An ethylenediamine extract of the *C. albicans* cell wall increased the respiratory burst and IL-6 production in murine resident peritoneal macrophages (255). The cell wall moieties were not identified in that study; however, IL-6 production could have been triggered by mannans, glucans, and other *C. albicans* cell wall components known to activate several macrophage functions. Castro et al., taking advantage of the capacity of soluble mannans and glucans to interfere with macrophage-*Candida* interactions, reported that these two constituents of the *C. albicans* cell wall stimulated the release of arachidonic acid, IL-1β, IL-6, IL-8, and TNF-α from human macrophages (36).

The response of macrophage to *C. albicans*, or products derived from fungal cell walls, can be complemented by the availability of serum factors. For example, production of the C3 complement component by human monocytes in response to *C. albicans* can be abrogated by granulocyte-macrophage CSF (GM-CSF) (97). Another example of how the presence of serum factors can modify the activation of macrophages in response to *C. albicans* is the observation that only *C. albicans* blastoconidia preincubated with normal serum produced an increase in the concentration of intracellular Ca^{2+} in mononuclear phagocytic cells (147) (presumably due to opsonization with complement components). Increased concentration of intracellular Ca^{2+} is an early event, which precedes the activation of multiple biochemical reactions, including killing of *C. albicans* by mononuclear phagocytic cells (65, 147, 151, 231).

Another interesting point in the activation of macrophages by *C. albicans* is the dimorphism that this fungus can exhibit. Blasi et al. (21) noted that in the absence of serum components, *C. albicans* blastoconidia did not enhance the expression and production of TNF-α in the ANA-1 macrophage cell line. Conversely, nonopsonized *C. albicans* hyphae stimulated the production of TNF-α by ANA-1 macrophages. The heterogeneous secretory responses to the different morphoforms of *C. albicans* also depends on the population of macrophages studied. Murine peritoneal macrophages secreted TNF-α in response to *C. albicans* hyphae but not in response to blastoconidia (22). In contrast, splenic macrophages and bone marrow-derived macrophages did not discriminate between the two morphoforms of *C. albicans* and produced TNF-α in response to either blastoconidia or hyphae (22). Production of TNF-α by macrophages in response to *C. albicans* may have diverse consequences during the course of the disease: whereas TNF-α regulates macrophage microbicidal activity (128, 184), this cytokine decreases the capacity of neutrophils to kill *C. albicans* hyphae (68). The autocrine and paracrine effects of TNF-α on macrophages and other cells that participate in the resolution of candidiasis deserve further investigation, especially in view of the mixed results reported in relation to TNF-α in candidiasis (see below).

How the coupling of *C. albicans* ligands to different receptors on phagocytic cells affects their activation is not well understood, and further study is needed to clarify these important biochemical events. Unraveling the biochemical phenomena that result from the interaction of *C. albicans* and macrophages may lead to rational ways to modify and direct prophylactic and therapeutic interventions in candidiasis.

MACROPHAGE CANDIDACIDAL MECHANISMS

Elimination of *C. albicans* from an infected host requires the cooperation of many immune cells and their products. Because

there is no evidence that antibody and complement can mediate the lysis of *C. albicans*, phagocytes are probably the prime effector cells in resistance to candidiasis. Phagocytosis of *C. albicans* by macrophages must be accompanied by killing; otherwise, macrophages could serve both as a vehicle for dissemination of the infection and as a privileged site where *C. albicans* can avoid other innate and acquired immune mechanisms. If we are going to understand how a host can control infections caused by this dimorphic fungus, it is important to clarify the mechanisms used by phagocytic cells to kill *C. albicans* blastoconidia and hyphae.

Methods

Some of the methods used to determine macrophage candidacidal activity have relied on microscopic examination of Giemsa-stained slides, in which living *Candida* cells stained blue whereas killed fungi remained colorless (ghost *Candida* cells) (72, 132). Other methods measured the incorporation of radioisotopes (e.g., [^{3}H]glucose, [^{3}H]leucine, and [^{3}H]uridine) as an indication of the candidacidal activity of mononuclear phagocytes (reviewed in reference 78). However, most of the studies designed to test the candidacidal activity of mononuclear phagocytes have used the classical microbiological method of plate counting (19, 27, 222, 248, 258). In these studies, the phagocytes are challenged with *C. albicans* at different effector-to-target-cell ratios for various incubation periods. At the end of the challenge, the killing of *C. albicans* is stopped by lysing the phagocytic cells by sonication or with a detergent (e.g., Triton X). Subsequently, the surviving *C. albicans* cells are enumerated after culture on Sabouraud's dextrose agar plates incubated at 37°C. Some of the advantages of quantifying the candidacidal activity of mononuclear phagocytes by the classical microbiological method include reproducibility, cost efficiency, the lack of a need for sophisticated machinery, and the large number of samples that can be processed simultaneously. One should be cautious when comparing data from the latter studies because of the many different methods and assay conditions that have been used in determining macrophage candidacidal activity.

Killing of *C. albicans* Yeasts by Mononuclear Phagocytic Cells

As is true for other microbes, phagocytic cells can apparently kill *C. albicans* via both oxygen-dependent and -independent mechanisms. Indirect proof of the involvement of oxygen-dependent and -independent mechanisms in killing *Candida* blastoconidia stems from observations that monocytes manifest a similar candidacidal activity under anaerobic and aerobic conditions (248). However, not all phagocytic cells are as efficient as monocytes in killing *C. albicans* blastoconidia under such disparate environments. For instance, macrophages and neutrophils have greater candidacidal activity under aerobic than anaerobic conditions (248), which suggests that macrophages and neutrophils kill *C. albicans* by mechanisms that mainly involve a respiratory burst. In fact, inhibitors of superoxide anion and hydrogen peroxide decreased intracellular killing of *C. albicans* yeasts by macrophages and neutrophils incubated in aerobic but not anaerobic atmospheres (248).

Oxygen-dependent candidacidal mechanisms. Monocytes and monocyte-derived macrophages from patients with X-linked chronic granulomatous disease had little capacity to kill *C. albicans* or *C. parapsilosis* yeasts (133, 147). These data suggest that a normal respiratory burst is necessary for the killing of *Candida* spp. by macrophages. More specifically, *C. albicans* blastoconidia have been shown to be susceptible to the following oxygen-dependent killing mechanisms of mononuclear phagocytic cells: superoxide anion, MPO-hydrogen peroxide-halide system, and reactive nitrogen intermediates (RNI).

(i) Superoxide anion-mediated killing. The candidacidal activity of mononuclear phagocytic cells has been associated with the production of superoxide anion, one of the products of reactive oxygen intermediate (ROI) metabolism that is essential for macrophage "oxidative killing" (146). One observation which indicates that different populations of mononuclear phagocytic cells can kill *C. albicans* by means of superoxide anion is the apparent correlation between their killing capacity and their capacity to generate superoxide anions. For example, human alveolar and resident peritoneal macrophages not only had lower candidacidal activity but also produced less superoxide anion than did Ca^{2+} ionophore-activated alveolar macrophages and peritoneal macrophages from individuals undergoing chronic dialysis, respectively (192, 261).

The importance of superoxide anion in killing *C. albicans* has also been suggested by studies with murine Kupffer cells which, when incubated with opsonized or nonopsonized *C. albicans*, did not produce superoxide anion and were unable to kill the yeast (201). Activation of Kupffer cells with IFN-γ in vitro resulted in increased killing of *C. albicans* compared to the killing by nonactivated Kupffer cells; however, activated Kupffer cells had both lower candidacidal activity and respiratory burst than did activated peritoneal macrophages (201). In contrast to the limited utilization of superoxide anion by Kupffer cells, macrophages from different species and with different states of activation appear to employ superoxide anion to kill *C. albicans*. For example, multinucleated giant cells, derived from human blood monocytes cultivated in vitro with IFN-γ and IL-3, exhibited greater candidacidal activity and superoxide anion production than did human monocytes (72). Similarly, murine resident peritoneal macrophages activated with IFN-γ in vitro used superoxide anion to kill *C. albicans* (27, 221, 258).

The production of superoxide anion by macrophages not only varies with their source but also is influenced by the *Candida* spp. with which the macrophages are infected. Studies with scavengers of ROI and luminol-dependent chemiluminescence suggest that nonactivated macrophages killed *C. parapsilosis*, but not *C. albicans*, by means of superoxide anion (27, 221). Similarly, activated murine macrophages not only exhibited a greater capacity to kill *C. parapsilosis* than to kill *C. albicans* but also showed greater production of ROI and consumption of oxygen during the killing of *C. parapsilosis* (221). Nevertheless, the killing of these two *Candida* spp. by human or murine mononuclear phagocytic cells appears to involve primarily superoxide anion (114, 147, 221). The relevance of superoxide anion in mediating candidacidal activity is further supported by other studies which show that monocyte-derived macrophages from patients with metastatic breast cancer and peritoneal macrophages from mice undergoing early postsurgical trauma showed both impaired candidacidal activity and impaired superoxide anion production (157, 200). Neoplasia and surgical trauma are recognized predisposing factors for systemic candidiasis (178).

The above observations emphasize the connection of superoxide anion and macrophage candidacidal activity; however, other observations indicate that superoxide anion cannot account for *C. albicans* killing by macrophages. Even though macrophages can kill *C. albicans* by means of superoxide anion, the low candidacidal activity of resident peritoneal macrophages cannot be explained by a lack of superoxide anion production, since resident peritoneal macrophages incubated

with *C. albicans* in vitro produce superoxide anion (27, 258). An unequivocal role for superoxide anion in the candidacidal activity of macrophages is also brought into question by the observation that concanavalin A, which can stimulate phagocytes to produce superoxide anion (48, 173), did not increase their candidacidal activity (26, 256).

The mechanism(s) by which superoxide anion exerts its toxic effect on *Candida* spp. is not known. One possibility is that superoxide anion oxidizes molecules essential for yeast survival, but superoxide anion is not likely to be candidacidal at the concentration that is produced by mononuclear phagocytes. Alternatively, studies with the scavenger benzoate suggest that superoxide anion may give rise to other oxidative metabolites such as hydroxyl radicals, which may be involved in the candidacidal activity of murine macrophages (221). The metal ion-catalyzed Haber-Weiss reaction is a pathway by which hydroxyl radicals can be formed from superoxide anion and H_2O_2 (equations 1 to 3):

$$O_2^{\cdot -} + Fe^{3+} \rightarrow O_2 + Fe^{2+} \quad (1)$$

$$O_2^{\cdot -} + Fe^{2+} + 2H^+ \rightarrow H_2O_2 + Fe^{3+} \quad (2)$$

$$H_2O_2 + Fe^{2+} \rightarrow {}^{\cdot}OH + OH^- + Fe^{3+} \text{ (Fenton reaction)} \quad (3)$$

However, whether the metal ion-catalyzed Haber-Weiss reaction takes place within the macrophage during killing of *C. albicans* is still an open question. Additionally, studies with mannitol, another hydroxyl radical scavenger, suggest that hydroxyl radicals are not candidacidal (221). Generation of highly candidacidal molecules from superoxide anion is more likely to occur by coupling this molecule to the MPO-hydrogen peroxide-halide system or to nitric oxide (NO).

(ii) Myeloperoxidase-hydrogen peroxide-halide-mediated killing. Hydrogen peroxide is another molecule that has been implicated in the killing of *C. albicans* by macrophages (221). Compared to macrophages from nontreated mice, the enhanced candidacidal activity of resident peritoneal macrophages from mice previously treated i.v. with BCG and intraperitoneally with purified protein derivative was paralleled by increased hydrogen peroxide production (252). However, the importance of hydrogen peroxide in mediating the killing of *C. albicans* by macrophages is not clear. The maximum candidacidal activity of activated macrophages did not correlate with peaks of hydrogen peroxide production (267).

It is possible that the candidacidal effects of hydrogen peroxide are amplified in the presence of iron and oxidizable halide. In a cell-free system, ferrous cation can catalyze the formation of hydroxyl radicals from hydrogen peroxide (equation 3). Studies with scavengers suggest that hydroxyl radicals have limited cytotoxicity for *C. albicans* (221, 258), but they can react with halides to form several oxidizing agents. This alternative mode of action of hydrogen peroxide is supported by the observations made by Yamada et al., who noted that *C. albicans* was killed during halogenation by an iron-hydrogen peroxide-halide system under cell-free conditions (276). Production of hydroxyl radicals by this chemical route has been proposed to be an important candidacidal mechanism in individuals with MPO deficiencies and in cells (e.g., macrophages) that lack MPO (134). The relevance of this iron-catalyzed production of hydroxyl radicals remains to be demonstrated in vivo. One limiting factor in the nonenzymatic production of hydroxyl radicals is iron, the availability of which is highly regulated in activated macrophages (29, 268). The capacity of iron, bound to iron-chelating proteins that catalyze the formation of hydroxyl radicals from hydrogen peroxide and superoxide anion (reviewed in reference 158), to affect the candidacidal activity of mononuclear phagocytes remains to be tested.

Alternatively, hydrogen peroxide can serve as a substrate of MPO for the generation of strong microbicidal oxidants. It is widely accepted that neutrophils utilize metabolites generated by the MPO-hydrogen peroxide-halide system to kill *Candida* spp. However, very little is known about the importance of the MPO-hydrogen peroxide-halide system in the candidacidal capacity of mononuclear phagocytic cells. The capacity of monocytes to synthesize MPO suggests that these cells are also able to kill *Candida* spp. by using metabolites generated in the MPO-hydrogen peroxide-halide system (146). In fact, the decreased killing activity of human monocytes aged in vitro was associated with decreased MPO activity (222). In addition to neutrophils and monocytes, killing of *Candida* spp. by the MPO-hydrogen peroxide-halide system has been noted to occur in murine macrophages. Resident peritoneal macrophages incubated with supernatants from concanavalin A-stimulated splenocytes killed *C. parapsilosis* and *C. albicans* by means of metabolites generated in the MPO-hydrogen peroxide-halide system (27). Since macrophages are deficient in MPO, these data suggest that the MPO-hydrogen peroxide-halide-mediated macrophage candidacidal activity may have been due to assimilation of MPO present in the concanavalin A supernatants. In support of this statement, it has been demonstrated that MPO can be taken up by macrophages through their mannose receptors (232). MPO incorporated into macrophages by this route has been shown to locate in lysosomal granules, to be enzymatically active, and to kill *C. albicans* (129, 232). The extent of the utilization of an MPO system incorporated into macrophages through the mannose receptor in vivo is unknown, but it may be of interest since both macrophages and neutrophils are present in *C. albicans*-infected tissues. In addition to incorporation of MPO released by neutrophils and monocytes for macrophage candidacidal activity, removal of MPO by macrophages may serve to reduce the oxidative tissue damage produced by free MPO.

Variations in the virulence of different *Candida* spp. may be explained by their differing susceptibility to various effector molecules generated by phagocytic cells. An interesting approach to the investigation of the participation of different effector molecules in the killing of *Candida* spp. is the use of cells from hosts with specific immunodeficiencies. Using this approach, Lehrer noted that neutrophils and monocytes from individuals deficient in MPO killed *C. parapsilosis* (130) but were unable to kill *C. albicans* and took longer to kill several bacterial species than did normal controls (130, 131).

Marodi et al. reported that *C. albicans* was more resistant than other, less pathogenic *Candida* spp. to halogenation by the MPO-hydrogen peroxide-halide system (146), which could account for the increased incidence of *C. albicans* in clinical candidiasis. Similarly, *C. tropicalis*, considered to be less pathogenic than *C. albicans* (101, 178, 239), was more resistant to killing by metabolites of the MPO-hydrogen peroxide-halide system than were other *Candida* spp. (146). Increased resistance of *C. tropicalis* to metabolites generated by the MPO system could also contribute to the high rate of *C. tropicalis* infections in certain patients. Resistance to oxidation is a well-recognized virulence factor in some other pathogens (177), but very little is known about this in *Candida* spp.

(iii) Reactive nitrogen intermediate-mediated killing. NO is synthesized by several NO synthases (NOS), enzymes that use arginine, molecular oxygen, and NADPH as substrates (171). RNI (e.g., NO, nitrogen dioxide, and nitrite) have been shown to participate in the killing of *Cryptococcus neoformans*, *Leish-*

mania major, *Schistosoma mansoni*, *Toxoplasma gondii*, *Mycobacterium leprae*, and *M. tuberculosis* by macrophages (41, 172). *C. albicans* blastoconidia are also susceptible to killing by NO-producing murine macrophages (38, 256, 259). The participation of NO in killing of *C. albicans* by macrophages is illustrated by the observation that the poor candidacidal activity of thioglycolate-elicited peritoneal macrophages from immunodeficient beige mice was associated with defective NO-mediated candidacidal activity (256); however, NO does not appear to be directly candidacidal (257). Although killing of *C. albicans* was impaired in macrophage cell lines and macrophages from immunodeficient beige mice, these cell lines both synthesized as much NO as did macrophages from immunocompetent mice that killed *C. albicans* (257). Evidence to support the notion that NO is not candidacidal was provided by the observation that a NO donor failed to kill *C. albicans* in vitro (257). Nevertheless, NO, most probably in the form of nitrosonium (NO^+), was candidastatic in vitro (257).

The poor candidacidal activity of some NO-producing macrophages could be explained if peroxynitrite production from NO and superoxide anion is taken into consideration. Peroxynitrite is formed by a dilution-limited reaction of NO and superoxide anion (equation 4) (the chemistry of peroxynitrite has been recently reviewed [195]):

$$NO^{\cdot} + O_2^{\cdot -} \rightarrow ONOO^- \quad (4)$$

In contrast to poorly candidacidal NO-producing macrophages, highly candidacidal NO-producing macrophages produced NO and superoxide anion in response to *C. albicans* (258). In addition, poorly candidacidal macrophages produced less peroxynitrite than did macrophages exhibiting enhanced candidacidal activity (258). The fact that the candidacidal activity of activated macrophages can be inhibited by scavengers of either NO or superoxide anion also suggests that peroxynitrite, which is formed from NO and superoxide anion, contributes to the candidacidal activity of activated murine macrophages.

Specificity is one of the problems that arises when radical scavengers and enzymatic inhibitors are used. Arginine is used by different NOS in the production of NO, as well as by ornithine decarboxylase in the production of polyamines, multifunctional metabolites that have been associated with microbicidal activity. Thus, it is possible that arginine analogs block the synthesis of both NO and polyamines. In an attempt to circumvent the problems in specificity that arise with the use of enzymatic inhibitors, we studied macrophages from inducible NOS knockout ($iNOS^{-/-}$) mice and observed that IFN-γ–lipopolysaccharide-activated macrophages from $iNOS^{-/-}$ mice, in contrast to macrophages from immunocompetent mice, produced no nitrite and only very small amounts of peroxynitrite and were unable to kill *C. albicans* blastoconidia (Fig. 1). Curiously, both macrophages from immunocompetent and $iNOS^{-/-}$ mice produced similar concentrations of superoxide anion when challenged with *C. albicans*. This supports the hypothesis that superoxide anion, at the concentrations produced by the macrophages, is not sufficient in and of itself to kill *C. albicans* but is necessary when coupled with NO to generate the strong candidacidal molecule peroxynitrite.

Additional proof that peroxynitrite is a molecule that is used by candidacidal macrophages comes from the observation that peroxynitrite is more potent than superoxide anion and NO at killing *C. albicans* in vitro (258). Under acidic conditions, peroxynitrite is protonated to peroxynitrous acid, which is very unstable and has a hydroxyl character (equation 5):

$$ONOO^- \rightarrow ONOOH \rightarrow NO_2^{\cdot} + {}^{\cdot}OH \quad (5)$$

The fact that hydroxyl radical scavengers did not decrease the candidacidal activity of a peroxynitrite donor suggests that the candidacidal activity of peroxynitrite does not depend on the generation of hydroxyl radicals (258). Thus, peroxynitrite synthesis from NO and superoxide anion may explain why NO- and superoxide anion-producing macrophages exhibit high candidacidal activity.

Oxygen-independent candidacidal mechanisms. Research efforts aimed at understanding candidacidal mechanisms of macrophages have focused mainly on the toxic effect of ROI generated by phagocytes during the respiratory burst; however, there is evidence that oxygen-independent mechanisms are also operative in the killing of *C. albicans*. First, although Kupffer cells have lower candidacidal activity in vitro than do other tissue macrophages (e.g., peritoneal macrophages), it is worth noting that the liver is one of the organs that manifest resistance to candidiasis. The liver can trap and kill *C. albicans* that disseminates from the alimentary tract (224, 226), and Kupffer cells residing within the hepatic sinusoidal lumen can ingest and kill *C. albicans* independently of ROI synthesis (60, 61, 227, 228). A role for ROI-independent mechanisms in macrophage killing is also suggested by the fact that Kupffer cells incubated with IFN-γ did not show an increased respiratory burst in response to *C. albicans* but were candidacidal (61, 201). The inability of Kupffer cells to produce ROI in vitro in response to *C. albicans* and the relative resistance of the liver to systemic candidiasis suggest that mechanisms other than ROI are important in the capacity of macrophages to control or eliminate *C. albicans*. Second, *C. albicans* is a less frequent pathogen in chronic granulomatous disease patients than are other microbes, such as *Aspergillus fumigatus* spores, *Staphylococcus aureus*, *Serratia marcescens*, and *Nocardia asteroides* (108, 164, 182). Because of mutations in the subunits that make NADPH oxidase, macrophages from chronic granulomatous disease patients are defective in generating ROI (52). Therefore, killing of *C. albicans* by macrophages that normally produce small amounts of ROI and by macrophages from chronic granulomatous disease patients suggests that macrophage oxygen-independent mechanisms must also be important in killing this common opportunistic fungus.

Oxygen-independent mechanisms have been reported to participate in the antimicrobial activity of macrophages against *Paracoccidioides brasilensis*, *Cryptococcus neoformans*, *T. gondii*, *Chlamydia psittaci*, BCG, *L. donovani*, and *S. mansoni* (24, 79, 165, 167, 168, 215, 230). The contribution of oxygen-independent mechanisms to the killing of microbes in general and *C. albicans* in particular has not been extensively studied. However, there is some evidence to indicate that lysosomal enzymes from macrophages may be important in the killing of *Candida* spp. Lysosomal extracts from rabbit alveolar macrophages reduced both the ability of *C. albicans* to incorporate methionine, valine, lysine, phenylalanine, and leucine and the viability of the fungus (191). Although the exact mechanism by which lysosomal extracts inhibited the uptake of these amino acids is not known, inhibition of a general amino acid permease in *C. albicans* could have occurred (191). Characterization of the lysosomal extracts from rabbit alveolar macrophages has shown that highly cationic proteins other than MPO had antimicrobial activity against *C. albicans*, *C. parapsilosis*, and several gram-positive bacteria in vitro (187). The microbicidal activity of these highly cationic proteins has been associated with two defensins, designated MCP-1 and MCP-2 (188). The candidacidal activity of MCP-1 and MCP-2 seems to be associated with damage to the cytoplasmic membrane of *C. albi-*

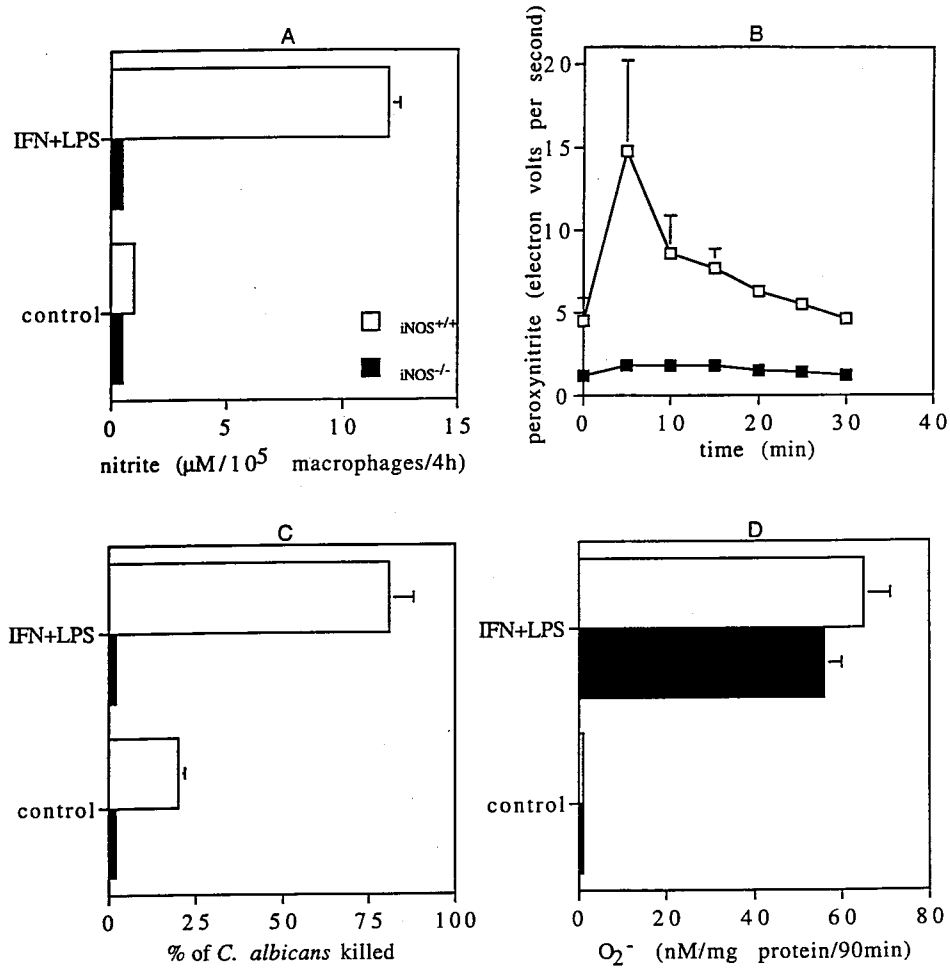

FIG. 1. Peroxynitrite production by macrophages from iNOS$^{-/-}$ mice. (A to C) IFN-γ-plus-lipopolysaccharide (LPS)-activated macrophages from iNOS$^{-/-}$ mice produced less nitrite (A) and peroxynitrite (B) and killed fewer *C. albicans* blastoconidia (C) than did control macrophages from normal controls (iNOS$^{+/+}$). (D) Macrophages from iNOS$^{-/-}$ and iNOS$^{+/+}$ mice produced similar amounts of superoxide anion when stimulated with IFN-γ, LPS, and *C. albicans*. The macrophages were incubated with 200 U of IFN-γ per ml for 18 h, 1 μg of LPS per ml for 2 h, and *C. albicans* for 2 h as previously described (258).

cans and suppression of oxygen consumption by the fungus (188). The high in vitro candidacidal activity (99.6% of *C. albicans* organisms were killed) of these cationic proteins indicates that they may be an effective mechanism for controlling candidiasis; however, they have been shown to be expressed only by rabbit alveolar macrophages (187, 188). Other cationic proteases cytotoxic for *C. albicans* that have not yet been as well characterized have been demonstrated to occur in human alveolar macrophages (261).

In addition to MCP-1 and MCP-2, other lysosomal proteins may participate in the killing of *Candida* by an oxygen-independent pathway. The increased candidacidal activity of murine macrophages incubated with IFN-γ in vitro was related to a noncationic heat-stable proteinaceous substance(s), active only at acidic pH (267). Although nonactivated and activated murine resident peritoneal macrophages contained this protein and exhibited similar percentages of phagolysosome fusion, only activated macrophages were candidacidal, and killing of *C. albicans* was correlated with acidification of the phagolysosome (267).

The contribution of other macrophage microbicidal mechanisms that are independent of oxygen metabolism (e.g., lysozyme, depletion of tryptophan, and deprivation of iron by downregulation of transferring receptors in the surface of the phagocytes) has not been studied in relation to *C. albicans*. However, it is likely that these microbicidal and microbiostatic mechanisms also contribute to the candidacidal and candidastatic capacity of mononuclear phagocytes.

Together with oxidative and nonoxidative mechanisms, macrophages can concentrate and use antimycotic drugs for killing *C. albicans* blastoconidia. Monocytes can accumulate amphotericin B, which is fungicidal by itself, to levels that are candidacidal (152), and they also can concentrate and synergize with fluconazole, which is fungistatic, to damage *C. albicans* (84, 272).

Killing of *C. albicans* Hyphae by Mononuclear Phagocytic Cells

As noted at the beginning of this review, the capacity of *C. albicans* to germinate and produce hyphal elements is considered to be one of its most important virulence factors. Several characteristics make *C. albicans* hyphae especially pathogenic. First, because of the size of the hyphae, phagocytes are unable to ingest them and so must rely on extracellular mechanisms to eliminate them from infected tissues. Second, multiple points

of blastoconidium production are possible from a single filament, thus increasing the number of infective elements. Third, *C. albicans* hyphae are more resistant to macrophage killing than are *C. albicans* blastoconidia (54). Therefore, elimination of *C. albicans* hyphae from an infected tissue is essential for the resolution of the disease. Neutrophils have been recognized as effectors in killing *C. albicans* hyphae (66, 68, 109); however, there is some evidence to indicate that human and murine mononuclear phagocytic cells are also capable of killing *C. albicans* hyphae.

Human monocytes seem to rely on oxygen-dependent mechanisms to kill *C. albicans* hyphae. The latter hypothesis is supported by the observation that inhibitors of the MPO-hydrogen peroxide-halide system, scavengers of hydrogen peroxide, and putative antagonists of hypochlorous acid or singlet oxygen decreased the capacity of human monocytes to damage *C. albicans* hyphae in vitro (67). In contrast to the utilization of ROI, the involvement of oxygen-independent mechanisms to kill *C. albicans* by normal human monocytes is not clear, since chelation of iron and neutralization of cationic proteins did not decrease the killing of *C. albicans* hyphae by human monocytes (67). However, studies with human monocytes from patients with chronic granulomatous disease suggest that killing of *C. albicans* hyphae by mononuclear phagocytic cells can be mediated by oxygen-independent mechanisms. Monocytes from patients with disorders in the production of reactive oxygen radicals (e.g., patients with chronic granulomatous disease or MPO deficiencies) could kill *C. albicans* hyphae (67).

Murine macrophages have also been reported to kill *C. albicans* hyphae. Killing of *C. albicans* hyphae by murine macrophages required close contact between the host cells and fungus and could not be abrogated by oxygen radical scavengers (93). The latter observations suggest that murine macrophages killed *C. albicans* hyphae by an oxygen-independent mechanism(s); however, the identity of a particular oxygen-independent mechanism able to participate in the killing of *C. albicans* hyphae by murine macrophages has not yet been discovered. The possibility that oxidative mechanisms are also involved in killing of *C. albicans* hyphae by murine macrophages cannot be excluded.

Although not studied in detail, it is possible that oxidative molecules can modify constitutively expressed lysosomal proteins of unstimulated macrophages. For example, at an acidic pH, NO is oxidized to NO^+, which is a potent nitrosating agent (162). NO is a very labile molecule that can be oxidized very quickly in the presence of oxygen. Therefore, a NO-protein aduct may serve as a store of NO within the phagolysosome and may endow constitutively expressed proteins with the capacity to kill *C. albicans*. Nitrosation of proteins by RNI has also been shown to be fungicidal for *C. albicans* hyphae (20). The growth of *Candida* hyphae, but not yeasts, was inhibited by extracellular mechanisms of macrophages that probably involved the production of a stable nitrogen-containing compound (20). An interaction between oxidative and nonoxidative mechanisms has also been described with extracts from neutrophils in vitro, where respiratory burst activity synergized with sublethal concentrations of purified neutrophil granule extracts to kill *C. albicans* hyphae (241).

Because of the difficulty in isolating fixed tissue macrophages from normal humans and peripheral blood monocytes from mice, it is hard to make a direct comparison of the capacity of a particular mononuclear phagocytic cell from these hosts to kill *C. albicans* hyphae. Thus, preferential utilization of oxygen-dependent and -independent mechanisms by mononuclear phagocytic cells to kill *C. albicans* hyphae (67, 93) could be due to the host that serves as donor of the mononuclear phagocytes (i.e., human versus murine) and/or the state of differentiation (monocytes versus macrophages) of the mononuclear phagocytic cells used in the studies. The activation state of mononuclear phagocytic cells is another factor that can affect their capacity to kill *C. albicans* hyphae. For example, cell culture medium-elicited peritoneal macrophages from *Listeria*-immune mice killed fewer *C. albicans* hyphae than did macrophages elicited by heat-killed *Listeria* (93).

Integrated View of Macrophage Candidacidal Mechanisms

As presented above, macrophages use an extensive array of oxidative and nonoxidative mechanisms to kill *C. albicans* blastoconidia and hyphae (Fig. 2). Most studies that address the mechanisms of macrophage candidacidal activity have explored the contribution of a single, independent factor. Macrophages produce a diverse arsenal of toxic components; synergistic interactions between single toxic molecules (e.g., NO and hydrogen peroxide) in killing *C. albicans* may also occur, as has been noted by others studying the killing of *Escherichia coli* in a cell-free system (185). Also, NO can enhance or stabilize the superoxide anion-mediated killing of *Staphylococcus aureus* by the hypoxanthine-xanthine oxidase system over prolonged periods of incubation (115). Independent of the possible stabilization of an oxidase by NO, reaction of NO and superoxide anion can generate a new fungicidal species, i.e., peroxynitrite, which is a more potent candidacidal molecule than either of its precursors alone (253).

Utilization of one or more of these candidacidal molecules by mononuclear phagocytic cells probably depends on at least some of the following factors: (i) the source of the cells (e.g., macrophages from rats and mice utilize NO during their microbicidal activity, whereas it is still controversial whether human macrophages, although able to synthesize large amounts of NO, use it preferentially to kill microbes [63; reviewed in reference 64]); (ii) the anatomical origin of the phagocytes (e.g., Kupffer cells do not generate as much superoxide anion as do peritoneal cells [reviewed in this article]); (iii) the state of activation of the macrophages (IFN-γ-activated but not unstimulated murine macrophages produce peroxynitrite in response to *C. albicans* challenge [reviewed herein]); and (iv) the morphoform of *C. albicans* studied (in contrast to blastoconidia, *C. albicans* hyphae are susceptible to a proteinaceous nitroso compound [20]). All of these factors deserve further consideration when the candidacidal activity of mononuclear phagocytic cells is studied.

Many of the metabolites produced by candidacidal macrophages (e.g., hydrogen peroxide, hydroxyl radical, NO, and peroxynitrite) are membrane soluble and can react with a number of molecular targets (reviewed in reference 158). Thus, in addition to killing *C. albicans*, these toxic metabolites have the capacity to damage key molecules of the host. To date, no information is available on the effect of free radicals, produced by the macrophages in response to *C. albicans*, on the host. Because *C. albicans* is such an efficient stimulator of oxidative metabolism, the potential deleterious effects of free radicals (synthesized in the course of an ongoing *C. albicans* infection) on the host and the ways that the host deals with these pernicious metabolites will be fruitful areas of research on this increasingly common opportunistic pathogen.

ACTIVATION OF ANTI-*CANDIDA* MACROPHAGE FUNCTION(S)

In contrast to neutrophils, the activation of macrophages is critical for stimulation of candidacidal activity. Therefore, un-

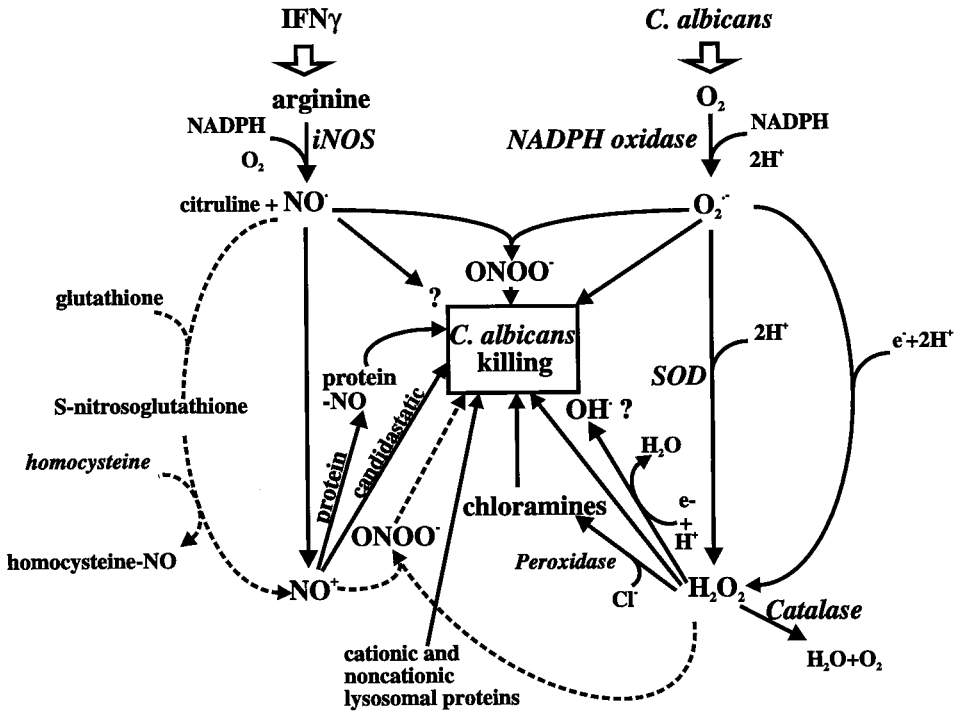

FIG. 2. Candidacidal mechanisms of mononuclear phagocytic cells. Mononuclear phagocytic cells generate multiple ROI that are candidacidal. Activated macrophages also generate RNI, which by themselves are candidastatic; however, when they are coupled with ROI, a strong candidacidal molecule, peroxynitrite, is formed. Mononuclear phagocytic cells constitutively express candidacidal molecules that are activated in response to low pH, ROI, and RNI. Enzymes and radical scavengers (62) are italicized. Solid line, reactions demonstrated empirically; broken line, hypothetical interactions. SOD, superoxide dismutase.

derstanding the mechanisms of macrophage activation not only is important to optimize candidacidal activity but also may identify possible therapeutic agents that can be used to activate macrophages, thereby maximizing treatment of clinical candidiasis.

Of all the cytokines that are known to activate macrophage functions, IFN-γ in particular has been studied the most in regard to activation of macrophage candidacidal activity. IFN-γ does not enhance the capacity of murine macrophages to kill poorly pathogenic *Candida* spp., such as *C. parapsilosis*, or other fungi, such as *Saccharomyces cerevisiae* (27, 258). The inability of IFN-γ to increase the capacity of macrophages to kill *C. parapsilosis* and *S. cerevisiae* may be because these two fungi stimulate the synthesis of large amounts of superoxide anion and are very susceptible to products of the oxidative burst (27, 222, 258). In contrast, with a few exceptions (149, 265), IFN-γ is considered to be an excellent stimulator of the capacity of macrophages to kill *C. albicans* (Table 3). IFN-γ-activated mononuclear phagocytic cells from different hosts, anatomical sites, and differentiation states show that enhanced killing of *C. albicans* coincides with the activation of multiple oxygen-dependent (ROI and RNI) and -independent mechanisms.

In addition, IFN-γ restores candidacidal activity to human alveolar macrophages that are aged in vitro (263) and induces morphological changes in the monocytes (72). Human blood monocytes incubated in vitro with IFN-γ and IL-3 matured into multinucleated giant cells that exhibited a greater candidacidal activity than did nonstimulated monocytes (72). The morphological changes induced in monocytes by IFN-γ may be relevant to the resistance to candidiasis, since multinucleated giant cells and granuloma formation are observed in hepatic, renal, and mucocutaneous candidiasis (156, 228; reviewed in reference 205); however, the role of activated multinucleated giant cells in the resistance to *Candida* has not been clarified. In cryptococcosis, the induction of multinucleated giant cells

TABLE 3. Cytokines that increase the candidacidal activity of mononuclear phagocytic cells

Cytokine	Host source of mononuclear phagocytes	Differentiation state of mononuclear phagocytes	Response of mononuclear phagocytes	Reference(s)
IFN-γ	Human	Alveolar macrophages	O_2^-	263
	Human	Monocyte-derived macrophages	MPO-H_2O_2-halide, O_2^-	149, 151
	Murine	Resident peritoneal macrophages	NO, $ONOO^-$, O_2^-	25, 27, 258
GM-CSF	Human	Monocytes	O_2^-	30, 234, 265
M-CSF	Murine	Exudate peritoneal macrophages	ND[a]	116
IL-1	Human	Alveolar macrophages	ND	263
	Human	Monocytes	ND	263
	Murine	GG2EE macrophage cell line	ND	19

[a] ND, not determined.

by $CD4^+$ T cells correlates with confinement of *C. neoformans* to the lungs (95). Similarly, the formation of multinucleated giant cells in vivo could be important in limiting *C. albicans* infection.

Besides IFN-γ, other cytokines can activate macrophages to an anticandidal state. GM-CSF increased the candidacidal activity of human monocytes and maintained the candidacidal activity of long-term-cultured macrophages in vitro (234, 265). The importance of cytokines other than IFN-γ, such as GM-CSF, in resistance to candidiasis is suggested by the observation that the GM-CSF, but not IFN-γ, induced the candidacidal activity of human monocytes that were aged in vitro and enhanced superoxide anion production in human monocytes (265).

Although less efficient than GM-CSF (265), M-CSF has been shown to improve the candidacidal activity of human monocytes and to enhance the candidacidal activity of murine exudate, but not murine resident peritoneal, macrophages (116). The enhanced candidacidal activity of exudate macrophages treated with M-CSF was not mediated by IFN-α (which is known to increase with M-CSF treatment) and did not involve increased superoxide anion production but, instead, coincided with a greater capacity to phagocytize *C. albicans* (116, 120). The capacity of M-CSF to enhance the candidacidal activity of mononuclear phagocytic cells in vitro has not been verified with enhanced resistance in vivo: M-CSF, administered before or after infection, enhanced the susceptibility of mice to *C. albicans* (100). Several reasons may explain why M-CSF enhances the susceptibility of mice to *C. albicans*. First, M-CSF could enhance the susceptibility of mice to candidiasis by increasing macrophage TNF-α production to levels that lead to an immunopathological reaction similar to toxic shock (100). Second, M-CSF could upregulate the expression of transforming growth factor β (TGF-β) receptors during monocytopoiesis (reviewed in reference 216); thus, macrophages could be downregulated in their effector functions (e.g., production of ROI and RNI) by TGF-β present in infected tissue.

IL-1 can also enhance the candidacidal activity of human and murine mononuclear phagocytic cells in vitro (19, 263). Of interest, GM-CSF, M-CSF, and IL-1 are monokines that can be produced in the absence of T-cell-mediated immunity, may play a role in the early resistance to infection with *C. albicans*, and are potential targets for immunotherapy in different presentations of the disease.

The effect of several other cytokines on the activation of macrophages to kill *C. albicans* has also been examined. For example, IL-4 had neither a positive nor a negative effect on the candidacidal activity of a macrophage cell line (19), but it inhibited the candidacidal activity and NO-producing capacity of IFN-γ-activated macrophages. IL-3 has been shown to either increase (265) or have no effect on (19) the candidacidal activity of human monocytes.

Granulocytopenic patients with disseminated candidiasis are frequently treated with amphotericin B. Long-term treatment with amphotericin B can have deleterious effects on the patient due to its gastrointestinal, renal, and bone marrow toxicity. Thus, treatment of candidiasis with amphotericin B might result in an increased risk of exacerbating the disease. Another factor that argues against the indiscriminate use of amphotericin B is the development of resistance to amphotericin B by *Candida* spp. (reviewed in reference 220). In cases where amphotericin B might be contraindicated, the use of an alternative anti-*Candida* therapy, such as activation of phagocyte candidacidal activity by cytokines, may improve the therapy of candidiasis. However, because so little information is available on the efficacy and safety of such therapy in animal models of candidiasis and in patients, we are still not able to treat candidiasis with selected cytokines that can enhance the candidacidal activity of macrophages in vivo.

MACROPHAGES AND RESISTANCE TO CANDIDIASIS

Several studies have demonstrated the importance of activated macrophages in controlling systemic and mucosal *C. albicans* infections.

Systemic Candidiasis

Macrophages play an important role in resistance to systemic candidiasis. Han et al. have shown that macrophages from the marginal zone of the spleen and macrophages from subcapsular and medullary sinuses of lymph nodes trap *C. albicans* yeast cells injected into mice (92). β-1,2-linked mannotetraose, present in the cell wall mannoproteins, mediates the binding of *C. albicans* to macrophages in the marginal zone of the spleen (138). More direct evidence for the role of macrophages in the resistance to systemic candidiasis comes from the observation that the selective elimination of macrophages with liposomes containing dichloromethylene diphosphonate results in a striking reduction of *C. albicans* binding to the spleen (197). In addition, euthymic and athymic mice that were depleted of macrophages with dichloromethylene diphosphonate removed *C. albicans* more slowly from the blood and caused the mice to die sooner than did the controls (197). In agreement with the latter observations of Qiar et al. (197), depletion of macrophages with silica enhanced the susceptibility of SCID mice to acute systemic candidiasis (104).

T-cell-independent mechanisms. In vivo studies suggest that resistance to systemic candidiasis can be independent of T-cell-mediated immunity (45, 53, 54, 189) and, furthermore, that enhancement of T-cell-mediated immunity may increase the susceptibility of mice to systemic candidiasis (206). Athymic mice, deficient in thymus-educated T cells, are more resistant to systemic candidiasis than are euthymic mice (53, 206). Coincident with the increased resistance of athymic mice to i.v. *C. albicans* challenge, macrophages from athymic mice were candidacidal whereas those from euthymic mice were candidastatic (54). The negative effect of either T cells or cytokines (e.g., IFN-γ) synthesized by T cells on the resistance of mice to systemic candidiasis is also indicated by the increased susceptibility to systemic candidiasis of athymic mice with a grafted thymus (206). In agreement with the suggestion that IFN-γ synthesized by T cells can impair resistance to systemic candidiasis, "*Candida*-naive" mice treated with IFN-γ were found to be more susceptible to systemic candidiasis than were untreated controls (86). Also, depletion of $CD4^+$ T cells was associated with resistance to an acute (i.v.) *C. albicans* challenge, which would otherwise have been lethal (45).

Several explications have been proposed to explain the susceptibility of euthymic mice to experimental murine candidiasis produced by i.v. inoculation. Suppressor T cells have been found in *Candida*-infected mice (50, 51, 85). According to this hypothesis, it should follow that elimination of $CD4^+$ suppressor T cells should enhance the activation of macrophages and increase the efficiency of killing *C. albicans*; however, the enhanced resistance of $CD4^+$-depleted mice was not paralleled by an obvious diminution of the *C. albicans* burden in the liver and kidneys (45). An alternative explanation for the increased susceptibility of euthymic mice to acute systemic candidiasis could be that IFN-γ produced by $CD4^+$ T cells induces an immunopathological reaction by, for example, overstimulating macrophages to produce TNF-α. It has been known for a long

time that *C. albicans* produces an endotoxin-like product (reviewed in reference 205). More recently, *C. albicans* and *C. albicans* mannoproteins have been noted to directly stimulate the synthesis of TNF-α by murine and human macrophages (28, 69, 87, 262). These *C. albicans* mannoproteins have been characterized as phospholipomannan and β-1,2-linked oligomannosides (112, 113).

Overproduction of TNF-α by macrophages could explain some of the clinical signs associated with systemic candidiasis in mice (e.g., ruffled fur and lethargy) (45). The involvement of TNF-α, produced by mononuclear phagocytic cells, in enhancing the susceptibility of mice inoculated i.v. with *C. albicans* is supported by several observations. Mice challenged i.v. with *C. albicans* exhibited increased TNF-α levels in serum (203). In addition, macrophages from *C. albicans*-immune mice exhibited enhanced TNF production (262), consistent with the observation that activated macrophages produce more TNF-α than do nonactivated macrophages (246). In fact, IFN-γ enhanced the production of TNF-α by macrophages challenged with *C. albicans* (262).

The detrimental effects of TNF-α in acute systemic candidiasis might be related to the transient expression of this cytokine during the infection. *C. albicans* induces IL-6, IL-1, and TNF-α production by macrophages in cerebral candidiasis (18). The latter three cytokines persisted at high levels during a lethal course of cerebral candidiasis, whereas IL-6 and TNF-α were downregulated in surviving mice that were treated with picolinic acid (18). Thus, TNF-α expression at the beginning of systemic *C. albicans* infections might be protective, whereas it might be detrimental if it is maintained throughout the course of the infection, a situation that may arise with overstimulation of macrophages by T cells or some T-cell-produced factors (e.g., IFN-γ). Allendoerfer et al. have presented evidence in favor of the concept that the expression of TNF-α is protective in systemic candidiasis induced by i.v. inoculation (3). Mice that were challenged systemically with *C. albicans* showed increased levels of TNF-α in serum. Treatment of these mice with neutralizing antibodies to TNF-α did not change the course of the infection; however, mice pretreated with TNF-α were more resistant to a subsequent lethal i.v. *C. albicans* inoculation than were untreated control mice (3). It is not known how pretreatment with TNF-α enhanced the resistance of mice to systemic candidiasis, but it is possible that it induced NOS expression (170) independently of T cells. Macrophage NO production has been associated with murine resistance to systemic candidiasis (38).

The participation of activated mononuclear phagocytes in the protection against systemic candidiasis is further suggested by the observation that IL-1α and IL-1β protected mice from an i.v. inoculation with *C. albicans* blastoconidia (159, 192, 254). The effect of IL-1 seemed to be due to activation of macrophages, since IL-1 enhanced the resistance of neutropenic mice (treated with cyclophosphamide or hydrocortisone) to experimental candidiasis (produced by i.v. inoculation) in comparison to non-IL-1-treated control mice (125, 190, 254). The resistance of cyclophosphamide- and IL-1-treated mice to *C. albicans* challenge was correlated with the appearance of a highly candidacidal population of splenocytes, which were described as being of a macrophage-monocyte lineage (9). However, no correlation was seen between the resistance of IL-1-treated neutropenic mice and the number of monocytes in blood or granulocytes in the kidneys (125). Although the number of monocytes in peripheral blood did not increase, it is also possible that IL-1 enhanced the resistance to candidiasis by activating fixed tissue macrophages.

The involvement of a T-cell-independent pathway in the activation of phagocytic cells in the resistance to *C. albicans* is underscored by studies of candidiasis in SCID mice. IFN-γ produced by natural killer (NK) cells activates macrophages and mediates protection against *Listeria monocytogenes* systemic infection (14, 270; reviewed in reference 13). The importance of NK cells or IFN-γ produced by NK cells in resistance to systemic candidiasis is not yet clear. Although NK cells from *C. albicans*-infected mice synthesized IFN-γ (210) (which can activate macrophages), depletion of NK cells did not alter the resistance of SCID mice to systemic candidiasis (90, 103). Because SCID mice respond to *C. albicans* by producing large numbers of neutrophils and macrophages, NK-cell function might not be critical in the resistance to acute systemic candidiasis in this murine model. Other studies with immunocompetent hosts have not shown a role for NK cells in the resistance to acute systemic candidiasis, despite their capacity to produce IFN-γ (210).

In agreement with the notion that T cells do not play a role in resistance to acute systemic candidiasis induced by i.v. inoculation, mice inoculated i.v. with *C. albicans* showed no dramatic fluctuations in either the αβ or the γδ T-cell responses (110). Overall, a number of studies strongly suggest that innate immunity, possibly mediated by neutrophils and macrophages, is critical for the clearance of acute systemic candidiasis. The contribution of macrophages to resistance to systemic candidiasis, independent of T cells, may be decisive in nonimmune hosts, in individuals with negative delayed-type hypersensitivity (DTH) responses to *C. albicans* antigens, and in heavily immunosuppressed hosts. It will be of great interest to dissect the innate mechanisms that operate in the resistance to systemic candidiasis so that they can be used to prevent acute systemic candidiasis and to control candidiasis in people with congenital, disease-induced, or iatrogenic defects in T-cell-mediated immunity.

T-cell-dependent mechanisms. In systemic fungal infections (e.g., histoplasmosis, blastomycosis, coccidioidomycosis, and cryptococcosis), activation of macrophages appears to be necessary for the full expression of resistance to the disease (42, 99, 140, 207). Macrophage activation in systemic fungal diseases appears to coincide with the development of CMI, wherein cytokines are produced by antigen-specific T cells that activate macrophages to limit or eradicate the fungal infection.

Macrophages and CMI participate in the immunity to systemic candidiasis in immune hosts (6). Mice infected i.v. with a low-virulence strain of *C. albicans* developed DTH and were resistant to a subsequent challenge with a high-virulence strain (39). The resistance of these mice to a systemic infection with *C. albicans* was associated with activated splenic macrophages that showed increased candidacidal activity in vitro (39). Furthermore, the resistance of low-virulence-*C. albicans*-infected mice could be adoptively transferred to naive mice via $CD4^+$ cells from the immunized mice (17, 39). The $CD4^+$ cells had a phenotype characteristic of T helper type 1 cells (Th_1); i.e., they produced IL-2, lymphotoxin, and IFN-γ (39). IFN-γ synthesized by immune $CD4^+$ cells serves as an activation signal for increasing the candidacidal capacity of effector cells such as macrophages (Table 3).

Evidence in favor of IFN-γ and macrophages in protecting against otherwise lethal i.v. *C. albicans* challenge has been demonstrated by abrogating the resistance of mice to candidiasis (adoptively transferred with $CD4^+$ cells) with either anti-IFN-γ or silica (39). Additional proof in favor of a protective role for Th_1 immune responses in systemic candidiasis comes from studies performed in mice immunized by maintaining a chronic infection of the kidneys with an attenuated strain of *C. albicans* (i.e., PCA) and subsequently challenged i.v. with a

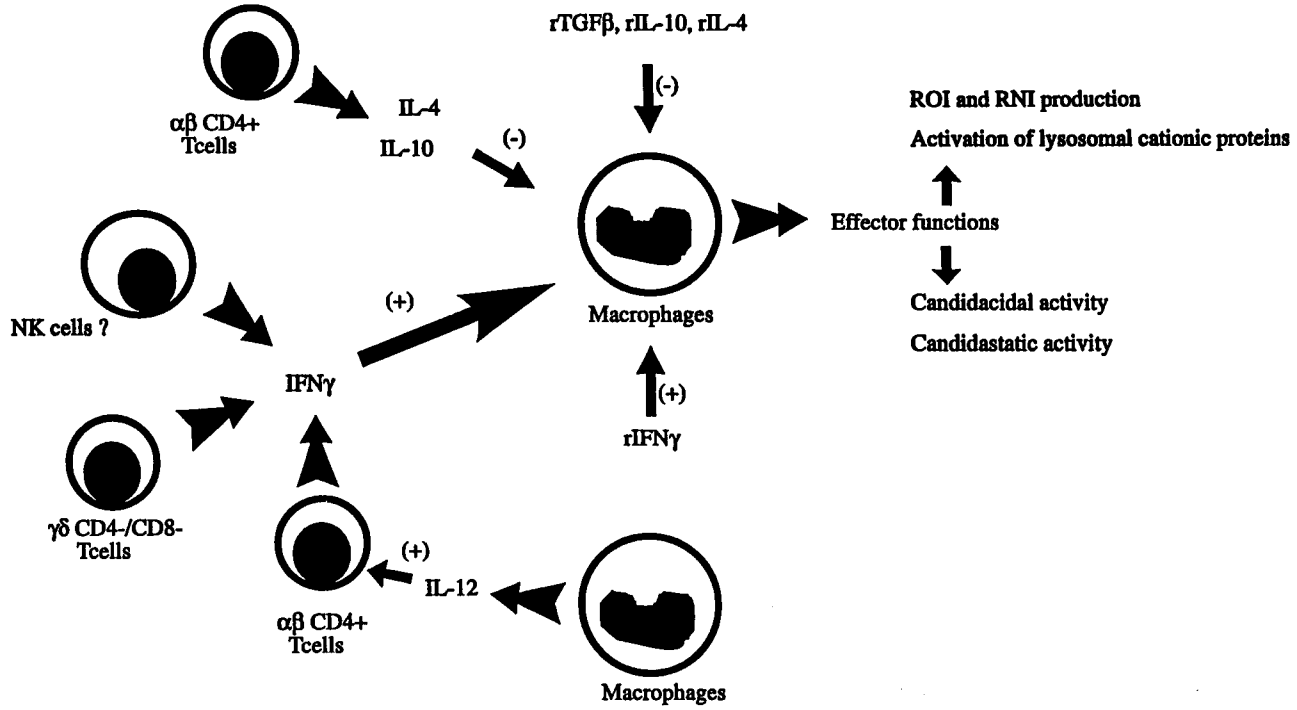

FIG. 3. Cellular source of IFN-γ for the activation of macrophage candidacidal activity. Several lymphocytes synthesize IFN-γ in response to *C. albicans*. $CD4^+$ αβ T cells that are differentiated to a Th_1 response produce IFN-γ that activates macrophage candidacidal mechanisms (dependent on the production of ROI and RNI, respectively) and resistance to systemic candidiasis. IFN-γ synthesized by γδ T cells also activates macrophage candidacidal mechanisms in vitro and may participate in the γδ T-cell-mediated resistance to mucosal candidiasis. NK cells are another source of IFN-γ when exposed to *C. albicans* in vitro; however, the participation of NK cells or IFN-γ produced by them in the activation of macrophage candidacidal mechanisms or in resistance to candidiasis is still not clear. IL-10, IL-4, and TGF-β synthesized by myeloid, lymphoid, and other somatic cells downregulate the activation of macrophage candidacidal mechanisms conferred by IFN-γ.

virulent strain of *C. albicans* (CA). Whereas depletion of $CD4^+$ cells and IFN-γ from "*Candida*-immune" mice enhanced their susceptibility to a *C. albicans* challenge (208), IFN-γ-treated mice showed increased resistance to *C. albicans* challenge (201). These studies (39, 201, 208) indicate the importance of IFN-γ synthesized by T cells for the activation of macrophages to an anticandidal state in resistance to systemic candidiasis.

Resistance to systemic candidiasis in immune hosts appears to be associated with a predominant IFN-γ Th_1 immune response, development of DTH specific for *C. albicans* antigens, and enhancement of fungicidal effector macrophages (Fig. 3) (209, 212; reviewed in references 196 and 207). A Th_1 immune response, associated with resistance to candidiasis, is characterized by the synthesis of IFN-γ and IL-2 by *Candida*-specific T cells. Romani et al. have shown an association of the Th_1 reaction with stimulation of iNOS and production of IL-12 by macrophages from resistant mice (211). In contrast, susceptible mice had a typical Th_2 immune response, which was characterized by the predominance of IL-4 and IL-10 (38). As has been demonstrated in other microbial systems, IL-4, IL-10, and TGF-β can reduce the capacity of macrophages to produce NO (38, 237). Neutralization of IL-10 in immunized mice increased their resistance to a subsequent challenge with *C. albicans*, and the resistance was associated with increased numbers of IFN-γ-secreting $CD4^+$ cells and decreased numbers of IL-10 and IL-4 transcripts (38). Conversely, administration of IL-4 or IL-10 exacerbated candidiasis in mice, and macrophages from anti-IL-10-treated mice produced larger amounts of NO than did those from untreated controls (249).

In addition to $CD4^+$ αβ T cells, NK cells and γδ T cells produce IFN-γ in response to *C. albicans* (Fig. 2) (111, 210, 213). The contribution of IFN-γ-producing NK cells to resistance of immune mice to systemic candidiasis is unclear. Depletion of NK cells did not increase the susceptibility of *C. albicans*-immune mice to an i.v. challenge with *C. albicans* and did not change the establishment of a Th_1 immune response (210). γδ T cells do not seem to participate in the resistance of naive or *C. albicans*-immune mice to systemic candidiasis (110). The latter statement is supported by the observation that although orally immunized mice had increased numbers of $CD4^+$ αβ and $CD8^+$ αβ T cells in response to a subsequent i.v. challenge with *C. albicans*, they did not show increased numbers of γδ T cells (110). In contrast to their apparent lack of a role in resistance to systemic candidiasis, γδ T cells do seem to be important in resistance to the mucosal form of the disease (111; also see below).

Mucosal Candidiasis

Few studies have assessed the role of phagocytic cells in resistance to mucosal candidiasis. Increases in the number of macrophages in the lamina propria and submucosa of the stomach of mice in response to oral colonization and infection with *C. albicans* suggest that these cells play a role in resistance to mucosal candidiasis (47, 259). In support of this hypothesis, Mohamed noted the presence of *C. albicans* inside macrophages in the oral mucosa of humans (161). Also, impairment of macrophage function with poly(I-C) increased the susceptibility of SCID mice to disseminated candidiasis of endogenous (gastrointestinal tract) origin (103, 105). Conversely, immunocompetent controls were resistant to mucosal candidiasis

after poly(I-C) treatment, and interference with both macrophage and neutrophil function was necessary to render these mice susceptible to this superficial form of the disease (105).

The importance of CMI in resistance to mucosal candidiasis has also been demonstrated by comparing T-cell-competent and T-cell-deficient animal models. Euthymic and athymic mice, raised under germfree conditions, can be colonized with *C. albicans* rapidly after oral inoculation with this fungus (11). Both euthymic and athymic mice developed orogastric candidiasis in the keratinized tissue of the tongue and stomach (11). After 10 days of colonization, euthymic mice acquired lymphoproliferation (in vitro) and DTH (in vivo) responses to *Candida* antigens; the onset of CMI correlated with the clearance of *C. albicans* hyphae from mucosal surfaces of euthymic mice (12). On the other hand, athymic, beige-athymic, and SCID mice manifested chronic orogastric infections with *C. albicans* and did not develop DTH responses to *Candida* antigens (12, 33, 90, 169). These observations suggest that *C. albicans*-specific lymphocytes, either by having direct anti-*Candida* activity (16, 135) or by being able to produce lymphokines and activate phagocytic cells, are essential for resistance to orogastric candidiasis.

Protection of mice from mucosal candidiasis appears to be mediated by $CD4^+$ lymphocytes (possibly via the production of IFN-γ) (34). However, a definitive protective role for IFN-γ in resistance to mucosal candidiasis is not supported by the observation that neutralization of IFN-γ or treatment with IFN-γ did not change the course of candidiasis in either resistant beige-euthymic or susceptible beige-athymic mice (34). The mechanisms by which $CD4^+$ lymphocytes mediate this immune protection are unknown, but phagocytic cells are likely to be an end point of the T-cell-mediated immunity that keeps *C. albicans* from invading orogastric tissues. The necessity for cooperation between phagocytes and T cells in limiting *C. albicans* infection is strongly suggested by the fact that mice with a combined beige-nude mutation are very susceptible to orogastric candidiasis (33). The relative contribution of macrophages and neutrophils in resistance to mucocutaneous candidiasis is difficult to assess from the work reported by Cantorna and Balish (33), since the beige mutation affects the candidacidal activity of both macrophages and neutrophils (109, 256).

NO appears to play an important role in resistance to orogastric candidiasis in T-cell-deficient mice (i.e., athymic and SCID mice) (109, 259). Gastrointestinal colonization of athymic and SCID mice with *C. albicans* stimulated the expression of iNOS in the gastric and oral mucosa. Inhibition of NO production in iNOS expressing SCID mice enhanced their susceptibility to orogastric candidiasis. In athymic mice, the expression of iNOS was controlled by γδ T cells, because depletion of these cells not only abrogated the expression of iNOS in the tongue and stomach but also increased the susceptibility of mice to orogastric candidiasis (111). In contrast to T-cell-deficient mice, immunocompetent mice did not express iNOS in the orogastric mucosa after they were monoassociated with *C. albicans*, and they did not show enhanced mucosal candidiasis after treatment with NOS inhibitors (111, 259). Studies with immunocompetent and immunodeficient mice suggest that NO is a T-cell-independent mechanism that protects against orogastric candidiasis. Other mechanisms, e.g., neutrophil-mediated and NO-independent macrophage-mediated immunity, may be the first line of defense against orogastric candidiasis in immunocompetent mice. Similar to the situation in mice, NO or some of its redox congeners may participate in resistance to orogastric candidiasis in humans. Benjamin and coworkers noted that bacteria colonizing the tongue reduced dietary nitrate to nitrite, which in an acidic environment similar to that found in the stomach, was candidacidal (15, 70).

One of the hallmarks of AIDS in HIV-infected people is the appearance of oral thrush (oral candidiasis) (122). Because HIV can infect macrophages, it has been postulated that macrophage dysfunction may be a predisposing factor for the establishment of AIDS; however, mixed results have been obtained on the anticandidal function of HIV-infected macrophages. Whereas some investigators have noted defective macrophage function, others have reported that HIV-infected macrophages produced superoxide anion and phagocytized and killed *C. albicans* as efficiently as did control, noninfected macrophages (176). Because HIV deletes αβ $CD4^+$ T cells, it is likely that oral candidiasis in HIV-infected people is the result of a decrease in αβ $CD4^+$ T cells (176). This interpretation is supported by the observation that colonization of germfree mice with *C. albicans* coincided with increases in the number of αβ $CD4^+$ T cells (40, 111); furthermore, elimination of $CD4^+$ T cells (i.e., depletion with antibodies or by infection with a retrovirus that mimics HIV infection in humans) enhanced the susceptibility of mice to orogastric candidiasis (34, 46). It is likely that $CD4^+$ T cells control anticandidal macrophage functions; however, the interaction of $CD4^+$ cells with cells other than macrophages should not be ruled out.

Peripheral blood monocytes from patients with chronic mucocutaneous candidiasis, while exhibiting normal phagocytic activity, had poor candidacidal activity in vitro (250) and poor oxidative burst in response to opsonized *C. albicans* (23). Such observations indicate that mononuclear phagocytic cells were defective and could predispose these individuals to chronic mucocutaneous candidiasis. A role for mononuclear phagocytic cells in resistance to chronic mucocutaneous candidiasis is further supported by the fact that granulocytes from these patients phagocytized and killed *C. albicans* normally (250).

It has been hypothesized that breast-fed children are less prone to infections than those who are artificially fed because breast milk contains humoral and cellular (neutrophils and macrophages) components with antimicrobial activity. The presence of mononuclear cells resembling macrophages in the curd recovered from the stomach of B-cell-deficient pups supports this hypothesis and suggests that macrophages transferred into breast milk participate in the resistance to mucosal candidiasis (264). Accordingly, macrophages transferred in human milk phagocytized *C. albicans* (204).

Because of the difficulty in isolating cells from the mucosa, little is known about the function of macrophages in resistance to mucosal candidiasis. Innovative cellular and molecular approaches will be necessary to study the function and regulation of macrophages in mucosae normally populated by *C. albicans*. The increased incidence in oral thrush due to the HIV epidemic, the fact that the mucosa is the most frequent source of local and systemic *C. albicans* infections, and our poor understanding of mucosal immunity to this opportunistic pathogen of the alimentary tract make the study of the cells (e.g., lymphocytes, neutrophils, and macrophages) that participate in the immunity to mucosal *C. albicans* infections a priority area for research on candidiasis.

Recurrent Chronic Vulvovaginal Candidiasis

C. albicans, a member of the normal flora of the female genital tract (247), can produce recurrent chronic vulvovaginal candidiasis (RCVC) in apparently healthy women (181, 274). Lack of DTH, lymphocyte blastogenesis, and lymphokine production in response to *C. albicans* antigens are all signs fre-

quently noted in patients with RCVC (244). Although numerous predisposing factors have been associated with RCVC (e.g., pregnancy, diabetes, antibiotics, oral contraceptives, and iron deficiency anemia [274]), the underlying innate or acquired immune defects that make these women susceptible to the disease are not known. Development of systemic, *Candida*-specific, Th_1-type CMI has a limited influence on protection from *Candida* vaginitis. The lack of a correlation between systemic immunity and protection of the vaginal mucosa against *C. albicans* infections could be explained by the unique composition of αβ and γδ T cells in this mucosa (76, 77). For example, and in contrast to systemic organs, γδ T cells are abundant in the vaginal mucosa and seem to play a role in resistance to vaginal candidiasis, as has been suggested by the increased susceptibility of γδ T-cell-depleted athymic mice (111). At present, it is not clear how αβ and γδ T cells contribute to resistance to vaginal candidiasis or whether these cells modulate or are modulated by mononuclear phagocytic cells at this mucosal surface.

It has been found that macrophages from women with RCVC produced prostaglandins and that lymphocyte proliferation to *Candida* antigen was supported if prostaglandin synthesis was inhibited in vitro (274). Furthermore, lymphocytes from women with RCVC proliferated in response to *C. albicans* antigens when incubated with macrophages from a normal (no RCVC) donor (274). Defective macrophage function, therefore, may contribute to the establishment of RCVC.

In some instances, RCVC is associated with oral candidiasis in individuals with AIDS (202). In contrast, congenitally immunodeficient beige athymic mice, which are susceptible to orogastric candidiasis (33) and have defective macrophages and neutrophils (109, 256), did not show overt susceptibility to vaginal candidiasis (32). These observations indicate that different subpopulations of phagocytic cells and/or selective phagocyte activation by T cells may dictate the mucosal tissue where *C. albicans* infection takes place. Therefore, treatment of different clinical presentations of candidiasis may require different immunologic and antifungal therapeutic procedures. Because antimycotic therapy very frequently fails in RCVC, unveiling the immune mechanisms that protect the vaginal mucosa from *C. albicans* infections will provide opportunities for innovative treatment for this common and fastidious form of the disease.

CONCLUSIONS

Macrophages are multifunctional immune cells. From data presented in this review, it is obvious that macrophages participate in many of the host responses to candidiasis. Much of our knowledge about the relationship of macrophages to opportunistic *C. albicans* is focused on the macrophage as an effector cell. Macrophages utilize a diverse array of opsonin-dependent and -independent mechanisms to recognize *C. albicans*. Macrophages are also able to kill *C. albicans* by several effector molecules, and their candidacidal state can be enhanced by activation with several cytokines. The heterogeneity of mononuclear phagocytic cells and their candidacidal mechanisms comprise a complex host defense system that must be maintained to prevent the overgrowth of *C. albicans* in the different mucosal sites colonized by this fungus.

Most of what is known about the biological response of macrophages to *C. albicans* comes from in vitro studies. Very little is known about the response of different populations of macrophages, located in different anatomic sites, to *C. albicans* challenge. The preferential utilization of human monocytes and murine peritoneal macrophages for analyzing the interactions between *C. albicans* and the phagocytes is a matter of convenience rather than the best cellular choice. Whether monocytes or macrophages (resident or exudate) participate in resistance to candidiasis is a question that needs to be answered. Such studies will allow unique insights into the role of mononuclear phagocytic cells in the different clinical manifestations of the disease. It is interesting that candidiasis is frequently a local problem that affects specific tissues (e.g., the oral cavity, esophagus, stomach, nails, skin, or vagina). The confinement of the *C. albicans* infection to a limited area suggests that defined populations or regulation of affector and effector cells, including macrophages, must be defective or impaired at one local site but intact at others. The presence of defective subpopulations of immune cells in resistance to *C. albicans* infections suggests the concept of immunologic microenvironments, a concept little understood but perhaps clinically well demonstrated in candidiasis.

One of the possible benefits that can be obtained from studying the activation of macrophage candidacidal activity is the identification of cytokines which, by themselves or in conjunction with chemotherapy, may improve the treatment and prognosis of this fungal disease. In addition to the positive side of cytokine treatment, studies with activators of macrophage anticandidal functions will have to consider possible negative side effects. If we take IFN-γ as an example, it is well documented that this lymphokine augments macrophage candidacidal activity and synergizes with *C. albicans* to trigger macrophage TNF-α production. An obstacle that will have to be resolved before IFN-γ is used in therapeutic regimes in candidiasis is the capacity of IFN-γ to potentiate macrophage TNF-α production to levels associated with toxic shock. Also, if IFN-γ is used together with antimycotic drugs such as amphotericin B, it is worth noting that amphotericin B has immunomodulatory properties (e.g., stimulation of macrophage TNF-α synthesis) that may contribute to its toxic characteristics (141, 277). Thus, the combination of amphotericin B and IFN-γ in treating candidiasis could lead to unwanted cytotoxic side effects.

Recent advances in molecular biology make it feasible to undertake studies that will unmask the interaction between different populations of macrophages with other cells of the immune system in *Candida*-susceptible tissues. Macrophages must have afferent functions such as antigen presentation and regulation of a particular immune response (e.g., a protective Th_1 versus a nonprotective Th_2 response), as well as efferent functions such as terminal differentiation and activation to an effector state (e.g., killing of *C. albicans*). A better understanding of macrophage function at specific *Candida*-susceptible sites, from the standpoint that macrophages are cells that can both regulate the immune response to *C. albicans* and act as effector cells to kill this fungus, will enhance our prospects to monitor and improve the clinical status of patients with candidiasis, as well as aid in the development of innovative therapeutic strategies for fighting this complex disease.

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