

Tumor Necrosis Factor-Inducing Activities of *Cryptococcus neoformans* Components

DEMETRIO DELFINO,¹ LUIGI CIANCI,¹ MARIA MIGLIARDO,¹ GIUSEPPE MANCUSO,¹
VITALIANO CUSUMANO,¹ CLAUDIO CORRADINI,² AND GIUSEPPE TETI^{1*}

*Istituto di Microbiologia, Facoltà di Medicina e Chirurgia, Università degli Studi di Messina, I-98122 Messina,¹
and Istituto di Cromatografia, Consiglio Nazionale delle Ricerche, I-00016 Rome,² Italy*

Received 24 June 1996/Returned for modification 30 August 1996/Accepted 30 September 1996

***Cryptococcus neoformans*-induced tumor necrosis factor alpha (TNF- α) production may lead to increased human immunodeficiency virus replication in patients with AIDS. In order to identify cryptococcal components that are predominantly responsible for stimulating TNF production, various concentrations of glucuronoxylomannan (GXM), galactoxylomannan (GalXM), mannoproteins (MP), and β (1-3) glucan were added to whole-blood cultures. All of the cryptococcal components tested, as well as whole heat-killed cryptococci, were capable of inducing TNF- α release in a dose-dependent manner. MP were significantly more potent than any of the other cryptococcal components tested or heat-killed cryptococci in stimulating TNF- α production ($P < 0.05$). GXM, in contrast, was significantly less potent in this activity than either GalXM or MP ($P < 0.05$). As little as 0.5 μ g of MP per ml was sufficient to produce moderate but significant elevations of TNF- α release. Maximal MP-induced TNF- α levels were similar to those induced by *Salmonella enteritidis* lipopolysaccharide, our positive control. Further experiments using isolated leukocytes suggested that monocytes were the cell population mainly responsible for TNF- α production, although the participation of other cell types could not be excluded. The presence of complement-sufficient plasma was a necessary requirement for TNF- α induction by GXM, GalXM, and low doses of MP. High MP concentrations (100 μ g/ml) were also capable of stimulating TNF- α production in the absence of plasma. These data indicate that soluble products released by *C. neoformans* are capable of inducing TNF- α secretion in human leukocytes. This may be clinically relevant, since high concentrations of such products are frequently found in the body fluids of AIDS patients infected with *C. neoformans*.**

Cryptococcus neoformans is a predominantly saprophytic yeast that can cause serious infections, mostly in individuals with compromised cellular immunity. Cryptococcosis is the fourth most common cause of mortality in patients with AIDS (7, 16). Currently, 4% of patients with AIDS in the United Kingdom (18), 5 to 10% of AIDS patients in the United States (10, 13), and an increasing percentage in the developing countries are known to have developed cryptococcosis (34, 42). The yeast, occurring as four serotypes, is surrounded by a capsule primarily composed of glucuronoxylomannan (GXM) (9) and composed, to a lesser extent, of galactoxylomannan (GalXM) and mannoproteins (MP).

Approximately 80% of the clinical isolates in the United States are of serotype A (21). Infection follows inhalation from environmental sources of poorly encapsulated yeasts, which are normally ingested and killed by pulmonary host defenses (4, 40). Increased capsule production and replication within the lung may lead to dissemination to other tissues, frequently resulting in fatal meningoencephalitis. It is likely that the capsule acts as a virulence factor by inhibiting opsonophagocytosis (19, 20, 30), although the specific mechanisms for this effect are not entirely clear.

Cytokines are low-molecular-weight proteins involved in a variety of specific and nonspecific responses to infection (6). Some of these mediators, including tumor necrosis factor alpha (TNF- α), granulocyte-macrophage colony-stimulating factor

(GM-CSF), and interleukin 1 (IL-1), may play a role in the pathogenesis of cryptococcosis (8, 23, 41). TNF- α could exert both beneficial and detrimental effects in AIDS patients infected with *C. neoformans*. Release of TNF- α and GM-CSF by macrophages upon interaction with acapsular *C. neoformans* can stimulate, by an autocrine mechanism, increased phagocytosis of encapsulated yeasts (8, 12). A beneficial role of TNF- α is also suggested by observations that TNF- α blockade can accelerate the progression of cryptococcosis in a murine model (11). In AIDS patients, however, TNF- α production may lead to increased replication of human immunodeficiency virus in latently infected cells and progression of the disease (17, 33). Little information is available on the identities of cryptococcal components responsible for cytokine release. Poorly encapsulated or acapsular cells of *C. neoformans* are potent inducers of TNF- α from freshly isolated human monocytes and alveolar macrophages in the presence of complement-sufficient serum (24). The presence of a large capsule or the addition of purified GXM down-regulates the production of TNF- α (41). Better understanding of the mechanisms whereby *C. neoformans* induces TNF- α production may lead to improved therapeutic approaches using specific antibodies or modifiers of the inflammatory response. Indeed, conventional chemotherapy is only partially successful in AIDS patients, and infection can be controlled only with continuous suppressive therapy (2, 5).

This study was undertaken to identify cryptococcal components responsible for stimulating TNF- α release. Specifically, a number of capsular and cell wall products, including GXM, GalXM, MP, and cell wall β (1-3) glucan were tested for their ability to stimulate human leukocytes to release TNF- α .

* Corresponding author. Mailing address: Istituto di Microbiologia, Piazza XX Settembre, 4, I-98122 Messina, Italy. Phone: 39-90-712110 or 672508. Fax: 39-90-719910. Electronic mail address: Teti@eniware.it.

MATERIALS AND METHODS

Cryptococcal strains. Encapsulated *C. neoformans* A 9759, serotype A, was kindly provided by E. Reiss, Centers for Disease Control, Atlanta, Ga. The acapsular strain CAP 67 was provided by E. Jacobson, Richmond, Va.

Purified cryptococcal components. Insoluble *C. neoformans* cell wall glucan was a kind gift of E. Reiss. The other components were obtained from culture supernatants of strains A 9759 (GXM) and CAP 67 (GalXM and MP). Late-log-phase cryptococcal cultures were obtained after incubation at 30°C under agitation for 4 days in a chemically defined, entirely dialyzable medium (3). After the removal of yeast cells by tangential filtration through 0.2- μ m-pore-size cassettes (Pellicon System; Millipore, Rome, Italy), culture supernatants were concentrated 20-fold by tangential filtration with 10,000-Da-cutoff cassettes and dialyzed against phosphate-buffered saline (PBS) (0.01 M phosphate, 0.15 M NaCl; pH 7.2). GXM was obtained from culture supernatants of serotype A 9759 by gel filtration chromatography. Before gel filtration, concentrated supernatants were heat inactivated (56°C for 30 min) and supplemented with 25 μ g of phenylmethylsulfonyl fluoride (Gibco BRL) per ml to inactivate lytic enzymes possibly present in these preparations.

Gel filtration was performed with a Sepharose CL-4B column (5 by 90 cm; Pharmacia S.p.A., Milan, Italy) at a flow rate of 1.5 ml/min in PBS supplemented with 0.02% sodium azide. Ten-milliliter fractions were collected and analyzed for carbohydrate and protein contents by the phenol-sulfuric acid method (15) and by measuring A_{280} , respectively. A 9759 supernatants were separated by Sepharose CL-4B gel filtration in two carbohydrate-containing peaks. Of these, the second peak contained protein in addition to carbohydrate material. The first peak was not detected in the elution pattern of supernatants from the acapsular CAP 67, which is unable to produce GXM, but is capable of producing GalXM and MP. Fractions from the first peak of A 9759 supernatants were pooled and precipitated with 75% ethanol. This material was identified as GXM on the basis of a compatible native polyacrylamide gel electrophoresis (PAGE) pattern (31) and reactivity with a GXM-specific monoclonal antibody (kindly provided by A. Casadevall, Yeshiva University, Bronx, N.Y.) in a cryptococcal agglutination inhibition assay.

GalXM and MP were obtained from CAP 67 supernatants by affinity chromatography on concanavalin A (ConA) Sepharose 4B (Pharmacia), exactly as described previously (14). Briefly, GalXM was recovered in the effluent from the ConA Sepharose column, whereas MP was eluted from the latter with a 0 to 0.3 M gradient of α -methyl-D-mannopyranoside. Both GalXM and MP were dialyzed against water and lyophilized.

The purity of GXM, GalXM, and MP was confirmed by PAGE analysis (29) of overloaded gels. GXM and GalXM preparations contained <1% proteins (Bio-Rad protein assay; Bio-Rad Laboratories, Milan, Italy). The lack of carbohydrate contamination in GXM and GalXM preparations was demonstrated by monosaccharide constituent analysis using anion-exchange high-performance liquid chromatography and a pulsed amperometric detector (27). In some experiments, the cytokine-inducing ability of purified components was compared with that of killed whole cryptococci. The latter were obtained from A 9759 or CAP 67 late log cultures, washed with water, heated at 80°C for 1 h, and lyophilized.

Detection of endotoxin. Cryptococcal components were tested for the presence of endotoxin with a *Limulus* amoebocyte lysate assay kit (E-Toxate; Sigma). The levels of endotoxin were <5 pg/mg in all of the materials.

Whole-blood cultures. Samples of whole peripheral blood were obtained from healthy volunteers and mixed with equal volumes of RPMI 1640 medium (Life Technologies, Milan, Italy) containing heparin at 20 U/ml. One-milliliter volumes were dispensed to 24-well plates and incubated at 37°C for 18 h in 5% CO₂ with the indicated amounts of cryptococcal constituents or heat-killed yeasts. MP, GXM, and GalXM were dissolved in PBS and filtered through 0.22- μ m-pore-size filters (Lida Manufacturing Corp. Kenosha, Wis.) before the addition to whole-blood or isolated leukocyte cultures. These compounds were fully soluble in PBS at all of the concentrations employed. Lipopolysaccharide (LPS) (100 ng/ml) from *Salmonella enteritidis* was used as a positive control. Cultures were then centrifuged (600 \times g for 15 min), and the supernatants were stored at -70°C until tested for TNF- α concentration.

Isolated leukocyte cultures. Whole blood, obtained as described above, was sedimented in the presence of 5% dextran, and leukocyte-rich plasma was harvested. Leukocytes were washed (200 \times g for 10 min) three times in RPMI 1640, resuspended, and centrifuged (400 \times g for 30 min) over Ficoll-Hypaque gradients (38). Peripheral blood mononuclear cells (PBMC) and polymorphonuclear leukocytes (PMN) were obtained from the interface and pellet, respectively. Pelleted cells were >95% neutrophilic granulocytes by morphology. In further experiments, PBMC were separated into adherent and nonadherent populations by incubation in plastic tissue culture dishes for 2 h at 37°C in serum-free RPMI 1640. Adherent and nonadherent PBMC were >95% monocytes and lymphocytes, respectively, by morphology. To assess cytokine production, 2 \times 10⁶ separated or unseparated leukocytes were cultured for 18 h in RPMI 1640 with 10% autologous plasma in the presence of cryptococcal components. In some experiments, autologous plasma was omitted or heated at 56°C for 30 min to inactivate complement. In selected experiments, the viability of leukocyte populations at the end of culture was >90% both in the presence and absence of stimuli, as assessed by the trypan blue exclusion test.

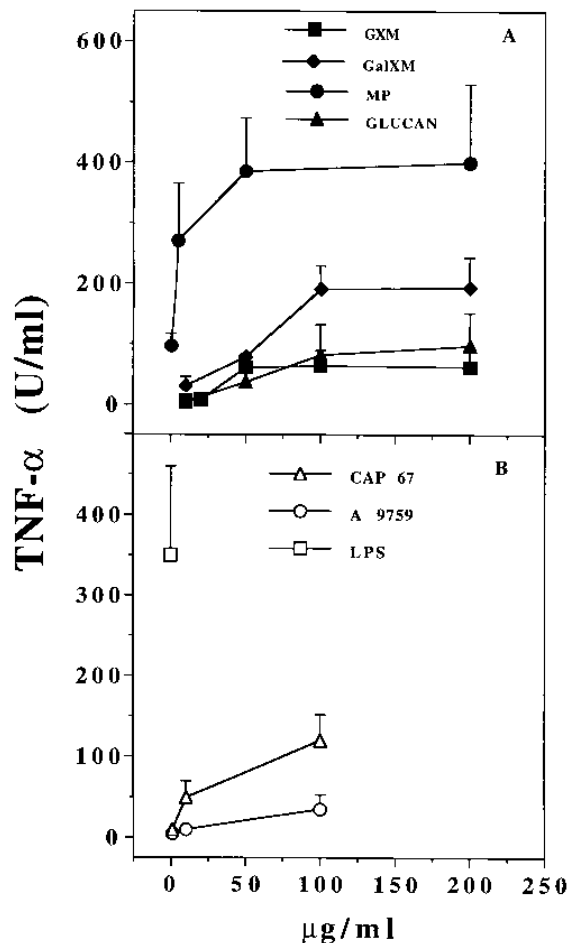


FIG. 1. TNF- α levels in supernatants of whole-blood cultures stimulated with *C. neoformans* components (A) or heat-killed yeast cells (B). *S. enteritidis* LPS (0.1 μ g/ml) was used as a positive control in panel B. Datum points are the means \pm standard deviations of five separate experiments conducted with blood samples from different donors.

Measurement of TNF- α . Measuring TNF- α was performed by a cytotoxicity assay (27) with minor modifications. TNF-sensitive WEHI 164 clone 13 cells (a kind gift of T. Espevik, Trondheim, Norway) were seeded in 96-microwell plates at 2 \times 10⁴ cells per well. After overnight incubation, nine serial twofold dilutions (1:8 to 1:2,048) for each sample were made in RPMI 1640 medium containing 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 2% fetal calf serum, and actinomycin D (Sigma, St. Louis, Mo.) at a final concentration of 1 μ g/ml. Sample dilutions (0.1 ml) were added to cell monolayers in triplicate. Cell control wells received 0.05% Nonidet P-40 lysis buffer. After 18 to 20 h, 5 μ g of the dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Sigma) per ml was added and the cells were incubated for an additional 4 h (38). Supernatants were removed without disturbing the dark blue crystals formed. These crystals were dissolved in dimethyl sulfoxide (Sigma) (100 μ l per well), and A_{550} was determined. One unit was defined as the amount of TNF causing 50% lysis of WEHI cells. TNF- α activity in selected plasma samples was totally inhibited by a 1:100 dilution of rabbit anti-TNF- α serum (Genzyme), but not by normal serum.

Data expression and statistical analysis. Data are expressed as means \pm standard deviations of separate experiments, each performed in duplicate with cells from different donors. Statistical significance was assessed by one-way analysis of variance and the Student-Keuls-Newman test.

RESULTS

TNF- α production in whole blood. GXM, GalXM, and MP, obtained from strain A 9759, as well as cell wall glucan, were added in increasing concentrations to whole-blood cultures, and the supernatants were tested for TNF- α (Fig. 1A). For

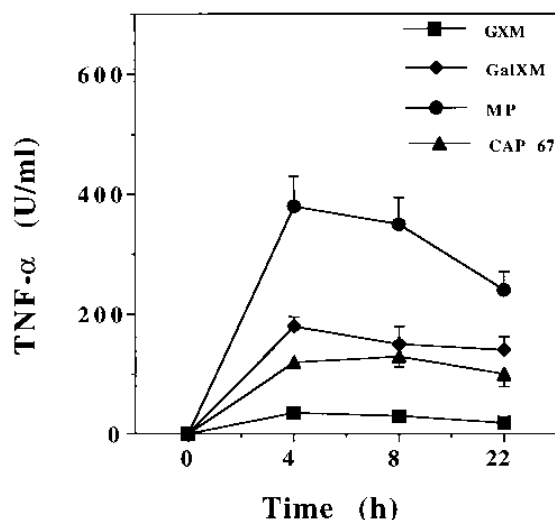


FIG. 2. Time course of TNF- α production in cultures of whole blood stimulated with MP, GXM, GalXM, or heat-killed CAP 67 cells. Data are from a representative experiment of three separate experiments conducted with blood samples from different donors.

comparative purposes, whole heat-killed cryptococci were also tested (Fig. 1B). TNF- α was not detected in unstimulated cultures. The acapsular CAP 67 strain induced significantly higher TNF- α secretion than the encapsulated A 9759 strain (Fig. 1B), confirming previous observations using leukocyte cultures (24). All of the cryptococcal components tested were capable of inducing TNF- α release. MP, however, were more potent in this activity than the other cryptococcal products or heat-killed cryptococci.

TNF- α levels induced by GalXM, GXM, or glucan were lower at all tested concentrations than those induced by MP ($P < 0.05$). As little as 0.5 μ g of MP per ml was sufficient to produce slight but significant elevations of TNF- α release over the level for unstimulated controls ($P < 0.05$). In contrast, the minimal concentration required to induce significant stimulation was 50 μ g/ml using GalXM, GXM, or glucan. Maximal TNF- α levels induced by MP were similar to those induced by *S. enteritidis* LPS, our positive control (Fig. 1). GXM induced slight, but significant, elevations in TNF- α concentrations ($P < 0.05$) over the level for unstimulated controls. TNF- α levels induced by GXM were significantly lower than those induced by GalXM ($P < 0.05$). In order to rule out contamination of cryptococcal preparations with endotoxin, the endotoxin-inactivating agent polymyxin B (25 μ g/ml) was added to whole-blood cultures in selected experiments. The addition of polymyxin B resulted in 75% inhibition of LPS-induced TNF- α but did not affect stimulation of TNF- α release by any of the cryptococcal components (not shown).

Time course of TNF- α production. Next, we determined the kinetics of TNF- α production in whole blood in response to purified components and whole killed acapsular *C. neoformans*. Figure 2 shows that significant TNF- α production was already detectable at 4 h after the addition of all the tested agents. Maximal levels were reached at 4 to 8 h and slowly declined thereafter.

Leukocyte types responsible for TNF- α release. Cytokine-producing cells in human blood comprise granulocytes, monocytes, lymphocytes, and natural killer cells. In order to identify the cell types which were predominantly responsible for the cytokine responses observed in whole blood, leukocytes were

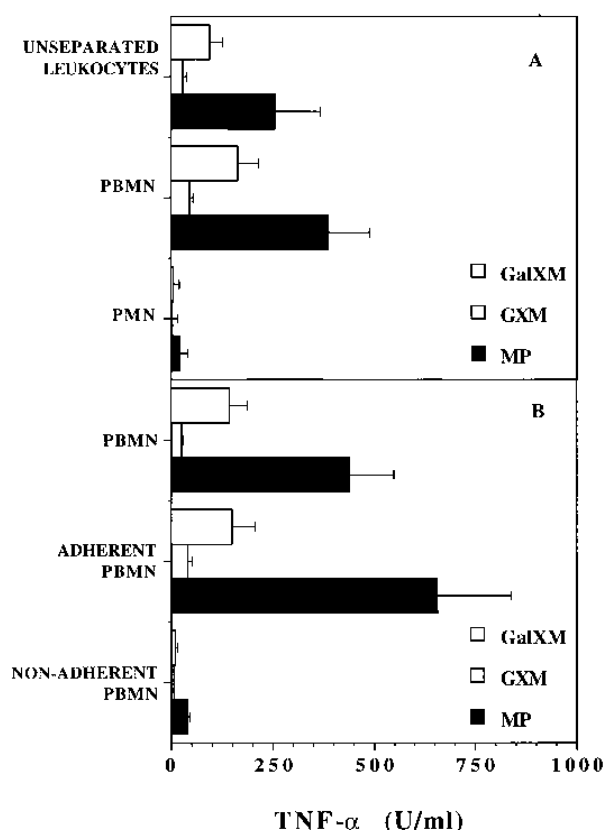


FIG. 3. TNF- α release from unseparated leukocytes, PBMN, and PMN stimulated with *C. neoformans* components (A). PBMN were further separated into adherent and nonadherent cells and stimulated with the same components (B). Bars represent the means \pm standard deviations of three separate experiments conducted with blood samples from different donors.

centrifuged over Ficoll-Hypaque gradients and PBMN were compared with PMN for their ability to produce TNF- α . Unseparated leukocytes, PBMN, and PMN were cultured in the presence of 10% autologous plasma with optimal stimulating concentrations of cryptococcal components (Fig. 3A). Under these conditions, PMN released considerably less TNF- α than unseparated leukocytes or PBMN did, in response to any of the tested agents.

Since these data indicated that the cell populations responsible for most of TNF- α production were contained in the PBMN fraction, the latter were further separated by adherence to plastic. Figure 3B shows that most of the TNF- α -producing activity was found in the adherent cell population. These data suggested that monocytes are predominantly responsible for cytokine release in response to *C. neoformans* components, although the contribution of other cell types could not be excluded.

Plasma factors affecting TNF- α release. Since it was previously shown that TNF- α production in response to whole heat-killed *C. neoformans* occurs only in the presence of complement-sufficient plasma (24), it was of interest to determine if the same requirement applied to purified cryptococcal components. Therefore, monocytes were cultured without plasma and in the presence of either heat-inactivated (56°C for 30 min) or unheated autologous plasma (Fig. 4). With GXM or GalXM, significant TNF- α production was observed only in the presence of heat-labile plasma factors (Fig. 4B and C). However, neither heat-labile nor heat-stable plasma factors were abso-

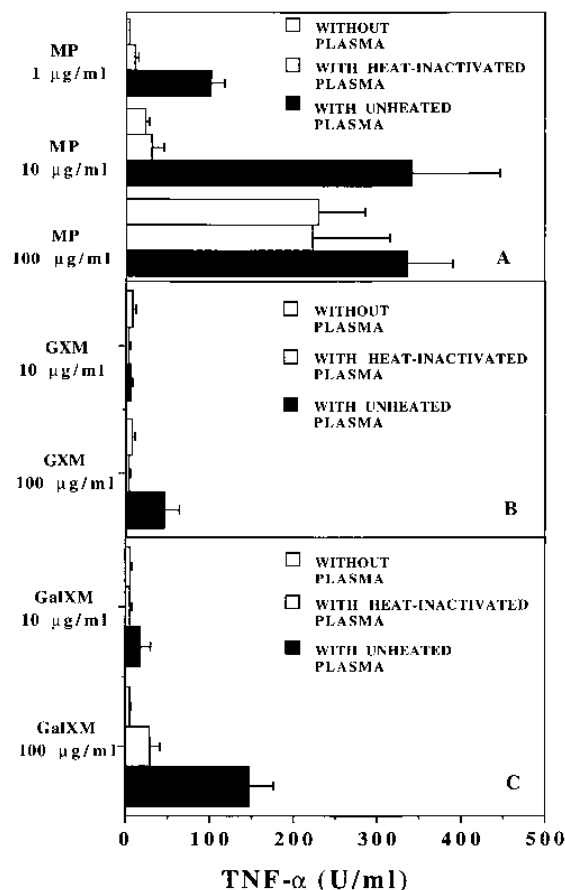


FIG. 4. TNF- α production from monocytes cultured without plasma or in the presence of heat-inactivated (56°C for 30 min) or unheated autologous plasma. Cells were incubated with the indicated concentrations of MP (A), GXM (B), and GalXM (C). Bars represent the means \pm standard deviations of three different experiments conducted with blood samples from different donors.

lute requirements for TNF- α responses with high (100- μ g/ml) concentrations of MP, since comparable levels of the cytokine were observed in the absence of plasma and in the presence of heat-inactivated or unheated plasma (Fig. 4A). With lower (10- μ g/ml) MP concentrations, however, TNF- α release was significantly higher in the presence of unheated plasma, relative to both heat-inactivated plasma and plasma-free cultures ($P < 0.05$).

DISCUSSION

Previous studies have indicated that TNF- α has an important role in host defenses against cryptococcosis in both naive and immunized mice (1). *C. neoformans*-induced TNF- α production, however, may result in accelerated human immunodeficiency virus replication in AIDS patients (28). Therefore, better understanding of the mechanisms whereby cryptococci induce TNF- α production could be useful to devise strategies to slow down the progression of AIDS in patients infected with this fungus. Since surface components and extracellular products are likely to be responsible for the cytokine-inducing activities of *C. neoformans*, we focused on the ability of those constituents to stimulate TNF- α production in human blood.

The present data indicate that several such components can stimulate TNF- α . MP, however, were significantly more potent than other components in this activity. The minimal MP dose

required to stimulate significant TNF- α production was 2 orders of magnitude lower than those of GXM, GalXM, and cell wall glucan. In addition, MP were significantly more potent than heat-killed yeasts in inducing the cytokine. These observations raise the possibility that MP are predominantly, although not exclusively, responsible for *C. neoformans*-induced TNF- α production. Additional *in vivo* studies using specific antibodies will be useful to test this hypothesis.

Our data indicate that GXM, the major capsular component, is a comparatively weak TNF- α inducer. These observations are in general agreement with those of a previous study showing that heat-killed, small-capsule or acapsular organisms, but not those with a large capsule, can induce significant TNF- α production (24). Moreover, the addition of GXM inhibited cytokine induction by the acapsular yeasts, but not by LPS (41). It is possible that GXM inhibits TNF- α production by masking cell wall components (20, 37) with high TNF- α -inducing activities. It is tempting to speculate that such components are indeed MP. It is presently unclear, however, if MP are expressed on the cryptococcal cell wall in sufficient amounts to stimulate TNF- α production. Fluorescein-conjugated ConA was shown to bind to the acapsular CAP 67 strain. However, labeling with ferritin-conjugated anti-MP antibodies demonstrated that these constituents are predominantly localized to the inner two-thirds of cryptococcal cell walls, with low-level surface expression (39).

A recent study reported the presence of exposed carbohydrate residues, which are masked by the polysaccharide capsule in the encapsulated form, on the cell walls of acapsular organisms (12). These exposed residues are recognized by various members of the collectin family, including mannose-binding protein (36). Such surface components are believed to stimulate mononuclear phagocytes for increased yeast phagocytosis and TNF- α production (25). It will be of interest to assess if at least some of the collectin-binding receptors on the surface of acapsular *C. neoformans* are MP.

Previously, phagocytosis of acapsular *C. neoformans* was shown to induce the release of TNF- α from mononuclear cells (12). We have shown here that some soluble cryptococcal products can also induce TNF- α secretion. It is unlikely that phagocytosis of insoluble particles present in the MP, GXM, and GalXM preparations employed was responsible for the observed release of TNF- α . First, all of these preparations were freely soluble in aqueous solutions. Second, these solutions were passed through 0.22- μ m-pore-size filters before being added to whole-blood or isolated leukocyte preparations. Therefore, apparently phagocytosis is not an obligatory event in cell activation mechanisms leading to TNF- α production. Experiments are under way to determine if similar receptors are involved in phagocytosis-dependent and -independent TNF- α release. The ability of soluble extracellular cryptococcal products to induce TNF- α production may be clinically relevant, since extracellular components are released *in vivo* during infection and may stimulate host cells at remote sites (16).

To study cytokine production, whole blood was initially used, since this is a highly sensitive system in which different cell populations and serum components are allowed to interact in near-physiological conditions (32). Since neutrophils, lymphocytes, and monocytes all interact with *C. neoformans*, it was of interest to determine which of these cell types produced TNF- α in response to cryptococcal components. Our data indicated that monocytes are likely to be the predominant cell type involved in TNF- α production in blood. This is in agreement with similar results obtained using whole heat-killed cryptococci (24). Our data do not exclude that leukocyte populations other than monocytes produce moderate amounts of

TNF- α upon interaction with *C. neoformans*. In fact, in the present study, low-level, but significant, cytokine production was detected in PMN or lymphocyte cultures stimulated with cryptococcal components. It was previously shown that the presence of complement is an obligatory requirement for TNF induction by whole cryptococci (24). Our data also indicate that heat-labile components such as complement are necessary for GXM- and GalXM-induced TNF- α production. With MP, this was true at low, but not high, stimulating doses. These data are consistent with the notion that GXM, GalXM, and MP all activate complement, which is, in turn, capable of inducing TNF- α production (14). Therefore, it appears that, with the exception of MP, cryptococcal components induce TNF- α production after interacting with heat-labile components and are incapable of directly stimulating monocytes for cytokine production. Experiments are under way to characterize the mechanisms of complement-independent cytokine induction by MP. Such mechanisms may be clinically relevant. Major foci of cryptococcal infection include serum-free sites such as the pulmonary alveoli and the central nervous system (22, 35). In addition, complement depletion, secondary to massive activation, can occur in overwhelming cryptococcosis (26).

In conclusion, our data indicate that cryptococcal MP are potent TNF- α inducers in human monocytes. Further studies will be needed to clarify the relevance of this finding to the pathogenesis of cryptococcosis.

ACKNOWLEDGMENT

This work was supported by Ministero della Sanità of Italy AIDS grant 931.

REFERENCES

- Aguirre, K., E. A. Havell, G. W. Gibson, and L. L. Johnson. 1995. Role of tumor necrosis factor and gamma interferon in acquired resistance to *Cryptococcus neoformans* in the central nervous system of mice. *Infect. Immun.* **63**:1725-1731.
- Bozette, S. A., R. A. Larsen, J. Chiu, et al. 1991. A placebo-controlled trial of maintenance therapy with fluconazole after treatment of cryptococcal meningitis in the acquired immunodeficiency syndrome. *N. Engl. J. Med.* **324**:580-584.
- Buchanan, K. L., and J. W. Murphy. 1993. Characterization of cellular infiltrates and cytokine production during the expression phase of the anti-cryptococcal delayed-type hypersensitivity response. *Infect. Immun.* **61**:2854-2865.
- Cameron, M. L., D. L. Granger, J. B. Weinberg, W. J. Kozumbo, and H. S. Koren. 1990. Human alveolar and peritoneal macrophages mediate fungistasis independently of L-arginine oxidation to nitrite or nitrate. *Am. Rev. Respir. Dis.* **142**:1313-1319.
- Casadevall, A., E. D. Spitzer, D. Webb, and M. G. Rinaldi. 1993. Susceptibilities of serial *Cryptococcus neoformans* isolates from patients with recurrent cryptococcal meningitis to amphotericin B and fluconazole. *Antimicrob. Agents Chemother.* **37**:1383-1386.
- Cavaillon, J. M., and C. Aznar. 1992. Cytokines et infections fongiques. *J. Mycol. Méd.* **2**:61-67.
- Chandler, F. W. 1985. Pathology of the mycoses in patients with the acquired immunodeficiency syndrome (AIDS). *Curr. Top. Med. Mycol.* **1**:1-23.
- Chen, G. H., J. L. Curtis, C. H. Mody, P. J. Christensen, L. R. Armstrong, and G. B. Toews. 1994. Effect of granulocyte-macrophage colony-stimulating factor on rat alveolar macrophage anticryptococcal activity in vitro. *J. Immunol.* **152**:724-734.
- Cherniak, R., L. C. Morris, B. C. Anderson, and S. A. Meyer. 1991. Facilitated isolation, purification, and analysis of glucuronoxylomannan of *Cryptococcus neoformans*. *Infect. Immun.* **59**:59-64.
- Chuck, S. L., and M. A. Sande. 1989. Infections with *Cryptococcus neoformans* in the acquired immunodeficiency syndrome. *N. Engl. J. Med.* **321**:794-799.
- Collins, H. L., and G. J. Bancroft. 1992. Cytokine enhancement of complement-dependent phagocytosis by macrophages: synergy of tumor necrosis factor- α and granulocyte-macrophage colony-stimulating factor for phagocytosis of *Cryptococcus neoformans*. *Eur. J. Immunol.* **22**:1447-1454.
- Cross, C. E., and G. J. Bancroft. 1995. Ingestion of acapsular *Cryptococcus neoformans* occurs via mannose and β -glucan receptors, resulting in cytokine production and increased phagocytosis of the encapsulated form. *Infect. Immun.* **63**:2604-2611.
- Currie, B. P., and A. Casadevall. 1994. Estimation of the prevalence of cryptococcal infection among HIV infected individuals in New York City. *Clin. Infect. Dis.* **19**:1029-1033.
- Dong, Z. M., and J. W. Murphy. 1993. Mobility of human neutrophils in response to *Cryptococcus neoformans* cells, culture filtrate antigen, and individual components of the antigen. *Infect. Immun.* **61**:5067-5077.
- Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28**:350-356.
- Eng, R. H. K., E. Bishburg, S. M. Smith, and R. Kapila. 1986. Cryptococcal infections in patients with acquired immune deficiency syndrome. *Am. J. Med.* **81**:19-23.
- Israel, N., U. Hazan, J. Alcamí, A. Munier, F. Arenzana-Seisdedos, F. Bachele-rie, A. Israel, and J. L. Virelizier. 1989. Tumor necrosis factor stimulates transcription of HIV-1 in human T lymphocytes, independently and synergistically with mitogens. *J. Immunol.* **143**:3956-3960.
- Knight, F. R., D. W. Mackenzie, B. G. Evans, K. Porter, N. J. Barrett, and G. C. White. 1993. Increasing incidence of cryptococcosis in the United Kingdom. *J. Infect.* **27**:185-191.
- Kozel, T. R., and E. C. Gotschlich. 1982. The capsule of *Cryptococcus neoformans* passively inhibits phagocytosis of the yeast by macrophages. *J. Immunol.* **129**:1675-1680.
- Kozel, T. R., and P. Mastroianni. 1976. Inhibition of phagocytosis by cryptococcal polysaccharide: dissociation of the attachment and ingestion phases of phagocytosis. *Infect. Immun.* **14**:62-67.
- Kwon-Chung, K. J., and J. E. Bennett. 1984. Epidemiologic differences between two varieties of *Cryptococcus neoformans*. *Am. J. Epidemiol.* **120**:123-130.
- Levitz, S. M., and D. J. DiBenedetto. 1989. Paradoxical role of capsule in murine bronchoalveolar macrophage-mediated killing of *Cryptococcus neoformans*. *J. Immunol.* **142**:659-665.
- Levitz, S. M., and T. P. Farrell. 1990. Growth inhibition of *Cryptococcus neoformans* by cultured human monocytes: roles of the capsule, opsonins, the culture surface, and cytokines. *Infect. Immun.* **58**:1201-1209.
- Levitz, S. M., A. Tabuni, H. Kornfeld, C. C. Reardon, and D. Golenbock. 1994. Production of tumor necrosis factor alpha in human leukocytes stimulated by *Cryptococcus neoformans*. *Infect. Immun.* **62**:1975-1981.
- Levitz, S. M., A. Tabuni, and C. Treseleer. 1993. Effect of mannose-binding protein on binding of *Cryptococcus neoformans* to human phagocytes. *Infect. Immun.* **61**:4891-4893.
- Macher, A. M., J. E. Bennett, J. E. Gadek, and M. M. Frank. 1978. Complement depletion in cryptococcal sepsis. *J. Immunol.* **120**:1686-1690.
- Mancuso, G., F. Tomasello, C. von Hunolstein, G. Orefici, and G. Teti. 1994. Induction of tumor necrosis factor alpha by the group- and type-specific polysaccharides from type III group B streptococci. *Infect. Immun.* **62**:2748-2753.
- Matsuyama, T., N. Kobayashi, and N. Yamamoto. 1991. Cytokines and HIV infection: is AIDS a tumor necrosis factor disease? *AIDS* **5**:1405-1417.
- McGuckin, W. F., and B. F. Mckenzie. 1958. An improved periodic acid fuchsin sulfite staining method for evaluation of glycoproteins. *Clin. Chem.* **4**:476-483.
- Murphy, J. W., and G. C. Cozad. 1972. Immunological unresponsiveness induced by cryptococcal capsular polysaccharide assayed by the hemolytic plaque technique. *Infect. Immun.* **5**:896-901.
- Murphy, J. W., R. L. Mosley, R. Cherniak, G. H. Reyes, T. H. Kozel, and E. Reiss. 1988. Serological, electrophoretic, and biological properties of *Cryptococcus neoformans* antigens. *Infect. Immun.* **56**:424-431.
- Nerad, J. L., J. K. Griffiths, J. W. M. Van der Meer, S. Endres, D. D. Poutsiaika, G. T. Keusch, M. Bennis, M. A. Salam, C. A. Dinarello, and J. G. Cannon. 1992. Interleukin-1 β (IL-1 β), IL-1 receptor antagonist, and TNF α production in whole blood. *J. Leukocyte Biol.* **52**:687-692.
- Osborne, L., S. Kunkel, and G. J. Nabel. 1989. Tumor necrosis factor alpha and interleukin 1 stimulate the human immunodeficiency virus enhancer by activation of the nuclear factor kB. *Proc. Natl. Acad. Sci. USA* **86**:2336-2340.
- Perfect, J. R. 1989. Cryptococcosis. *Infect. Dis. Clin. N. Am.* **3**:77-102.
- Perfect, J. R., M. M. Hobbs, D. L. Granger, and D. T. Durack. 1988. Cerebrospinal fluid macrophage response to experimental cryptococcal meningitis: relationship between in vivo and in vitro measurements of cytotoxicity. *Infect. Immun.* **56**:849-854.
- Schelenz, S., R. Malhotra, R. B. Sim, U. Holmskov, and G. J. Bancroft. 1995. Binding of host collectins to the pathogenic yeast *Cryptococcus neoformans*: human surfactant protein D acts as an agglutinin for acapsular yeast cells. *Infect. Immun.* **63**:3360-3366.
- Small, J. M., and T. G. Mitchell. 1986. Binding of purified and radioiodinated capsular polysaccharides from *Cryptococcus neoformans* serotype A strains to capsule-free mutants. *Infect. Immun.* **54**:742-750.
- Teti, G., G. Mancuso, and F. Tomasello. 1993. Cytokine appearance and effects of anti-tumor necrosis factor alpha antibodies in a neonatal rat model of group B streptococcal infection. *Infect. Immun.* **61**:227-235.
- Vartivarian, S. E., G. H. Reyes, E. S. Jacobson, P. G. James, R. Cherniak, V. R. Mumaw, and M. J. Tingler. 1989. Localization of mannoprotein in *Cryptococcus neoformans*. *J. Bacteriol.* **171**:6850-6852.

40. **Vecchiarelli, A., M. Dottorini, D. Pietrella, C. Monari, C. Retini, T. Todisco, and F. Bistoni.** 1994. Role of alveolar macrophages as antigen presenting cells in *Cryptococcus neoformans* infection. *Am. J. Respir. Cell Mol. Biol.* **11**:130–137.
41. **Vecchiarelli, A., C. Retini, D. Pietrella, C. Monari, C. Tascini, T. Beccari, and T. R. Kozel.** 1995. Downregulation by cryptococcal polysaccharide of tumor necrosis factor alpha and interleukin-1 β secretion from human monocytes. *Infect. Immun.* **63**:2919–2923.
42. **Zuger, A., E. Louie, R. S. Holzman, M. S. Simberkoff, and J. J. Rahal.** 1986. Cryptococcal disease in patients with the acquired immunodeficiency syndrome: diagnostic features and outcome of treatment. *Ann. Intern. Med.* **104**:234–240.

Editor: T. R. Kozel