INFECTION AND IMMUNITY, Sept. 1996, p. 3446–3450 Vol. 64, No. 9
0019-9567/96/$04.00+0
Copyright © 1996, American Society for Microbiology

Opsonization of *Cryptococcus neoformans* by Human Anticryptococcal Glucuronoxylomannan Antibodies

ZHAOJING ZHONG[1] AND LIISE-ANNE PIROFSKI[1,2]*

Departments of Medicine[1] and Microbiology and Immunology,[2] Albert Einstein College of Medicine, Bronx, New York 10461

Received 4 April 1996/Returned for modification 30 April 1996/Accepted 31 May 1996

Cryptococcal meningitis occurs in 6 to 8% of human immunodeficiency virus-infected individuals. Despite the availability of powerful antifungal agents that are active against *Cryptococcus neoformans*, these drugs generally fail to cure cryptococcal infections in immunocompromised hosts. Alternative approaches to prevention and therapy of cryptococcosis are urgently needed. Complement promotes phagocytosis of *C. neoformans*, but human antibodies to cryptococcal capsular polysaccharide have not been shown to function as complement-independent opsonins. The goal of our studies was to characterize the in vitro biological function of human antibodies to glucuronoxylomannan (GXM) from individuals immunized with a GXM-tetanus toxoid (GXM-TT) vaccine. We studied sera from nine vaccinees that manifested good serologic responses to GXM-TT. The results indicate that GXM-TT-elicited antibodies promote phagocytosis of *C. neoformans* by both murine J774 cells and human peripheral blood mononuclear cells (PBMCs). The two sera with the highest titers of anti-GXM immunoglobulin G2 antibodies were the most opsonic. When PBMC FcγRIIa receptors were blocked, a 75% decrease in phagocytosis occurred following incubation of the PBMCs with *C. neoformans* opsonized with these sera. Our data indicate that, in the absence of complement, human anti-GXM-TT antibodies are opsonic and that antibodies of the immunoglobulin G2 isotype are effective opsonins.

Infection with the capsulated pathogenic fungus *Cryptococcus neoformans* is a profound problem in human immunodeficiency virus-infected individuals; in New York City, 6 to 8% of patients with AIDS develop cryptococcal meningitis (5). Treatment of *C. neoformans* infections presents a significant challenge because presently available antifungal agents do not completely eradicate the organism in individuals such as AIDS patients with severe underlying immunologic defects (38). One potential approach to reducing the incidence of cryptococcal infections in susceptible individuals is immunization. This entails active immunization with a vaccine to prevent infection and/or passive immunization with antibody-based therapies derived from immunization of healthy individuals. Based upon recent experience with murine cryptococcosis models, the success of the aforementioned approaches is likely to depend upon the capacity of human antibodies against cryptococcal capsular polysaccharide to enhance effector cell function against *C. neoformans* (11, 32, 36).

The glucuronoxylomannan (GXM) capsule of *C. neoformans* is antiphagocytic and essential for virulence (22). Opsonins for capsulated *C. neoformans* have been identified in many experimental systems (16, 21, 24, 36), and human serum containing active complement components opsonizes *C. neoformans* (6, 11). Anticryptococcal capsular antibodies from several species including mice, rats, and rabbits can promote antibody-dependent Fc-mediated phagocytosis of *C. neoformans* in vitro (16, 17, 36, 42). However, proof of the opsonic activity of human cryptococcal capsular antibodies has been hard to establish because it has been difficult to discriminate the contribution of antibody itself from that of serum complement-derived opsonins. In addition, high-affinity human cryptococcal capsular immunoglobulin G (IgG), which is necessary to evaluate antibody-mediated complement-independent phagocytosis, has not been available for study. Despite the presence of naturally occurring anticryptococcal capsular antibodies (7, 13, 18), heat-inactivated human sera fail to opsonize *C. neoformans* (6); however, the concentration of capsular IgG antibodies appears to be insufficient to mediate phagocytosis (18). Nonetheless, in one system, disparities between the antifungal activity mediated by complement-containing versus heat-inactivated sera could be partially reconstituted with purified serum immunoglobulin (11).

In the pre-AIDS era, cryptococcal meningitis patients with demonstrable serum cryptococcal capsular antibodies had an improved prognosis (10). Protection of humans against bacterial pathogens with polysaccharide capsules is mediated by capsular antibodies (40), and the mechanism of protection is usually enhancement of effector cell function by antibody-mediated phagocytosis (14, 15, 31, 40). The latter activity has been demonstrated for murine monoclonal GXM antibodies (MAbs) (36) elicited by GXM-tetanus toxoid (GXM-TT), which is a conjugate vaccine that contains a serotype A GXM (8). GXM-TT is immunogenic in mice, and murine GXM-TT-elicited antibodies are protective in models of murine cryptococcosis in vivo (33–35). The goal of the work presented below was to determine the opsonic activity of human antibodies elicited in response to GXM-TT.

MATERIALS AND METHODS

Sera and antibodies. Pre-GXM-TT immunization (preimmune) and post-GXM-TT immunization (immune) sera containing GXM antibodies from human volunteer recipients of GXM-TT at the National Institutes of Health, Bethesda, Md., were kindly provided by J. Robbins and R. Schneerson (National Institutes of Health). Sera did not contain anticoagulants or preservatives. Sera were stored at −20°C, thawed at room temperature, and heat inactivated at 56°C for 30 min prior to use. Table 1 lists the subjects studied. Because of the small amount of serum available from each subject all experiments could not be performed with each sample.

GXM ELISA. Costar enzyme-linked immunoassay (ELISA) plates (Corning Glass Works, Corning, N.Y.) were coated overnight with 50 μl of purified GXM from serotype A *C. neoformans* (strain 371) per well which was used at a

* Corresponding author. Mailing address: Division of Infectious Disease, Room 402 Forchheimer Building, 1300 Morris Park Ave., Bronx, NY 10461. Phone: (718) 430-2372. Fax: (718) 430-8968. Electronic mail address: pirofski@aecom.yu.edu.

concentration of 10 μg/ml as described previously (7). The plates were incubated for 1 h at 37°C with serial dilutions of preimmune or immune serum. After washing, bound antibodies were incubated with mouse anti-human IgG1 (HP 6069) or IgG2 (HP6002) (Sigma Chemical Co., St. Louis, Mo.), washed, and incubated for 1 h at 37°C with alkaline phosphatase-labeled goat anti-mouse IgG (Fisher Biotech, Fisher Scientific, Orangeburg, N.Y.). Then, the plates were washed and developed with *p*-nitrophenyl phosphate substrate (Sigma). The optical densities of the wells were read at A_{405} in a Ceres 900 ELISA reader (BioTek Instruments, Inc., Winooski, Vt.). Binding curves were drawn using Quattro Pro 4.0 software (Borland International, Inc., Scotts Valley, Calif.). The serum anti-GXM antibody titers were defined as the dilutions that produced a signal two times above background. For sera from subjects 14, 15, and 16, total anti-GXM IgG and IgM were determined using the same ELISA except that alkaline phosphatase-labeled goat anti-human IgG (Fisher Biotech) was used to detect IgG antibodies and alkaline phosphatase-labeled goat anti-human IgM (Fisher Biotech) was used to detect IgM antibodies.

GXM and organisms. Purified GXM from *C. neoformans* serotype A (strain 371) was provided by R. Schneerson (National Institutes of Health). Cells of *C. neoformans* serotype D (24067) were used for all phagocytosis experiments because this strain has been used extensively for the in vitro and in vivo evaluation of murine GXM-TT-elicited antibodies (33–36). Human MAbs generated from a GXM-TT-immunized individual bind the 24067 strain (39). Yeast cells were grown in Sabouraud dextrose broth (Difco Laboratories, Detroit, Mich.) for 72 h prior to use.

Phagocytic cells. Peripheral blood mononuclear cells (PBMCs) were separated from venous blood from a healthy volunteer by density-gradient centrifugation on Ficoll-Hypaque (Pharmacia Biotech, Piscataway, N.J.) using published techniques (27). T lymphocytes were removed by rosetting with AET (2-aminoethylisothiouronium bromide)-treated sheep erythrocytes (BioWhittaker, Walkersville, Md.). The remaining mononuclear cells were resuspended in RPMI medium containing 10% heat-inactivated human serum from the donor, 1% essential amino acids, 1% sodium pyruvate, 1% HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), and 50 IU of penicillin/50 μg of streptomycin per ml. All cell culture reagents were obtained from Mediatech, Washington, D.C., unless otherwise stated. The cells were plated on Lab Tek 8 chamber tissue culture slides (Nunc, Inc., Naperville, Ill.) or 96-well plastic tissue culture plates (Nunc). Nonadherent cells were washed away with phosphate-buffered saline (PBS), and the remaining cells were left to form monolayers. PBMC monolayers were used after 8 days of culture. Only wells or slides with confluent monolayers were used. All experiments were performed with cells from one immunocompetent donor.

Murine J774 cells cultured in tissue culture dishes (Nunc) were plated on Lab Tek tissue culture slides (Nunc) or 96-well tissue culture plates (Nunc) at a density of 10^5 per well. For some phagocytosis experiments, the J774 cells were preincubated with 500 U of recombinant mouse gamma interferon (Genzyme, Boston, Mass.) per ml for 18 h prior to treatment with *C. neoformans*. The monolayers were washed with cell culture medium two times before addition of medium containing the opsonized organisms and (in some experiments) 500 U of gamma interferon per ml.

Phagocytosis. Organisms treated with PBS (used as a control) and preimmune and immune sera were used for phagocytosis experiments at a yeast cell:phagocyte ratio of 10:1. Aliquots of 10^6 *C. neoformans* cells were incubated for 1 h at 37°C with PBS or a 1:20 dilution of heat-inactivated preimmune or immune serum. All of the PBS- and serum-treated organisms were then added to monolayers consisting of 10^5 murine J774 cells or human PBMCs, and the mixtures were incubated for 4 h at 37°C. After incubation, the cells were washed in PBS three times, stained with Giemsa, and visualized by light microscopy. The number of cells in the monolayers was confirmed by counting Giemsa-stained cells on the slide at the end of the experiment. Each experiment was performed two times, in duplicate. For experiments with murine J774 cells and fluorescein isothiocyanate (FITC)-labeled *C. neoformans* (see below), the phagocytic index (PI) was defined as the mean number of attached and internalized *C. neoformans* cells per 200 macrophages in each of the four different wells. The human PBMCs adhered to the wells in a more heterogeneous manner than the J774 cells. Therefore, in order to assess a larger number of cells in the experiments using PBMCs, the PI was defined as the mean number of attached and internalized *C. neoformans* cells in eight discontinuous 40× microscope fields (500 to 900 cells) divided by the number of mononuclear cells, to yield the number of organisms per mononuclear cell.

For some experiments, the FcγRIIa receptor was blocked by pretreatment of human PBMCs with the anti-FcγRIIa IgG2b murine MAb IV.3 (Medarex Inc., Annandale, N.J.) (10). Murine IgG2b (Fisher Biotech), a myeloma protein of unknown specificity, was used as an irrelevant isotype control. Human PBMCs were preincubated for 30 min at 4°C with 5 μg of either MAb IV.3 or murine IgG2b per ml. Then, the cells were washed and phagocytosis experiments were done with PBS or serum treated, FITC-labeled *C. neoformans*. This method evaluates phagocytosis of killed organisms, a standard technique for determining phagocytosis of fungi (2). FITC labeling was performed according to published methods (12). Briefly, heat-killed *C. neoformans* cells were washed with PBS and incubated for 1 h at room temperature with 0.1 mg of FITC isomer 1 (Sigma Chemical Co., St. Louis, Mo.) per ml in 0.1 M $NaHCO_3$, pH 9.0. FITC-labeled organisms were indistinguishable from unlabeled *C. neoformans* with respect to the binding of anti-GXM antibodies by ELISA. Organisms and PBMCs were visualized with fluorescence and bright-field microscopy using a Zeiss Axiophot microscope and photographed with a Sony video color printer, both of which are part of the Analytical and Ultrastructural Facility of the Cancer Center of the Albert Einstein College of Medicine. The percent inhibition of phagocytosis was calculated by comparison of the PIs obtained using cells with and without FcγRIIa blockade. Statistical analyses were performed using the two-sample Student *t* test with the assistance of C. J. Chang, Department of Epidemiology and Biostatistics, Albert Einstein College of Medicine.

TABLE 1. Anti-GXM antibody titers following GXM-TT immunization and relative opsonic activity of sera for phagocytosis of *C. neoformans* by murine J774 cells and human PBMCs

Donor (serum)	ELISA anti-GXM IgG2 titer	ELISA anti-GXM IgG titer	ELISA anti-GXM IgG1 titer	ELISA anti-GXM IgM titer	Rank order of opsonic activity
14	ND[a]	1:1,000	ND	1:180	2[b]
15	ND	1:540	ND	1:540	Not opsonic
16	1:500	1:9,720	1:100	1:100	1[b]
31	1:200		1:100		4[c]
30	1:100		<1:10		4[c]
12	1:100		1:200		3[c]
9	1:100		1:20		3[c]
32	1:400		<1:10		2[c]
29	1:2,000		1:90		1[c]

[a] ND, not determined.
[b] J774 cells, Fig. 1.
[c] Human PBMCs, Fig. 2.

RESULTS

ELISA. Anti-GXM IgM and IgG titers were determined for preimmune and immune sera from subjects 14, 15, and 16. The GXM binding titers for the immune sera are shown in Table 1. The relative titers of sera from these individuals was 16 > 14 > 15 for IgG and 15 > 14 ≥ 16 for IgM antibodies. Anti-GXM IgG1 and IgG2 titers were determined for only the immune sera from seven of the vaccinees because the anti-GXM IgG levels in preimmune sera were very low and essentially undetectable in the other sera studied (data not shown). Table 1 shows that all tested vaccinated subjects had detectable titers of anti-GXM IgG2, and five of seven had anti-GXM IgG1. Subjects 32 and 30 had no detectable anti-GXM IgG1 at a serum dilution of 1:10.

Phagocytosis. The opsonic activities of sera from GXM-TT recipients were examined with both murine J774 cells and human PBMCs. Since the murine J774 cell system has been extensively used for evaluation of murine anti-GXM MAb-mediated phagocytosis, we first evaluated sera from subjects 14, 15, and 16 according to published methods (36). These experiments revealed differences in the opsonic activity of preimmune and immune sera and in phagocytosis by unstimulated and gamma interferon-stimulated J774 cells. Compared with preimmune sera, immune sera from subjects 14 and 16 were opsonic. Following opsonization of *C. neoformans* by these sera, phagocytosis by both untreated and gamma interferon-stimulated J774 cells was significantly enhanced ($P < 0.05$). Without gamma interferon stimulation of J774 cells, organisms treated with preimmune and immune sera from subjects 14 and 16 produced significantly higher PIs than those treated with PBS, whereas with gamma interferon stimulation of organisms treated with immune serum only enhanced phagocytosis (Fig. 1). Preimmune-serum-treated organisms from subject 15 enhanced phagocytosis compared with PBS, but immune serum from subject 15 was not opsonic (Fig. 1). Given that the titers of anti-GXM IgG in preimmune sera were very low (data not shown) and the fact that two of three immune

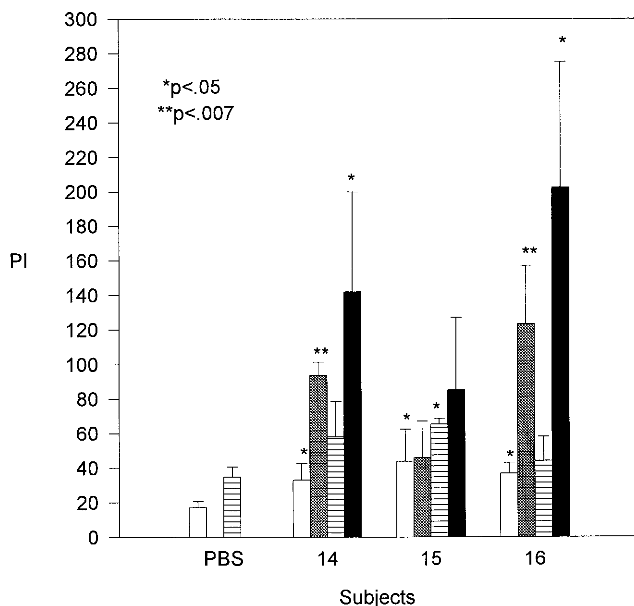

FIG. 1. Phagocytosis of *C. neoformans* by unstimulated and gamma interferon-stimulated murine J774 cells treated with preimmune and immune serum-opsonized organisms. The *y* axis shows the PIs, which were determined as indicated in the text. The *x* axis indicates gamma interferon stimulation of J774 cells and the numerical designations for the sera from the subjects studied. The open bars represent unstimulated J774 cells treated with preimmune serum or PBS-treated *C. neoformans*. The cross-hatched bars represent unstimulated J774 cells treated with immune serum-treated *C. neoformans*. The open bars with horizontal stripes represent gamma interferon-stimulated J774 cells treated with preimmune serum or PBS-treated *C. neoformans*. The closed bars represent gamma interferon-stimulated J774 cells treated with immune serum-treated *C. neoformans*. * and **, PIs that are significant in comparison with value for PBS (Student *t* test).

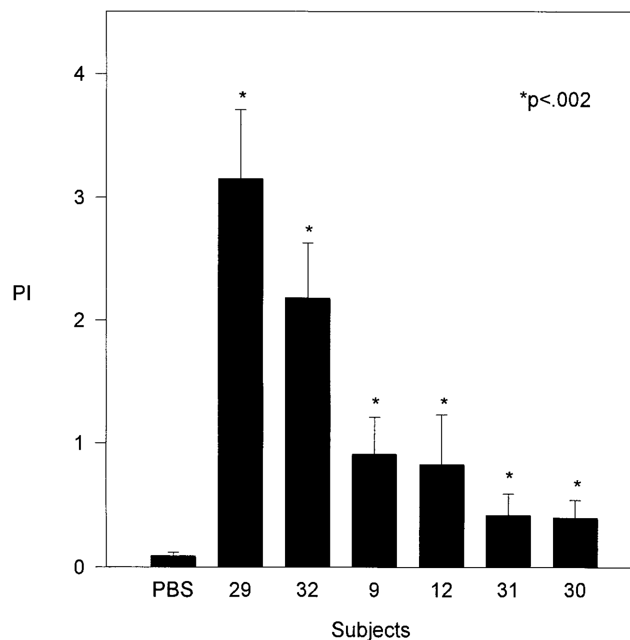

FIG. 2. Phagocytosis of immune serum-opsonized *C. neoformans* by human PBMCs with and without FcγRIIa blockade. The *y* axis shows the PIs. The PI, shown as the number of organisms per mononuclear cell, was determined as described in the text. The *x* axis indicates the numerical designations for the immune sera from the subjects studied. *, significant (Student *t* test), compared with the PBS control. The PIs following opsonization: serum 29 > serum 32 > serum 9 = serum 12 > serum 30 = serum 31 ($P < 0.05$ [one-way analysis of variance, paired Student *t* test).

sera were opsonic, preimmune sera were not used in subsequent experiments. Six immune sera from different subjects were studied in phagocytosis experiments with human PBMCs. Treatment of human PBMCs with *C. neoformans* treated with all six sera significantly enhanced phagocytosis compared with cells pretreated with PBS (Fig. 2). In comparison with one another, there were significant differences in the opsonic activities of the individual sera (Fig. 2).

To evaluate the role of anti-GXM IgG2 in opsonization, the two sera with the highest anti-GXM IgG2 titers, sera 16 and 29, were studied after PBMC Fc receptor blockade with MAb IV.3 (2). This murine IgG2b Ab blocks the human FcγRIIa receptor. There was a >75% decrease in phagocytosis when PBMCs treated with MAb IV.3 were incubated with organisms opsonized with sera from subjects 16 (Fig. 3) and 29 (data not shown). Incubation of the PBMCs with murine IgG2b as an irrelevant isotype control (Fig. 3A and B) resulted in a PI similar to that obtained with PBS-treated phagocytes (data not shown).

DISCUSSION

Our data demonstrate that human sera containing GXM-TT-elicited anti-GXM antibodies opsonize a capsulated serotype D strain of *C. neoformans* and promote phagocytosis by murine J774 cells and human PBMCs. Immune sera contained markedly higher titers of anti-GXM IgG than preimmune sera, and immune sera from subjects 14 and 16 were at least three times more opsonic than preimmune sera (Fig. 1). Therefore, anti-GXM IgG is the most likely functional mediator of phagocytosis. Anti-GXM IgM is an unlikely opsonin because our experiments were performed without complement and other heat-labile serum components; in addition, an Fc receptor for human IgM has not been identified on human phagocytes (37). The differences between the PIs of preimmune and immune sera support the findings of others that naturally occurring human serum anti-GXM antibodies are not opsonic (6, 18, 26, 28). In unstimulated murine J774 cells, opsonization of *C. neoformans* with preimmune sera from subjects 14, 15, and 16 enhanced phagocytosis compared with treatment with PBS alone. In contrast, for gamma interferon-stimulated cells, PBS treatment produced a higher PI, preimmune sera from subjects 14 and 16 were not opsonic, and the PIs for immune sera were higher (Fig. 1). These experiments support the hypothesis that the activation state of effector cells influences human antibody-mediated phagocytosis of *C. neoformans* (4, 27, 36).

Phagocytosis of *C. neoformans* by murine and human phagocytes was enhanced after treatment with organisms opsonized with immune sera from eight of nine subjects (Fig. 1 and 2). Murine anti-GXM MAbs and lapine polyclonal capsular antibodies promote complement-independent effector cell function against *C. neoformans* (9, 27, 36). The PIs following opsonization of *C. neoformans* with immune sera were comparable to the PIs reported following opsonization with murine anti-GXM MAbs that are protective or alter the course of infection in vivo (33–36, 41, 42). Immune serum from subject 15 was not opsonic, and the relative proportions of anti-GXM IgM and IgG were different for this serum. Compared with subjects 14 and 16, subject 15 had a higher titer of anti-GXM IgM and a lower titer of anti-GXM IgG (Table 1). Biologically functional antibodies may be diluted or masked by antibodies of different isotypes (3, 25). Therefore, could anti-

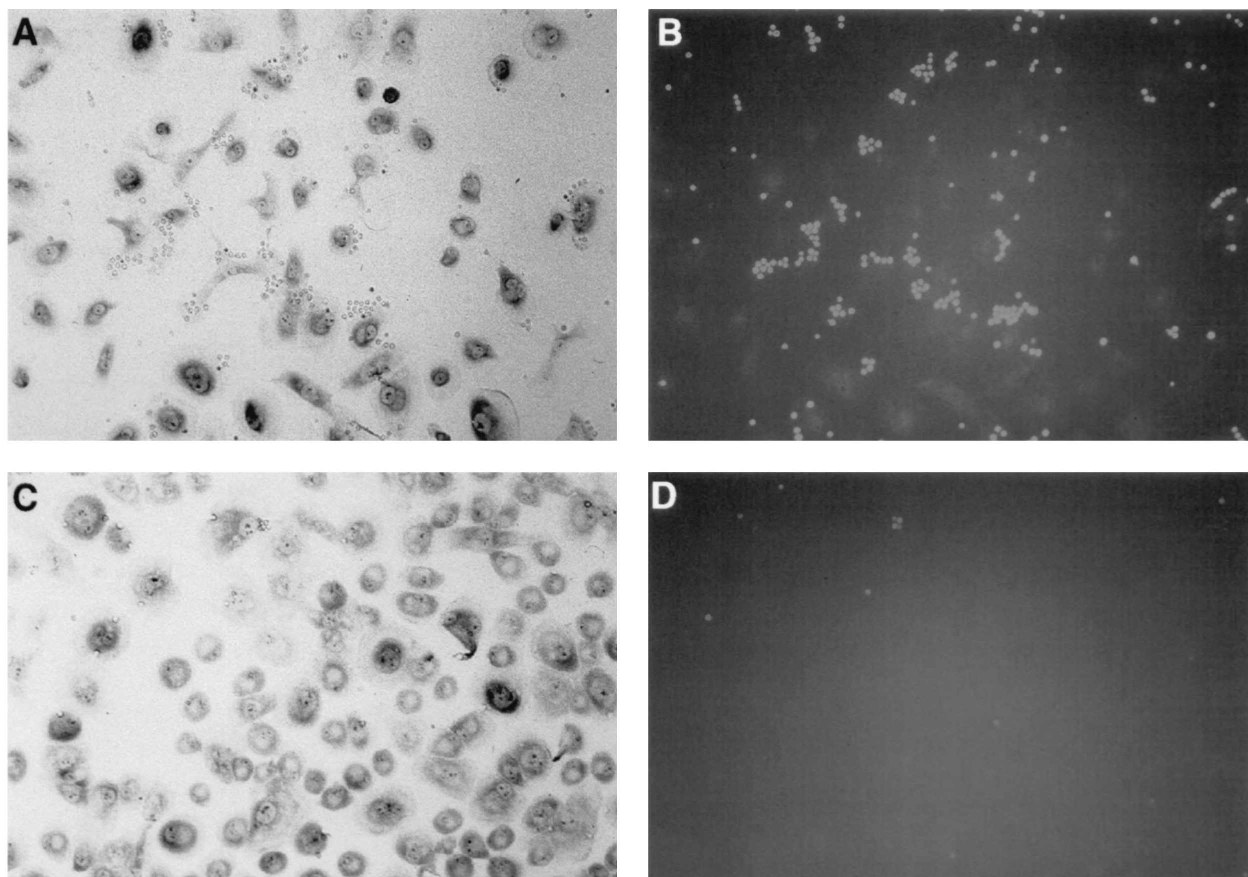

FIG. 3. Phagocytosis of *C. neoformans* by untreated and FcγRIIa-treated human PBMCs following opsonization with immune serum from subject 16. (A) PBMCs preincubated with isotype control murine IgG2b and then treated with *C. neoformans* opsonized with immune serum (light microscopy, 40× magnification). (B) Fluorescence microscopy of the field shown in panel A. (C) PBMCs preincubated with MAb IV.3 treated with *C. neoformans* opsonized with immune serum (light microscopy, 40× magnification). (D) Fluorescence microscopy of the field shown in panel C.

GXM IgM block Fc-receptor mediated phagocytosis by anti-GXM IgG when complement opsonins are absent? Deleterious anti-GXM antibodies have been identified (38, 44), and epitope blocking by antibodies unable to fix complement has been reported for other pathogens (20, 25). Individual differences in the opsonic power of serum anti-GXM antibodies will be better understood when presently undefined variables are elucidated for human anti-GXM antibodies, such as their fine specificity, idiotype, and isotype.

The predominant subclass of naturally occurring anti-GXM IgG antibodies is IgG2 (7, 18). Although these antibodies have not been shown to promote phagocytosis of *C. neoformans* in vitro, this may be attributable in part to the lack of high-titer antibodies (18). For our phagocytosis experiments performed with human PBMCs, the *C. neoformans* cells were opsonized with immune sera that contained anti-GXM IgG2 antibodies (Table 1). The highest PIs were observed following treatment of the phagocytes with organisms opsonized with immune serum from the individuals that had the highest IgG2 titers, subjects 16 and 29 and 32 (Table 1; Fig. 1 to 3). There was no correlation between anti-GXM IgG1 titers and PI; serum from subject 32 had the second highest PI and contained no detectable anti-GXM IgG1, and sera from subjects 9 and 12 had statistically similar PIs despite a 10-fold difference in anti-GXM IgG1 titer (Table 1, Fig. 2). There was a greater than 75% reduction in phagocytosis by FcγIIa-blocked PBMCs for phagocytes treated with *C. neoformans* opsonized with immune sera from subjects 16 (Fig. 3) and 29 (data not shown). This suggests that FcRγIIa, the only human Fc receptor that binds IgG2 (19), mediates antibody-dependent phagocytosis of *C. neoformans* by human PBMCs. In this regard, effector cell function against *C. neoformans* may resemble that against serotype 14 *Streptococcus pneumoniae* in that only pneumococcal anticapsular IgG2 is opsonic in heat-activated serum (29). Our data demonstrating that anti-GXM IgG2 can mediate complement-independent phagocytosis of *C. neoformans* supports a correlation between the opsonic activity of immune serum and serum anti-GXM IgG2 antibody titer.

The determinants of human PBMC activation for phagocytosis of *C. neoformans* in vitro and in vivo are incompletely defined. Phagocytosis is influenced by the expression and specificity of the opsonin receptors on effector cells (1, 43) and cellular activation factors that induce pathogen binding and internalization (4, 23). A critical determinant of host defense against *C. neoformans* is likely to be the presence of the appropriate opsonin(s) for the available effector cell receptors. Complement-dependent phagocytosis of *C. neoformans* is a regulated event that requires cytokine activation (4, 16) whereas Fc receptor-mediated phagocytosis by macrophages is not (16). HIV-associated abnormalities in cell-mediated immunity could result in decreased production of cytokine mediators necessary for complement-dependent antifungal host defense (4). Such abnormalities would compound the problem of complement depletion that occurs during cryptococcosis

(30). Anti-GXM antibodies might help enhance host defense against *C. neoformans* by augmenting effector cell function through a mechanism which is independent of T-cell-mediated stimulation. In this regard, the ability of anti-GXM IgG2 to promote complement-independent phagocytosis of *C. neoformans* has important ramifications for antibody therapy of cryptococcosis in individuals with AIDS.

ACKNOWLEDGMENTS

This work was supported in part by NIH grants K11AI80087 (L.A.P) and RO1AI35370 (L.A.P). We gratefully acknowledge this support.

We also thank A. Casadevall and M. Scharff for their intellectual contributions to this work which came in the form of many discussions.

REFERENCES

1. **Berger, M., T. M. Norvell, M. F. Tosi, S. N. Emancipator, M. W. Konstan, and J. R. Schreiber.** 1994. Tissue-specific Fc gamma and complement receptor expression by alveolar macrophages determines relative importance of IgG and complement in promoting phagocytosis of Pseudomonas aeruginosa. Pediatr. Res. **35:**68–77.
2. **Bjerknes, R., C. F. Bassoe, H. Sjursen, O. D. Laerum, and C. O. Solberg.** 1989. Flow cytometry for the study of phagocyte functions. Rev. Infect. Dis. **11:**16–24.
3. **Casadevall, A., and M. D. S. Scharff.** 1994. Serum therapy revisited: animal models of infection and the development of passive antibody therapy. Antimicrob. Agents Chemother. **38:**1695–1702.
4. **Collins, H. L., and G. J. Bancroft.** 1992. Cytokine enhancement of complement-dependent phagocytosis by macrophages: synergy of tumor necrosis factor-alpha and granulocyte-macrophage colony-stimulating factor for phagocytosis of Cryptococcus neoformans. Eur. J. Immunol. **22:**1447–1454.
5. **Currie, B. P., and A. Casadevall.** 1994. Estimation of the prevalence of cryptococcal infection among patients infected with the human immunodeficiency virus in New York City. Clin. Infect. Dis. **19:**1029–1033.
6. **Davies, S. F., D. P. Clifford, J. R. Hoidal, and J. E. Repine.** 1982. Opsonic requirements for the uptake of *Cryptococcus neoformans* by human polymorphonuclear leukocytes and monocytes. J. Infect. Dis. **145:**870–874.
7. **DeShaw, M., and L. Pirofski.** 1995. Antibodies to *Cryptococcus neoformans* capsular polysaccharide glucuronoxylomannan are ubiquitous in the serum of HIV+ and HIV− individuals. Clin. Exp. Immunol. **99:**425–432.
8. **Devi, S. G. N., R. Schneerson, W. Egan, T. J. Ulrich, D. Bryla, J. B. Robbins, and J. E. Bennett.** 1991. *Cryptococcus neoformans* serotype A glucuronoxylomannan-protein conjugate vaccines: synthesis, characterization, and immunogenicity. Infect. Immun. **59:**3700–3707.
9. **Diamond, R. D.** 1974. Antibody-dependent killing of *Cryptococcus neoformans* by human peripheral blood mononuclear cells. Nature (London) **247:**148–150.
10. **Diamond, R. D., and J. E. Bennett.** 1974. Prognostic factors in cryptococcal meningitis. Ann. Intern. Med. **80:**176–181.
11. **Diamond, R. D., J. E. May, M. A. Kane, M. M. Frank, and J. E. Bennett.** 1974. The role of the classical and alternative complement pathways in host defenses against *Cryptococcus neoformans* infection. J. Immunol. **112:**2260–2270.
12. **Drevets, D. A., and P. A. Campbell.** 1991. Macrophage phagocytosis: use of fluorescence microscopy to distinguish between extracellular and intracellular bacteria. J. Immunol. Methods **142:**31–38.
13. **Dromer, F., P. Aucouturier, J. Clauvel, G. Saimot, and P. Yeni.** 1988. *Cryptococcus neoformans* antibody levels in patients with AIDS. Scand. J. Infect. Dis. **20:**283–285.
14. **Fothergill, L. D., and J. Wright.** 1933. Influenzal meningitis: relation of age incidence to the bactericidal power of blood against the causal organism. J. Immunol. **24:**273–284.
15. **Goldschneider, I., E. C. Gotschlich, and M. S. Artenstein.** 1969. Human immunity to the meningococcus. I. The role of humoral antibodies. J. Exp. Med. **129:**1307–1326.
16. **Griffin, F. M., Jr.** 1981. Roles of macrophage Fc and C3b receptors in phagocytosis of immunologically coated Cryptococcus neoformans. Proc. Natl. Acad. Sci. USA **78:**3853–3857.
17. **Hobbs, M. M., J. R. Perfect, D. L. Granger, and D. T. Durack.** 1990. Opsonic activity of cerebrospinal fluid in experimental cryptococcal meningitis. Infect. Immun. **58:**2115–2119.
18. **Houpt, D. C., T. Pfrommer, B. Young, T. A. Larson, and T. R. Kozel.** 1994. Occurrences, immunoglobulin classes, and biological activities of antibodies in normal human serum that are reactive with *Cryptococcus neoformans* glucuronoxylomannan. Infect. Immun. **62:**2857–2864.
19. **Indik, Z. K., J. G. Park, X. Q. Pan, and A. D. Schreiber.** 1995. Induction of phagocytosis by a protein tyrosine kinase. Blood **85:**1175–1180.
20. **Jarvis, G. A., and J. M. Griffis.** 1991. Human IgA1 blockade of IgG-initiated lysis of *Neisseria meningitidis* is a function of Fab binding to the polysaccharide capsule. J. Immunol. **147:**1962–1967.
21. **Kozel, T. R.** 1993. Opsonization and phagocytosis of *Cryptococcus neoformans*. Arch. Med. Res. **24:**211–218.
22. **Kozel, T. R., and E. C. Gotschlich.** 1982. The capsule of *Cryptococcus neoformans* passively inhibits phagocytosis of the yeast by macrophages. J. Imunol. **129:**1675–1680.
23. **Kozel, T. R., and T. G. McGaw.** 1979. Opsonization of *Cryptococcus neoformans* by human immunoglobulin G role of immunoglobulin G in phagocytosis by macrophages. Infect. Immun. **18:**701–707.
24. **Kozel, T. R., G. S. T. Pfrommer, A. S. Guerlain, B. A. Highison, and G. J. Highison.** 1988. Role of the capsule in phagocytosis of *Cryptococcus neoformans*. Rev. Infect. Dis. **10:**S436–S439.
25. **Kozlowski, P. A., B. P. Kelley, L. Shen, and S. Jackson.** 1996. High prevalence of serum IgA HIV-1 infection-enhancing antibodies in HIV-infected persons. J. Immunol. **154:**6163–6173.
26. **Levitz, S. M., and T. P. Farrell.** 1990. Growth inhibition of *Cryptococcus neoformans* by cultured human monocytes: role of the capsule, opsonins, the culture surface, and cytokines. Infect. Immun. **58:**1201–1209.
27. **Levitz, S. M., T. P. Farrell, and R. T. Maziarz.** 1990. Killing of *Cryptococcus neoformans* by peripheral blood mononuclear cells stimulated in culture. J. Infect. Dis. **163:**1108–1113.
28. **Levitz, S. M., and A. Tabuni.** 1991. Binding of Cryptococcus neoformans by human cultured macrophages. Requirements for multiple complement receptors and actin. J. Clin. Invest. **87:**528–535.
29. **Lortan, J. E., A. S. Kanuik, and M. A. Monteil.** 1993. Relationship of *in vitro* phagocytosis of serotype 14 *Streptococcus pneumoniae* to specific class and IgG subclass antibody levels in healthy adults. Clin. Exp. Immunol. **91:**54–57.
30. **Macher, A. M., J. E. Bennett, J. D. Gadek, and M. M. Frank.** 1978. Complement depletion in cryptococcal sepsis. J. Immunol. **120:**1686–1690.
31. **MacLeod, C. M., R. G. Hodges, M. Heidelberger, and W. G. Berhnard.** 1945. Prevention of pneumococcal pneumonia by immunization with specific polysaccharides. J. Exp. Med. **82:**445–465.
32. **Mozaffarian, N., J. W. Berman, and A. Casadevall.** 1995. Immune complexes increase nitric oxide production by interferon-gamma-stimulated murine macrophage-like J774.16 cells. J. Leukocyte Biol. **57:**657–662.
33. **Mukherjee, J., L. Pirofski, M. D. Scharff, and A. Casadevall.** 1993. Antibody-mediated protection in mice with lethal intracerebral *Cryptococcus neoformans* infection. Proc. Natl. Acad. Sci. USA **90:**3636–3640.
34. **Mukherjee, J., M. D. Scharff, and A. Casadevall.** 1992. Protective murine monoclonal antibodies to *Cryptococcus neoformans*. Infect. Immun. **60:**4534–4541.
35. **Mukherjee, S., S. Lee, J. Mukherjee, M. D. Scharff, and A. Casadevall.** 1994. Monoclonal antibodies to *Cryptococcus neoformans* capsular polysaccharide modify the course of intravenous infection in mice. Infect. Immun. **62:**1079–1088.
36. **Mukherjee, S., S. C. Lee, and A. Casadevall.** 1995. Antibodies to *Cryptococcus neoformans* glucuronoxylomannan enhance antifungal activity of murine macrophages. Infect. Immun. **63:**573–579.
37. **Ohno, T., H. Kubagawa, S. K. Sanders, and M. D. Cooper.** 1990. Biochemical nature of an Fc mu receptor on human B-lineage cells. J. Exp. Med. **172:**1165–1175.
38. **Pirofski, L., and A. Casadevall.** 1996. *Cryptococcus neoformans*: paradigm for the role antibody in immunity. Zentralbl. Bakteriol., in press.
39. **Pirofski, L., R. Lui, M. DeShaw, A. B. Kressel, and Z. Zhong.** 1995. Analysis of human monoclonal antibodies elicited by vaccination with a *Cryptococcus neoformans* glucuronoxylomannan capsular polysaccharide vaccine. Infect. Immun. **63:**3005–3014.
40. **Robbins, J. B., R. Schneerson, and S. C. Szu.** 1995. Perspective: hypothesis: serum IgG antibody is sufficient to confer protection against infectious diseases by inactivating the inoculum. J. Infect. Dis. **171:**1387–1398.
41. **Sanford, J. E., D. M. Lupan, A. M. Schalgeter, and T. R. Kozel.** 1990. Passive immunization against *Cryptococcus neoformans* by a family of isotype-switch variant antibodies specific for the capsular polysaccharide. Infect. Immun. **58:**1919–1923.
42. **Schlageter, A. M., and T. R. Kozel.** 1990. Opsonization of *Cryptococcus neoformans* by a family of isotype-switch variant antibodies specific for the capsular polysaccharide. Infect. Immun. **58:**1914–1918.
43. **Wallace, P. K., A. L. Howell, and M. W. Fanger.** 1994. Role of Fc(gamma) receptors in cancer and infectious diseases. J. Leukocyte Biol. **55:**816–826.
44. **Yuan, R., A. Casadevall, G. Spira, and M. D. Scharff.** 1995. Isotype switching from IgG3 to IgG1 converts a nonprotective antibody murine antibody to *Cryptococcus neoformans* to a protective antibody. J. Immunol. **154:**1810–1815.

Editor: T. R. Kozel