Antibody-mediated Modulation of *Cryptococcus neoformans* Infection Is Dependent on Distinct Fc Receptor Functions and IgG Subclasses

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Summary

Coupling of an antibody response to effector cells through the Fc region of antibodies is a fundamental objective of effective vaccination. We have explored the role of the Fc receptor system in a murine model of *Cryptococcus neoformans* protection by infecting mice deleted for the common γ chain of FcRs. Passive administration of an IgG1 mAb protects FcRγ$^{+/-}$ mice infected with *C. neoformans*, but fails to protect FcRγ$^{-/-}$ mice, indicating that the γ chain acting through FcγRI and/or III is essential for IgG1-mediated protection. In contrast, passive administration of an IgG3 mAb with identical specificity resulted in enhanced pathogenicity in γ chain–deficient and wild-type mice. In vitro studies with isolated macrophages demonstrate that IgG1-, IgG2a-, and IgG2b-opsonized *C. neoformans* are not phagocytosed or arrested in their growth in the absence of the FcRγ chain. In contrast, opsonization of *C. neoformans* by IgG3 does not require the presence of the γ chain or of FcRII, and the internalization of IgG3-treated organisms does not arrest fungal growth.

Cryptococcus neoformans is an encapsulated fungus that infects immunosuppressed individuals and is responsible for the death of 6–8% of AIDS patients (1). Antibodies to the glucuronoxylomannan (GXM)[1] portion of the capsular polysaccharide modulate the infection (2). We have previously demonstrated that anticryptococcal IgG3 mAbs are not protective in mouse models of cryptococcal infection (3–5). However, when the nonprotective IgG3 mAbs were switched in vitro to other downstream IgG isotypes, these antibodies became protective (4, 5). Since the IgG3 antibodies and their switch variants have identical antigen binding sites (5), this observation underscores the importance of Fc-mediated functions for antibody efficacy against *C. neoformans*. In principle, the Fc domain of IgG immune complexes can interact with a variety of soluble and cell-bound molecules that may be involved in mediating the protective capacity of this antibody. Thus, complement C1q binding can lead to the activation of C3, which leads in turn to either association with CR1/2 or generation of a membrane attack complex through C5 (6). Direct interaction of IgG1 complexes with cognate FcRs could mediate either fungal killing, or the arrest of fungal growth through NK or macrophage-mediated antibody-dependent cytotoxicity (ADCC) (7), macrophage phagocytosis, or neutrophil activation (8). Finally, IgG1, through its interaction with the FcRn transporter (for review see reference 9), could be involved in clearance of opsonized cells.

In this study, we have begun the dissection of the mechanisms by which IgG subclasses mediate their biological responses by comparing the capacity of the IgG3 and the IgG1 isotypes to (*a*) modulate cryptococcal infection in FcR-deficient mice in vivo and (*b*) mediate macrophage phagocytosis and the arrest of fungal growth in vitro. Three different classes of murine Fc receptors for IgG (FcγRI, FcγRII and FcγRIII) have been identified on immune effector cells (for review see references 10–12). Activation by cross-linking of FcγRI and III on macrophages, NK cells, mast cells, neutrophils, and other myeloid cells by antigen–antibody complexes leads to activation of diverse biological responses including phagocytosis, ADCC (for review see references 10, 12, 13), and release of inflammatory mediators (for review see reference 14). The γ subunit of Fc receptors is an essential component of type I and III receptors for IgG antibodies and is required for both receptor assembly and signal transduction (15, 16). FcRγ chain deletion leads to impaired macrophage phagocytosis of IgG-coated SRBCs despite persistent binding, and leads to defects in NK cell–

[1] *Abbreviations used in this paper:* ADCC, antibody-dependent cytotoxicity; GXM, glucuronoxylomannan.

mediated ADCC (17). In contrast, cross-linking of FcγRII does not elicit a biological response on effector immune cells. However, when coligated to an activation receptor, such as FcγRIII, B cell receptor, or TCR, FcγRII inhibits the activation signal generated in those cells by inhibiting calcium influx to the cell through the recruitment of the SH2 inositol polyphosphate phosphatase SHIP (18, 19). Deletion of FcγRII results in mice with hyperresponsive B cells, mast cells, and macrophages (20).

Here, we report that the FcRγ chain–deficient mice and heterozygous littermate controls are equally susceptible to cryptococcal infection and that γ chain is essential for IgG1-mediated passive protection against this pathogen. In addition, IgG3 mediated the phagocytosis of *C. neoformans* and SRBCs in the absence of FcγRI, II, or III function, suggesting that it either uses a different FcR for IgG3 or a signal transduction pathway that is different from the other FcγRs. In contrast to the ability of IgG1 and IgG2a mAbs to inhibit fungal growth, IgG3-mediated phagocytosis is not associated with the arrest of fungal replication. The findings in this study provide a rationale for the different protective efficacies of IgG3 and IgG1 mAbs in mice infected with *C. neoformans*.

Materials and Methods

C. neoformans. Strain 24067 (serotype D) was obtained from the American Type Culture Collection (Rockville, MD) and maintained on Sabouraud dextrose agar (Difco, Detroit, MI) at 4°C. For murine infection, *C. neoformans* was grown at 37°C in Sabouraud dextrose broth (Difco) for 24 h. Yeast cells were washed three times with PBS and the inoculum was determined by counting in a hemocytometer and by scoring the CFU plated on Sabouraud dextrose agar plates.

Monoclonal Antibodies. The 3E5 IgG3 was made in response to immunization with the GXM fraction of the *C. neoformans* capsular polysaccharide conjugated to tetanus toxoid (21). The IgG1, IgG2a, and IgG2b switch variants of 3E5 IgG3 were generated by in vitro isotype switching (5). The variable region sequence of the IgG1 switch variant was sequenced and is identical to 3E5 IgG3 and all of the switch variants bind GXM (5). Ascites was obtained by injecting 10^7 hybridoma cells into the peritoneal cavity of Pristane-primed (Sigma Chemical Co., St. Louis, MO) BALB/c mice. Antibody concentration was determined by an ELISA relative to isotype matched standards of known concentration. For in vitro studies, antibodies were purified by protein G chromatography (Pierce Chemical Co., Rockford, IL), and were sterilized by filtering through a 0.2-mm-pore-size membrane (Sigma Chemical Co.). For some in vitro experiments, the purified antibodies were incubated at 56°C for 30 min to inactivate complement. Purified anti-SRBC mAbs (anti-SRBC IgG1, IgG2a, IgG2b, and IgG3) for rosetting assays were supplied by Dr. B. Diamond (Albert Einstein College of Medicine; reference 22).

Animal Experiments. Female C57BL/6J mice were purchased from The Jackson Laboratory (Bar Harbor, ME). FcRγ chain knockout ($FcR\gamma^{-/-}$) mice and wild-type ($FcR\gamma^{+/+}$) or heterozygous ($FcR\gamma^{+/-}$) controls had been backcrossed to C57BL/6J for eight generations. Mice kept under specific pathogen-free conditions were used for protection experiments at 6 wk of age. 10 mice per group were given 1 mg of IgG1, IgG3, or PBS as a control. mAbs were administered via intraperitoneal injection 24 h before intravenous challenge with 5×10^6 *C. neoformans*, and mouse deaths were recorded daily.

Organ CFUs and Serum GXM Levels. Groups of five mice were treated with 1 mg of IgG1, IgG3, or PBS 1 d before infection. CFUs were determined 14 d after infection by plating homogenized brain or lung tissue on Sabouraud dextrose agar (5). CFUs are expressed as the mean ± SD.

Serum GXM levels were determined by capture ELISA. Before ELISA, serum obtained 14 d after infection was diluted 1:25 with PBS, incubated overnight at 37°C with 0.2 mg/ml of proteinase K, and then heated for 20 min at 100°C (5). Serum GXM concentration was determined relative to GXM standards of known concentration.

In Vitro Macrophage Phagocytosis. Bronchoalveolar macrophage cells were obtained from C57BL/6J, $FcR\gamma^{+/+}$, $FcR\gamma^{-/-}$, or FcRγ-chain and FcγRII double knockout ($FcR\gamma^{-/-}$ /$RIIB^{-/-}$) mice (Clynes, R., unpublished observations). For the phagocytosis assay, 10^5 cells were plated per well in 96-well tissue culture plates (Falcon; Becton Dickinson, Mountain View, CA) and cultured overnight at 37°C in the presence of 500 U of IFN-γ per ml (Genzyme, Cambridge, MA). Phagocytosis was measured in the presence or absence of purified mAbs.

Phagocytosis of *C. neoformans* by macrophages without IFN-γ pretreatment was studied in the same manner. After the addition of *C. neoformans* (E/T ratio of 1:5), the cells were incubated at 37°C for 4 h, washed three times with sterile PBS to remove nonadherent yeast cells, fixed with cold absolute methanol, and stained with a 1:20 solution of Giemsa stain (Sigma Chemical Co.). Phagocytic indices were determined with a microscope at a magnification of ×600 (Nikon Diaphot; Nikon Inc., Melville, NY). The phagocytic index is the number of macrophages with two or more internalized yeast cells/total number of macrophages in each field. For each experiment, eight fields were counted (23).

In Vitro Macrophage Antifungal Activity. The antifungal efficacy of primary macrophage cells was determined by counting the *C. neoformans* CFU after coculturing with macrophage cells (E/T = 5:1) in the presence and absence of mAb as previously described (24). In brief, the macrophage cells were mixed with *C. neoformans* in the presence of 5 μg of mAb per milliliter as described for the phagocytosis assay, and the mixture was incubated for 2 or 24 h. The supernatants from each well were then removed and the cells were lysed with sterile water for 30 min at 37°C, then vigorously aspirated and ejected in order to completely disrupt them. The supernatant was added back to the lysate, diluted with PBS, and plated on Sabouraud dextrose agar plates. Results are expressed as mean ± SD.

Rosetting Assay. SRBCs were purchased from Colorado Serum Company (Denver, CO). The preparation of Ig-coated SRBC and Fc rosetting was done as previously described (25, 26). In brief, 1–5 μg of mAb was incubated with 1 ml of SRBCs (2×10^7 cells) for 30 min at 37°C, washed, and then diluted 1:10. The Ig-coated SRBCs were added to the adherent cells in 96-well plates (E/T = 1:20), incubated for 30 min at 37°C, and then washed and assayed for rosettes. Attachment of three or more SRBCs signified a rosette. For phagocytosis, the cells were incubated for 1 or 2 h at 37°C, then free SRBCs were lysed with distilled water and intracellular SRBCs were assayed using phase contrast microscopy. SRBCs incubated in the absence of mAbs were used as controls. Results are expressed as the mean ± SD.

Statistics. Data were analyzed with statistical software for Macintosh (Instat version 2.01; GraphPDA Software for Science, San Diego, CA) using the unpaired Student *t* test. Results were

Table 1. *Survival in Days of FcRγ$^{+/-}$ and FcRγ$^{-/-}$ Mice Challenged with C. neoformans*

	FcRγ$^{+/-}$	$P <$	FcRγ$^{-/-}$	$P <$
mAbs (3E5)				
IgG1	270 ± 130	0.02‡	112 ± 29	0.21‡
IgG3	102 ± 56	0.14§	64 ± 22	0.004§
PBS control	147 ± 74	0.02*	159 ± 18	0.20*
Controls				
PBS FcRγ$^{+/+}$	156 ± 22			
PBS (C57BL/6)	159 ± 22	0.8‖		

*PBS versus IgG1.
‡IgG1 versus IgG3.
§IgG3 versus PBS.
‖PBS (FcRγ$^{+/-}$) versus other PBS controls (C57BL/6J, FcRγ$^{+/+}$, FcRγ$^{-/-}$) (unpaired Student's *t* test).

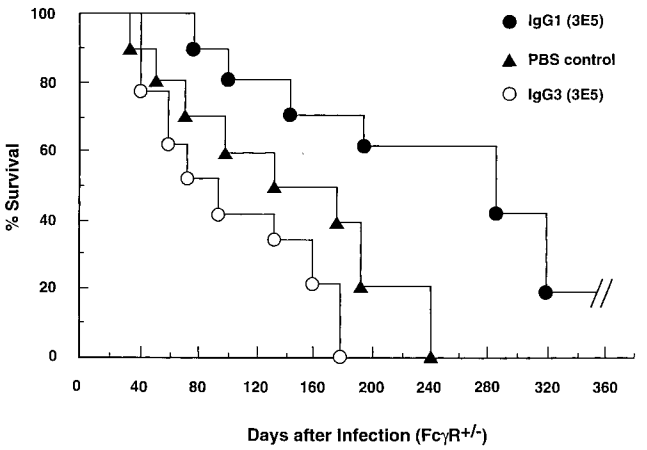

Figure 1. Survival of FcRγ$^{+/-}$ mice given either mAbs IgG1, IgG3, or PBS. 1.0 mg of each antibody was given intraperitoneally 24 h before intravenous challenge with 5×10^6 *C. neoformans*. Average survival and standard deviation for the IgG1, IgG3, and PBS groups were 270 ± 130, 101 ± 56, and 147 ± 74 d, respectively.

also analyzed by the unpaired Wilcoxon test, which gave similar results (4, 27).

Results

Effects of mAbs on Survival of FcRγ$^{+/-}$ Mice. We have previously demonstrated that 3E5 IgG3 mAb was not protective against lethal infection of immunocompetent C57BL/6J mice with *C. neoformans*, whereas its IgG1 switch variant prolonged the life of the infected animals (27). C57BL/6J and the FcRγ$^{+/+}$ and FcRγ$^{+/-}$ litter mates of FcRγ$^{-/-}$ homozygous mice were infected with a lethal dose of *C. neoformans* to quantify their susceptibility to infection. All four mouse strains had similar susceptibility to cryptococcal infection ($P = 0.8$; Table 1). To determine the effect of the IgG3 and IgG1 isotypes on FcRγ$^{+/-}$ mice, we treated groups of 10 mice with IgG3, IgG1, or PBS and then challenged them with *C. neoformans*. Consistent with previous results (27), IgG3 mAb tended to reduce animal survival when compared to PBS controls (Fig. 1), although the difference in survival time was not statistically significant ($P = 0.14$; Table 1). FcRγ$^{+/-}$ mice given IgG1 mAb were significantly protected compared to PBS controls ($P < 0.02$; Table 1 and Fig. 1). Animal survival in 3E5 IgG3– and IgG1-treated C57BL/6J and FcRγ$^{+/+}$ mice was comparable to that in FcRγ$^{+/-}$ mice (data not shown). Thus, the in vivo data confirm that the isotype of the mAb is an important determinant of protection against *C. neoformans* and that the heterozygous FcRγ$^{+/-}$ mice do not differ in their susceptibility to infection or response to passive antibody from wild-type mice.

Effects of mAbs on Survival of FcRγ$^{-/-}$ Mice. To determine whether FcγRs play a role in antibody-mediated immunity against cryptococcal infection, FcRγ$^{-/-}$ mice were challenged with *C. neoformans* as described above. Interestingly, FcRγ$^{-/-}$ mice had similar susceptibility to cryptococcal infection compared to FcRγ$^{+/-}$ mice indicating that lack of the FcRγ chain does not reduce natural resistance to cryptococcal infection ($P > 0.9$; Fig. 2, *inset*). Passive administration of IgG1 mAb failed to protect FcRγ$^{-/-}$ mice against infection, and it even appeared to lessen animal survival at early intervals after infection compared to PBS controls, but there was not a statistically significant shortening of animal survival overall (Fig. 2 and Table 1). However, IgG3 mAb did significantly reduce animal survival ($P < 0.004$). These results indicate that the FcRγ chain is crucial for IgG1-mediated protection against cryptococcal infection, and suggest that the accelerated infectious process provoked by IgG3 is mediated through a different pathway.

Effects of mAbs on Organ CFUs and Serum GXM Levels of FcRγ$^{-/-}$ Mice. The animal survival data for FcRγ$^{-/-}$ mice were extended by quantifying organ *C. neoformans* CFUs and serum GXM antigen levels. Table 2 shows that 14 d after infection lung and brain CFU number and serum GXM antigen in IgG1-treated FcRγ$^{-/-}$ mice were similar to PBS-treated controls. In contrast, mice treated with IgG3 had many more organisms in their lungs compared to controls or IgG1-treated mice ($P < 0.02$), but no increase in brain CFUs was found. The level of circulating GXM antigen in IgG3-treated mice was also elevated, suggesting a higher fungal burden ($P < 0.03$, Table 2). These results are consistent with the animal survival studies and indicate that the FcRγ chain is necessary for IgG1-mediated protection.

Effects of mAbs on Macrophage Phagocytosis In Vitro. Phagocytosis by macrophages derived from wild-type, FcRγ$^{-/-}$ and FcRγ$^{-/-}$/RIIB$^{-/-}$ mouse strains was studied to further define the roles of FcRs in this model. In the absence of capsule-specific mAbs, there was little or no phagocytosis of *C. neoformans* by lung macrophages (Fig. 3 *A*). Addition of IgG1, IgG2b, or IgG2a mAbs to the normal macrophage assay significantly increased the phagocytic index, especially after IFN-γ stimulation ($P < 0.0001$), whereas

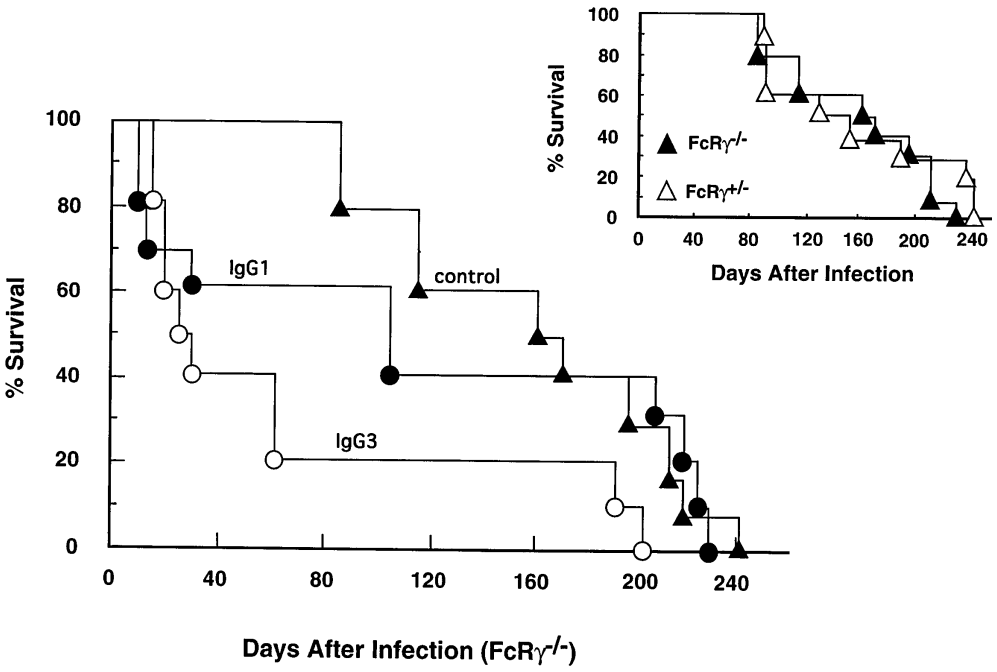

Figure 2. Survival of FcR$\gamma^{-/-}$ mice infected with *C. neoformans* after the administration of mAbs. Average survival and standard deviation of FcR$\gamma^{-/-}$ mice for the IgG1-, IgG3-, and PBS-treated groups were 112 ± 95, 64.0 ± 70, and 158 ± 57 d, respectively. The 3E5 IgG1 mAb failed to protect FcR$\gamma^{-/-}$ mice (P = 0.20), whereas the IgG3 mAb decreased survival (P <0.004). (*inset*) FcR$\gamma^{-/-}$ mice were similarly susceptible to cryptococcal infection as FcR$\gamma^{+/-}$ mice (P <0.8).

the IgG3 mAb induced less phagocytosis (9%; P <0.0001). Addition of IFN-γ significantly increased IgG3-mediated phagocytosis (19%) indicating that IFN-γ caused an increase in expression of the FcγR responsible IgG3-mediated phagocytosis. When IgG1, IgG2a, or IgG2b mAbs were added to FcR$\gamma^{-/-}$ macrophages, little or no phagocytosis was observed even after stimulation with IFN-γ (Fig. 3 *B*), and analysis of macrophages from either the bone marrow or the peritoneal cavity gave similar results (data not shown). Organisms treated with IgG3 were poorly phagocytosed by FcR$\gamma^{-/-}$ macrophages without IFN-γ stimulation. However, IFN-γ pretreatment of FcR$\gamma^{-/-}$ macrophages dramatically increased IgG3-mediated phagocytosis (P <0.0001). IgG3 mAb pretreated by heating at 56°C to inactivate complement still provoked similar phagocytosis by the macrophages from FcR$\gamma^{-/-}$ or FcR$\gamma^{-/-}$/RIIB$^{-/-}$ mice (data not shown). To exclude the possibility that the phagocytosis was mediated through FcγRIIB, we assayed macrophages from FcγRIIB$^{-/-}$ (20) and FcR$\gamma^{-/-}$/RIIB$^{-/-}$ mice and similar results were obtained (data not shown).

To confirm the data obtained from the macrophage assay with anticryptococcal mAbs, we also examined mAb-mediated rosetting of SRBCs. Fig. 4 *A* shows that there is little rosetting in the absence of mAbs in FcR$\gamma^{+/+}$ mouse macrophage cells. The anti-SRBC IgG1, IgG2a, and IgG2b induced high levels of rosetting, which was consistent with ear-

Table 2. *Organ CFUs and Serum GXM Levels 14 d after Infection*

	Lung CFUs (× 10^4)	$P \leq$	Brain CFUs (× 10^4)	$P \leq$	GXM	$P \leq$
					mg/ml	
FcR$\gamma^{-/-}$						
IgG1	47 ± 27	0.01‡	71 ± 33	0.72‡	0.68 ± 0.27	0.13‡
IgG3	196 ± 80	0.02§	81 ± 49	0.61§	1.95 ± 0.95	0.03§
PBS	56 ± 43	0.74*	66 ± 32	0.82*	0.51 ± 0.18	0.29*
FcR$\gamma^{+/-}$						
PBS	64 ± 21	0.82‖	78 ± 51	0.67‖	0.47 ± 0.12	0.70‖

*PBS versus IgG1.
‡IgG1 versus IgG3.
§IgG3 versus PBS.
‖PBS (FcR$\gamma^{+/-}$) versus PBS (FcR$\gamma^{-/-}$) (unpaired Student's *t* test).

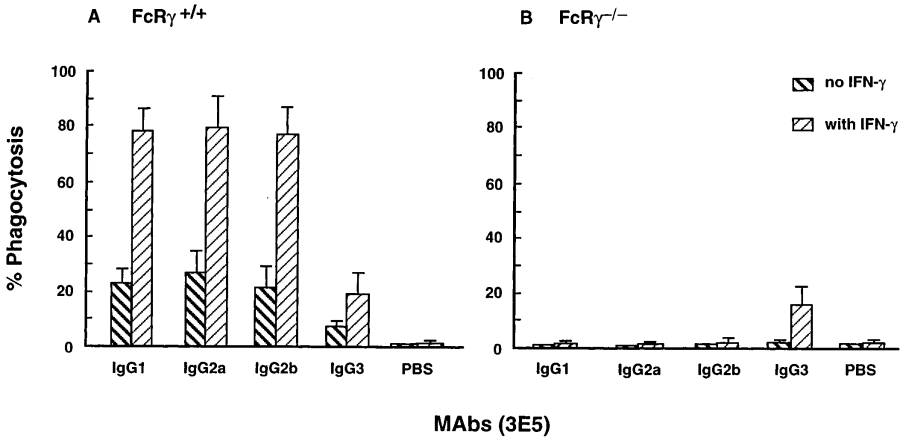

Figure 3. Effects of 3E5 mAbs on phagocytosis by bronchoalveolar macrophage cells from FcR$\gamma^{+/+}$ and FcR$\gamma^{-/-}$ mice. Phagocytic index was determined at an E/T ratio of 1:1 after 4 h of incubation. Each bar represents the average of eight fields, and brackets denote standard deviation.

lier reports (26). As with *C. neoformans*, IgG3 induced lower rosetting levels compared to other IgG isotypes ($P < 0.001$). When the same mAbs were added to macrophages from the FcR$\gamma^{-/-}$ mice, IgG1 and IgG2b mAbs induced lower frequencies of rosetting, and only 30% of the cells rosetted with IgG2a (Fig. 4 *B*). Again, IgG3-treated SRBCs had a relatively low frequency of rosetting (13–17%) with both macrophages from the FcR$\gamma^{-/-}$ and the FcR$\gamma^{-/-}$/RIIB$^{-/-}$ double knockout mice (Fig. 4, *B* and *C*). Very limited rosetting was observed in IgG1-, IgG2a-, or IgG2b-treated FcR$\gamma^{-/-}$/RIIB$^{-/-}$ macrophages (Fig. 4 *C*). Prolonged incubation (2 h) with IgG1-, IgG2b- or IgG2a-treated SRBCs did not result in phagocytosis (data not shown). Consistent with the results from the macrophage phagocytosis assay using *C. neoformans*, IgG3-treated SRBCs had a 10–12% phagocytic index with the FcR$\gamma^{-/-}$/RIIB$^{-/-}$ macrophages (data not shown). Taken together, these observations suggest that an IgG3 Fc receptor expressed on the cell surface of mouse primary macrophages differs from the other FcγRs or IgG3-mediated phagocytosis is dependent upon a different pathway of activation than the other FcRs.

Effects of mAbs on Macrophage on the Growth of C. neoformans In Vitro. We next determined whether binding this FcγR-IgG3 would lead to fungal killing after phagocytosis. Antifungal activity was quantified by measuring the CFU after incubating antibody, *C. neoformans*, and macrophages pretreated with IFN-γ. Table 3 (FcR$\gamma^{+/+}$) shows that IgG1 mAb significantly reduced the CFUs when incubated with wild-type alveolar macrophages at both early (2 h) and later (24 h) coculture periods. In contrast, IgG3 mAb did not restrain the growth of the organism after 2 or 24 h of coculture. Macrophages obtained from FcR$\gamma^{-/-}$ mice (FcR$\gamma^{-/-}$) had little or no effect on CFU number in the presence of IgG1 mAb ($P < 0.6$). The CFUs of the IgG3-treated organisms increased the same as the controls ($P > 0.6$) when they were cocultured with FcR$\gamma^{-/-}$ macrophages. These results strongly suggest that the FcγRs for IgG1 mediate phagocytosis and the release of factors leading to the arrest of fungal growth. In contrast, IgG3 induced phagocytosis of the organism but failed to inhibit fungal replication.

Discussion

The interaction of antibody–antigen complexes with effector cells results in a variety of different immune responses that are initiated through the binding of the antibody Fc region to cell surface Fc receptors. As a result, both the structural heterogeneity of FcRs on effector cells and the different antibody isotypes may contribute to the diversity of the response of effector cells (28, 29). Passive 3E5 IgG1 administration significantly prolongs survival of normal FcR$\gamma^{+/+}$ and FcR$\gamma^{+/-}$ mice challenged with a lethal dose of *C. neoformans*, whereas IgG3 with the identical variable region does not protect and even shows a tendency to reduce animal survival. The different protective efficacy mediated by this pair of IgG1 and IgG3 mAbs confirms our previous findings (5, 27) and strongly suggests that Fc-mediated effector functions play an important role in protection against *C. neoformans*.

The role of the FcγRs in natural resistance against cryptococcal infection or any microbial pathogen is unknown.

Table 3. *Effects of mAbs on C. neoformans CFU after Coculture with Bronchoalveolar Macrophages for 2 and 24 h*

	2 h ($\times 10^3$)	$P \leqslant$	24 h ($\times 10^3$)	$P \leqslant$
FcR$\gamma^{-/-}$				
IgG1	18 ± 4	0.58‡	281 ± 39	0.44‡
IgG3	20 ± 5	0.45§	309 ± 79	0.67§
Control	19 ± 4	0.75*	293 ± 30	0.53*
FcR$\gamma^{+/+}$				
IgG1	7 ± 2	0.001‡	159 ± 27	0.0002‡
IgG3	15 ± 4	0.76§	263 ± 35	0.79§
Control	14 ± 4	0.008*	269 ± 32	0.0001*

*PBS versus IgG1.
‡IgG1 versus IgG3.
§IgG3 versus PBS (unpaired Student's *t* test).

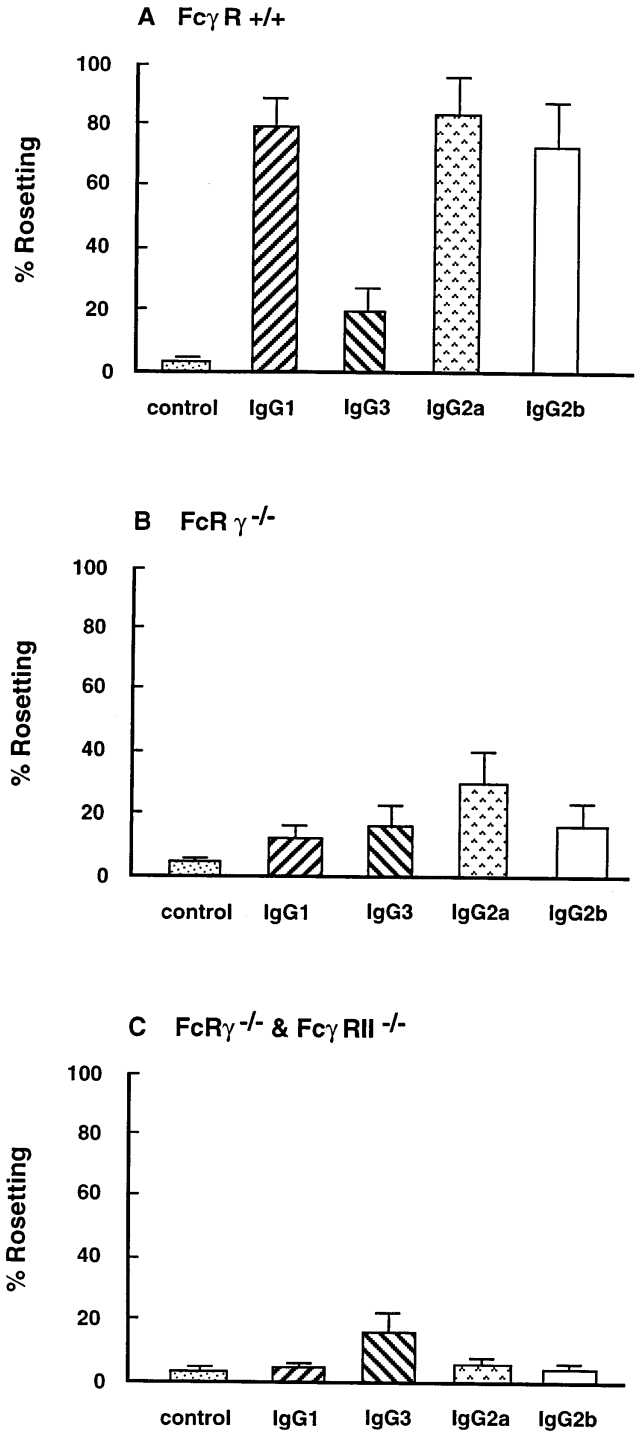

Figure 4. Rosetting of anti-SRBC IgGs by macrophages from FcR$\gamma^{+/+}$ (*A*), FcR$\gamma^{-/-}$ (*B*), and FcR$\gamma^{-/-}$/RIIB$^{-/-}$ (*C*) mice. Anti-SRBC mAbs (5 μg/ml) were incubated with SRBCs (2×10^7 cells) for 30 min before adding to the adherent macrophages (E/T = 1:20). Attachment of three or more SRBCs signified a rosette. SRBCs incubated in the absence of mAbs were used as controls. Results are expressed as the mean ± SD.

Our finding that FcRγ chain–deficient mice, which are unable to phagocytose through FcγRI and III, have similar susceptibility to *C. neoformans* to normal C57BL/6J or FcR$\gamma^{+/-}$ mice, suggests that FcγRI and III are not essential for natural resistance against cryptococcal infection. Most wild-type mice infected with *C. neoformans* do not develop an antibody response and antibody-mediated clearance is not believed to play a significant role in natural resistance (30). Even in those animals that do produce antibodies, there is a mixture of protective and nonprotective antibodies, and the latter may block the effects of the former (4). It is likely that FcRγ chain–deficient mice have a similar response, and therefore the presence or absence of FcγRs has no significant consequence for natural resistance.

The observation that passively administered 3E5 IgG1 fails to protect FcR$\gamma^{-/-}$ mice against cryptococcal infection clearly demonstrates that the FcRγ chain is essential for IgG1-mediated protection. An analogous situation has been observed in a passive protection model to metastatic melanoma. Administration of an IgG2b mAb to the melanosome protein gp75 is able to protect wild-type mice from significant lung metastasis. This protective effect is abolished in FcRγ chain–deficient mice, demonstrating the essential role of FcγR-mediated ADCC in the mechanism of antibody protection (30a). Deletion of FcRγ chain leads to defects of macrophage phagocytosis, ADCC, and other cytotoxic activities (13, 17), offering a likely explanation for the lack of protection by passive administration of IgG1, IgG2a, or IgG2b mAbs. The loss of the γ chain in vivo has also been shown to result in a decreased ability to clear RBCs opsonized with rabbit IgG anti–mouse RBCs, induce thrombocytopenia with an IgG1 mAb, or mount an Arthus reaction to IgG1, IgG2a, or IgG2b immune complexes (17, 31). IgG3 immune complexes, in contrast, retained the ability to induce the cutaneous inflammatory response of an Arthus reaction (31). Those results are consistent with the data presented here in which macrophages derived from FcRγ chain–deficient mice are unable to phagocytose IgG1-, IgG2b-, and IgG2a-treated particles, but retain the ability to bind and phagocytose IgG3-treated particles in vitro and enhance infection in FcR$\gamma^{-/-}$ mice in vivo. Our studies show that interaction of the IgG1–*C. neoformans* complex with Fc receptors on normal macrophages significantly inhibits replication of *C. neoformans*. However, the in vitro fungal inhibitory activity of IgG1 mAb is abolished completely when FcRγ chain–deficient macrophages are tested. Consistent with the in vivo study, IgG3 treatment permitted the organisms to replicate when tested with both normal and FcR$\gamma^{-/-}$ macrophages.

Our data extends the earlier finding by Diamond et al. (26) and suggests that there is either a separate receptor for IgG3 or a different pathway to activate phagocytosis on primary mouse macrophages and macrophage-like cell lines. IFN-γ stimulates expression of FcγRs (32), which was confirmed in our in vitro studies using normal mouse macrophages. More importantly, treatment with IFN-γ and IgG1 mAb had a synergistic effect on promoting phagocytosis and inhibiting proliferation of *C. neoformans* within normal macrophages, which is abolished in FcRγ deficient macrophages. In contrast, exposure to IFN-γ significantly promotes IgG3 FcR expression in both normal and FcR$\gamma^{-/-}$ macrophages. The interaction of this receptor with IgG3

mAb failed to inhibit *C. neoformans* replication. We previously demonstrated that IFN-γ plays an important role in stimulating both IgG1-mediated protection and IgG3-mediated enhancement of infection (27). It is possible that IgG3-mediated phagocytosis actually provides an intracellular sanctuary for *C. neoformans* that allows the fungus to escape killing by other effector cells and serum factors. Fungi could then proliferate freely within macrophage cells and be disseminated, finally killing the host.

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Note added in proof: While this paper was in press, Gavin et al. reported that FcγRIα bound and mediated phagocytosis of IgG3 SRBCs. Gavin, A.L., N. Barnes, H.M. Dijstelbloem, and P.M. Hogarth. 1998. *J. Immunol.* 160:20–23.

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