Investigation of Anti–WI-1 Adhesin Antibody-Mediated Protection in Experimental Pulmonary Blastomycosis

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Infection with Blastomyces dermatitidis elicits strong antibody responses to the surface adhesin WI-1. The antibodies are directed chiefly against the adhesive domain, a 25-amino-acid repeat. Tandem-repeat–specific monoclonal antibodies (mAbs) were studied for their opsonic activity in vitro and their capacity to adoptively transfer protection in murine experimental blastomycosis. mAbs to WI-1 enhanced binding and entry of B. dermatitidis yeasts into J774.16 cells but did not enhance killing or growth inhibition of the yeast. Passive transfer of 8 mAbs to WI-1 into 3 different inbred strains of mice also did not improve the course of experimental infection and sometimes worsened it. μ-deficient mice were more resistant to experimental blastomycosis than were intact littermates, and passive transfer of the mAbs into these mice did not protect them against experimental infection. Thus, antibody to WI-1 does not appear to improve the outcome of murine blastomycosis and may enhance the infection.

Blastomyces dermatitidis is a dimorphic fungal pathogen that infects the host through inhalation of conidia [1]. On transformation into the pathogenic yeast phase, yeasts multiply within the lung and may disseminate via the bloodstream and lymphatics to cause disease in the lung, skin, bone, genitourinary tract, and brain [1, 2]. Inflammatory reactions occur at the initial site of infection and at these metastatic foci. Cellular immunity is believed to be the principal host response that protects against progressive blastomycosis [3–6]. In contrast, humoral immunity is thought to exert no protective benefit, but it has not been investigated extensively [7]. A growing body of evidence suggests that antibodies may confer protection against infections with medicinally important fungi. For example, passive transfer of monoclonal antibodies (mAbs) have been shown to ameliorate the course of experimental cryptococcal, candidal, and Pneumocystis infections [8–19]. These recent observations prompted us to reconsider the role of antibodies in protective immunity to B. dermatitidis infection.

WI-1, a 120-kDa protein antigen and adhesin displayed on B. dermatitidis yeasts, evokes antibody and T cell responses during natural infection of people [20] and dogs [21]. When WI-1 is used to immunize mice, it evokes IgG antibody responses, which resemble the subclass profile of natural infection (IgG1>IgG2b>IgG2a>IgG3) and evince a mixed T-helper 1 and T-helper 2 immune response. Immunization with WI-1 also enhances resistance of mice against experimental pulmonary infection with B. dermatitidis [22]. Natural and vaccine-induced antibodies to WI-1 are directed chiefly against the 25-amino-acid tandem repeat of the protein, which is the adhesive domain [23]. Gene targeting and disruption of WI-1 has shown that the adhesin is indispensable for pathogenicity of B. dermatitidis. Yeasts lacking it are unable to bind lung tissue or macrophages in vitro and cannot establish a lethal experimental infection [24]. Thus, antibodies that interfere with WI-1–mediated binding might modulate the course of infection with B. dermatitidis.

In the present study, we investigated whether mAbs directed against the 25-amino-acid tandem repeat of WI-1 significantly affect the host-pathogen interaction in vitro and in vivo. We tested whether the antibodies influence the following: (1) binding and uptake of yeasts by macrophages; (2) survival of opsonized yeasts in macrophages; and (3) course of the experimental infection. We report that mAbs to WI-1 do increase binding and entry of B. dermatitidis yeasts into macrophages, but this does not enhance killing of the yeast. Furthermore, systemic administration of the antibodies or their Fab fragments does not improve the course of experimental B. dermatitidis and in some instances exacerbates the infection.
Methods

Fungal strains and growth conditions. Strains used in this study included American Type Culture Collection (ATCC) 60636, originally isolated from soil and patients during an outbreak of blastomycosis in Wisconsin [25], and ATCC 26199, originally isolated from a human patient in South Carolina [26]. Isolates were maintained in the yeast form on Middlebrook 7H10 agar slants with oleic acid-albumin complex (Sigma, St. Louis) at 37°C.

Mouse strains. Inbred C57BL/6, A/J, BALB/c, and γ-deficient C57BL/6-Igh-6mαμαα (stock 002288) [27, 28] strains of mice were obtained from Jackson Laboratories (Bar Harbor, ME). Male mice that were 5–6 weeks of age at the time of purchase were housed and cared for throughout these experiments according to guidelines of the University of Wisconsin Animal Care Committee, which approved all aspects of this work.

Ascites production. Hybridomas were generously provided by the Centers for Disease Control and Prevention, Atlanta (Drs. Christine Morrison and Errol Reiss). mAbs were prepared in BALB/c mice that were immunized with the A antigen of B. dermatitidis and had their spleen cells fused with SP2/0 mouse myeloma cells [29]. All mAbs showed strong reactivity with both WI-1 and the 25-aminooic acid tandem repeat, by radioimmunoassay and ELISA [30]. Antibodies used in this study were BD6-BC4, DD5-AD9, DD5-AD11, DD5-AE5, CA5-AA3, DD5-BC4, and CA5-BC12. Antibody-secreting hybridomas were cloned by limiting dilution and were assessed for antibody production before ascites was produced.

Characterization and quantitation of mAbs. Ascites was induced by intraperitoneal injection of 1.6 × 10^6 hybridoma cells into BALB/c female ex-breeders after they were primed and mAbs to WI-1 were analyzed and detected by ELISA, as described in detail elsewhere [22]. The concentration of mAbs in ascites fluid was determined by quantitative ELISA by using standards of the same subclass of mouse antibodies and by following the ELISA protocol as mentioned earlier.

Preparation of mAbs and Fab fragments. mAbs to WI-1 were purified from ascites on protein A/G agarose according to manufacturer’s specifications for the isolation and purification of IgG (product 21001, Pierce Chemical, Rockford, IL). Ascites fluid was diluted 1 : 1 with IgG binding buffer and centrifuged at 3000 g for 10 min to remove fibrin clots. Two milliliters of sample (1 mL ascites fluid and 1 mL binding buffer) per mL of packed gel was applied to the column. The column was washed with 10–15 column volumes of binding buffer and equilibrated with 5 column volumes of the buffer. Two-milliliter samples were eluted with a total of 3–5 column volumes of elution buffer (product 21004, Pierce). Immediately after elution, the pH of samples was adjusted to neutral with 1 M PBS, pH 7.5. The concentration of purified mAbs was quantified by absorbance at 280 nm. Eluted samples of mAbs were pooled and analyzed for purity by SDS-PAGE.

Fab fragments were made by papain digestion of purified WI-1–reactive mAbs BD5-CB4 by using a commercial kit and the manufacturer’s instructions (Pierce). After the mAbs was digested, the Fab fragments were purified by binding them 3 times over a protein A column. Fab fragments were analyzed by SDS-PAGE to show that they were free of contamination from intact IgG or Fc fragments.

Phagocytosis assays. Murine macrophage cell line J774.16 [31], generously provided by Dr. Arturo Casadevall (Yeshiva University, NY), was used for in vitro binding and phagocytosis assays. Macrophages were grown in Dulbecco’s modified Eagle medium (Life Technologies, Grand Island, NY) with 10% heat-inactivated fetal calf serum (HyClone Laboratories, Logan, UT), 10% NCTC-109 medium, and 1% nonessential amino acids (Bethesda Research Laboratories, Gaithersburg, MD) and plated at 5 × 10^6 cells per well in 16-well tissue-culture chamber slides (Nunc, Naperville, IL). Cells were stimulated with 500 U/mL of recombinant murine interferon-γ (IFN-γ; Boehringer Mannheim, Germany). After overnight incubation at 37°C in 8% CO_2, medium in each well was replaced with fresh medium containing 500 U of IFN-γ/mL, 3 μg/mL of lipopolysaccharide (LPS; Sigma), and B. dermatitidis yeasts.

Binding and phagocytosis of yeasts was analyzed in vitro as described elsewhere [32–34]. In brief, yeasts were heat-killed for 45 min at 65°C and stained with rhodamine isothiocyanate (10 μg/mL). Phagocytosis was measured in the presence or absence of opsonins: purified mAb, Fab fragments, complement, and inactivated complement (obtained from fresh serum of BALB/c mice). Assays done in the presence and absence of complement used 10% normal mouse serum (NMS) and heat-inactivated NMS, respectively. Complement was inactivated by heating NMS at 56°C for 30 min. Macrophages and yeasts were incubated at an effector:target ratio of 1 : 4 for varying periods at 37°C in 8% CO_2. Unattached yeasts were removed by washing wells 3 times with PBS. Attached but uningested yeasts were stained with 0.1% Uvitex 2B (Specialty Chemicals for Medical Diagnostics, Kandern, Germany) for 30 s. Cells were fixed in 1% paraformaldehyde for 15 min. After fixation, gels were added to the slide. To quantify binding and phagocytosis, we counted the number of yeasts attached to and ingested by 100 macrophages at 600x magnification by using a U-MWU fluorescence cube in an Olympus BX60 microscope (Leeds Precision Instruments, Minneapolis). The association index is defined as the number of attached and ingested yeasts divided by the number of macrophages counted. The ingestion index is defined as the number of yeasts ingested per macrophage. Results are expressed as the mean ± SE of the mean of at least 3 experiments.

Analysis of in vitro growth inhibition of B. dermatitidis. Murine macrophage cell line J774.16 was plated at 10^5 cells/well in a 96-well flat-bottom tissue culture plate (Corning, Corning, NY). Macrophages were grown as described earlier. J774.16 cells were stimulated with 500 U/mL of recombinant murine IFN-γ. After overnight incubation at 37°C in 8% CO_2, medium in each well was replaced with fresh medium containing 500 U of IFN-γ/mL, 3 μg/mL of LPS, and B. dermatitidis yeasts. Yeasts were preopsonized with 5 μg of purified DD5-CB4 or BD6-BC4 mAbs/10^6 of strain 26199 yeast for 30 min at 37°C. Macrophages and yeasts were incubated at an effector:target ratio of 4 : 1 for 4 days at 37°C in 8% CO_2. Growth inhibition of yeast grown alone or cocultured with J774.16 cells in the presence or absence of mAbs was assessed by colony-forming unit (CFU) plating on brain-heart infusion (Difco, Detroit) agar plates.

Passive transfer of mAbs to WI-1. mAbs were administered to mice intraperitoneally in a dose of 1 mg in 1 mL of saline 16–18 h before infection, by using a 0.4 × 13 mm (27G × 1/2") needle. Some animals were given an intraperitoneal boost with a second
Figure 1. Monoclonal antibody (mAb) to WI-1 enhances the association and ingestion of *Blastomyces dermatitidis* yeasts on interaction with macrophages in vitro. ATCC 26199 yeasts were preopsonized with mAb DD5-CB4 in varying concentrations or with mAb UPC10 as a control. Association and ingestion were measured as described in the Methods section. A. Association and ingestion of *B. dermatitidis* by J774.16 macrophage-like cells was monitored in vitro after coculture for 1 h. B. Influence of mAbs to WI-1 was measured in the presence and absence of complement opsonins, by using 10% normal mouse serum (NMS) and heat-inactivated mouse serum, respectively.

dose of 1 mg of the mAbs at indicated times postchallenge. In some experiments, yeasts were preopsonized with 10 μg of mAbs/10⁶ yeast and delivered intranasally as the means of antibody administration.

To monitor the concentrations of mAbs in serum and in the alveolus, we killed 3 mice at different intervals after intraperitoneal injection and collected fresh serum and bronchoalveolar lavage (BAL) fluids. BAL fluids were harvested by repetitive intratracheal administration of 0.5 mL PBS to yield a total of 1.8–2.1 mL lavage fluids. The concentrations of mAbs in these fluids were determined by quantitative ELISA by using known concentrations of the same mAbs as a standard. The amount of mAbs in a BAL fluid was calculated by multiplying the concentration of mAbs detected and the volume of the fluid. To investigate anti-WI-1 Fab fragments in passive protection, we preopsonized yeasts with 50 μg anti-WI-1 DD5-CB4 Fab fragments per 10⁶ yeasts for 30 min at 37°C with shaking. As controls in these experiments, yeasts were opsonized with 20 μg intact DD5-CB4 mAbs per 10⁶ yeasts. Mice were chal-
lenged intranasally with a lethal inoculum of opsonized yeasts on 2 consecutive days, as described later. Infected mice were boosted intranasally with 4 μg Fab, 2 μg intact mAb DD5-CB4, or 20 μL PBS (according to what they received initially) 4 and 8 days postinfection, to maintain amounts of mAbs in the alveolus that are achieved at that site after intraperitoneal administration. In addition, mice were given an intraperitoneal boost by using 250 μg Fab anti–WI-1, 500 μg intact IgG, or 0.5 mL PBS 4 and 11 days postinfection.

Experimental infection. Approximately 18 h after mice (BALB/c, C57BL/6, A/J, and μ-deficient C57BL/6) received 1 mg of mAbs by intraperitoneal injection, they were infected with B. dermatitidis yeasts intranasally. Mice were anesthetized with inhaled Metafane (McAllinckrodt Veterinary, Mundelein, IL), and a suspension of yeast cells was administered in a volume of 25 μL dropwise into their nares. This procedure was repeated on each of 2 consecutive days to ensure optimal delivery of B. dermatitidis yeasts into the lungs. The minimal number of yeast needed to achieve a lethal pulmonary infection under these conditions was established in preliminary experiments as 102 cells for both ATCC strains 60636 and 26199. To assess protective efficacy of mAbs to WI-1, we assessed passively immunized mice and control mice daily for survival after infection. In addition, some groups of infected mice were killed at various intervals postinfection and their lungs monitored for the number of yeasts.

Statistical analysis. Kaplan-Meier [35] survival curves were generated for mice that received a lethal infection. Survival times of mice that were alive by the end of the study were regarded as censored. Time data were analyzed by the log-rank statistic, and exact P values were computed by using the statistical package Stat Xact-3 by CYTEL Software Corporation (Cambridge, MA). The survival of 2 groups are considered to be significantly different if the 2-sided P value is <.05. Differences in number of CFUs in tissue were analyzed statistically by using the Wilcoxon rank test for nonparametric data [35].

Results

mAbs to WI-1 mediate binding and uptake of yeasts by J774.16 macrophage-like cells. Natural infection in humans [20] and dogs [21] with B. dermatitidis is accompanied by a strong antibody response to WI-1, which is directed chiefly against the tandem repeat. To investigate whether tandem-repeat-specific antibodies opsonize B. dermatitidis and promote binding and internalization of yeasts into macrophages, we performed immunofluorescent staining and phagocytosis assays by using murine macrophage-like J774.16 cells. Incubation of 4 mAbs to WI-1 (DD5-CB4, CA5-AA3, CA5-BC12, and DD5-AD11) with 5 wild-type ATCC isolates (26199, 60636, 26198, 66136, and 32090) revealed bright fluorescent staining with relative fluorescent intensities of 19.8- to 131.4-fold over background. In the phagocytosis assay, opsonization of yeasts with mAb DD5-CB4 resulted in a concentration-dependent increase of binding and internalization by macrophages (figure 1A). Both association and ingestion indices were substantially augmented with mAb concentrations as low as 100 ng/mL and reached their maximum values of ~3 yeasts associated per macrophage and 2 yeasts internalized per macrophage at an mAb concentration of 10 μg/mL. Enhanced binding and internalization of yeasts also was observed when yeasts were opsonized with complement components in 10% NMS (figure 1B). mAbs to WI-1 and complement together had an additive effect on association and ingestion of yeast by macrophages. Similar findings were observed with other mAbs to WI-1 (data not shown).

Because mAbs to WI-1 enhanced binding and internalization of B. dermatitidis, we investigated whether these opsonic properties influenced the number of CFUs after coculture of B. dermatitidis with the murine macrophage-like cell line J774.16. Preopsonized yeast cocultured with J774.16 macrophages were completely phagocyted by 12 h of incubation. After 5 × 103 yeasts were either cultured alone or with J774.16 cells in the absence or presence of anti-WI-1 mAb DD5-CB4 for 4 days, the respective CFU values were 113,800 ± 8092, 98,800 ± 15,130, and 108,600 ± 6633. Use of another mAb, BD6-BC4, gave similar results in these experiments, showing similar values for these 3 groups (data not shown).

Adoptive transfer of mAbs to WI-1 does not protect against experimental blastomycosis. To determine whether mAbs to WI-1 can modify the course of experimental blastomycosis, we passively transferred 8 mAbs (2 IgG1 and 6 IgG2a) into mice. The influence of these mAbs in 3 inbred strains of mice infected with various isolates of B. dermatitidis at multiple doses is summarized in table 1. None of the mAbs protected mice. In experiments 1–3, C57BL/6 mice were infected with a lethal dose of 108 ATCC strain 60636 yeast. The median survival time of control animals had a range of 11–14 days. None of 7 mAbs tested in these experiments prolonged survival significantly. In experiment 2, mAb DD5-AD11 shortened survival, but the result was not statistically significant. In experiment 3, mAb DD5-CB4 also shortened survival marginally, and this result was statistically significant.

We adjusted variables of the model to attempt to uncover a protective effect of antibody. We reduced the inoculum to 103 organisms because infection with 104 yeast produces a rapid, overwhelming infection. We also selected a “less virulent” wild-type isolate ATCC strain 26199 (based on the relative time to onset of illness and death; unpublished observations). We considered that mAb affinity for WI-1 may vary from isolate to isolate because the tandem-repeat protein sequence varies by 13% between isolates [36]. However, each of the mAbs to WI-1 (tested at 1 μg/105 yeast cells) bound yeast of both ATCC isolates 60636 and 26199, yielding brilliant fluorescence staining of similar intensity between isolates (data not shown). Thus, in experiments 4–6, a lethal dose of 103 ATCC 26199 yeasts was used to challenge C57BL/6, BALB/c, and A/J mice. The median survival time of control mice in these experiments was increased to 56–72 days. However, no protection was achieved with the...
### Table 1. Summary of survival studies after adoptive transfer of 8 different monoclonal antibodies (mAbs) to WI-1.

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Mouse strain</th>
<th>Yeast strain</th>
<th>Dose</th>
<th>Groups treated with 1 mg of mAbs (n)</th>
<th>Subclass of mAb</th>
<th>Median survival, days</th>
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NOTE. Mice received 1 mg of tandem-repeat-specific mAbs intraperitoneally and were subsequently challenged intranasally with 10^3 or 10^4 Blastomyces dermatitidis yeasts as described in the Methods section. Mice were followed up for survival daily and assessed for median survival time. The statistical significance of each experiment is indicated by a 2-sided P value.

* ATCC 60636 yeast were preopsonized with mAb DD5-CB4 shortly before used for infection, as described in the Methods section.

4 mAbs tested. Thus, lowering the infectious dose and changing the infecting B. dermatitidis isolate did not improve the protective efficacy of mAbs. In fact, 2 mAbs, DD5-AD11 in experiment 5 and DD5-CB4 in experiment 6, continued to exacerbate the infection.

The limited protective efficacy of mAbs could be due to a problem of distribution of mAbs. We therefore measured mAb concentrations in lung BAL fluid and serum after administration. The mean total amounts of anti–WI-1 IgG2a (DD5-CB4) measured in BAL fluid of 3 mice tested 2 and 7 days after intraperitoneal administration of 1.7 mg were 0.84 ± 0.18 and 0.34 ± 0.15 µg, respectively. The mean serum concentrations at these time intervals were 590 ± 86 and 230 ± 87 µg/mL, respectively. These values are similar to values observed for passively transferred mAbs that protect against experimental Cryptococcus neoformans infection [37]. Nevertheless, we modified mAb treatment in some experiments to enhance antibody levels in lung tissue. In experiment 5, mice received 1 mg mAbs before infection and received a booster injection 3 weeks later; the timing of this booster was based on the half-life of murine IgG in serum, which is 8–10 days for IgG1 and IgG2a [37, 38]. This modification did not prolong survival. In experiment 3, mAb DD5-CB4 was used to treat yeasts shortly before infection, to maximally opsonize the organisms with mAb. As before, this mAb significantly shortened the survival of the mice.

μ-Deficient mice are more resistant to experimental blastomycosis. Our results suggested that some anti–WI-1 antibodies enhance infection rather than protect against it, raising the possibility that antibodies are harmful rather than helpful in blastomycosis. We therefore investigated the course of experimental infection in μ-deficient mice, which have B cells but do not produce immunoglobulins [27, 28], and their intact littermates. At 12 days postinfection, μ-deficient mice showed a tendency toward a lower burden of lung infection (P = .19; figure 2A). By 20 days postinfection, the burden of infection was significantly reduced in μ-deficient mice, compared with controls (P = .012). μ-deficient mice also lived significantly longer than intact littermate controls (P = .032) after a lethal infection (figure 2B). Thus, the sum total of antibodies enhanced experimental B. dermatitidis infection. Findings in μ-deficient mice suggested that enhancing antibodies generated during infection of an intact host could negate the beneficial effect of anti–WI-1 (or other) protective antibodies. To address this possibility, we tested whether mAbs to WI-1 transferred into μ-deficient mice could mediate protection in the absence of other competing antibodies. We administered...
Figure 2. \(\mu\)-Deficient mice are more resistant to experimental pulmonary blastomycosis. A, Burden of infection at 2 time points after challenge with \(10^6\) strain 26199 yeasts in \(\mu\)-deficient mice and their intact littersmates. B, \(\mu\)-Deficient mice and their intact littersmates (C57BL/6; mice/group) were infected intranasally with \(10^4\) strain 26199 yeasts and followed up for survival.

2 mAbs indifferent to WI-1 (CA5-BC12 and CA5-AA3) or PBS as a control into \(\mu\)-deficient mice 18 h before infection and gave booster injections to the mice with 1 mg of mAbs or PBS 6 days later. None of the mAbs protected the mice as assessed by survival (data not shown).

*Fab anti–WI-1 inhibit binding of B. dermatitidis yeasts to macrophage-like cell line J774.16 but do not passively transfer protection in vivo.* Because WI-1 knockout yeasts lose pathogenicity partly because of impaired adherence to lung tissue and cells [24], we postulated that anti–WI-1 Fab might interfere with WI-1–mediated binding to host cells and ameliorate the course of infection. We first investigated whether Fab fragments of mAbs to WI-1 block the adherence of yeast to phagocytes in vitro. Yeasts (ATCC strain 26199) were preincubated for 30 min at 37°C with Fab anti–WI-1 from mAb DD5-CB4. Saturating concentrations \(\geq 25 \mu g/mL\) of Fab fragments inhibited binding of yeast to J774.16 cells by \(\leq 56\%\) and uptake by \(\leq 63\%\) (figure 3). Some residual binding and ingestion remained in Fab-treated yeasts, compared with untreated yeasts and with isogenic WI-1 knockout yeasts [24]. Mice were infected with yeasts that had been preopsonized with anti–WI-1 Fab or intact mAbs to WI-1, as described earlier, and boosted with Fab fragments or intact mAbs. Intranasal boosts included 4 \(\mu g\) Fab, 2 \(\mu g\) intact mAb DD5-CB4, or 20 \(\mu L\) PBS (according to what they received initially) at 4 and 8 days postinfection; intraperitoneal boosts included 250 \(\mu g\) Fab anti–WI-1, 500 \(\mu g\) intact IgG, or 0.5 mL PBS 4 and 11 days postinfection. At 18 days postchallenge, mice that received Fab fragments had a burden of infection (mean CFU = 18,602) comparable to those that received PBS (mean CFU = 14,058; \(P = .571\)). Mice that received intact mAbs had a greater burden of infection (mean CFU = 22,586), but the difference was not statistically significant, compared with the PBS group (\(P = .241\)).

**Figure 3.** Fab fragments of tandem-repeat–specific monoclonal antibodies (mAbs) diminish WI-1–mediated binding to macrophage-like J774.16 cells. Yeasts were opsonized in the presence of varying concentrations of Fab fragments (from mAb DD5-CB4) for 30 min at 37°C and then cocultured with activated J774.16 cells for 4 h. Unbound yeasts were washed away, and association and ingestion were assessed by counting 100 macrophages. Inhibition is defined as: \([1 – (association or ingestion in the presence of Fab)/association or ingestion in the absence of Fab)] \times 100\). WT, wild-type strain ATCC 26199; WI-1 KO, isogenic WI-1 knockout strain 55 [23].

Discussion

It has generally been thought that cell-mediated immunity (T cells) and nonspecific cellular immunity (i.e., macrophage, NK cells, and neutrophils) provide the main defenses against fungi, including *B. dermatitidis* [39]. In contrast, passive transfer of antibodies during experimental cryptococcosis, candidiasis, and *Pneumocystis* infection have been shown to mediate protection in naive mice [40]. The importance of humoral immunity and particularly anti–WI-1 antibodies in blastomycosis has not been studied systematically. Because immunization with WI-1 enhances resistance to infection, and because immunization and natural infection elicit a strong antibody response to WI-1, we postulated that WI-1–reactive antibodies might benefit the host, either by promoting opsonic clearance of the fungus or by interfering with the adhesive property of WI-1.
We first tested the opsonophagocytic activity of mAbs to WI-1. Incubation of 4 mAbs to WI-1 with 5 different wild-type ATCC isolates revealed bright fluorescent staining, indicating that the antibodies bind the yeast surface. Opsin consequences of binding were studied further by coculturing B. dermatitidis and murine macrophage-like J774.16 cells. mAbs to WI-1 enhanced the binding and uptake of B. dermatitidis but did not promote killing or growth inhibition of the fungus by J774.16 cells. These cells have many characteristics of murine macrophages, including the capacities for phagocytosis, oxidative burst, nitric oxide synthase activity, and production of microbicidal proteins [31, 41]. J774 cells have been used to study the interaction of murine macrophages with other pathogens, including Mycobacterium tuberculosis [31], Bacillus anthracis [42], Brucella abortus [43], and C. neoformans [34]. Antibody enhancement of macrophage function is one mechanism by which anticyryptococcal antibodies appear to modify infection [34]. Addition of anti-GXM mAbs to C. neoformans and J774.16 cells results in a substantial reduction in the number of CFUs. It is unclear why opsonophagocytosed B. dermatitidis yeast are not killed or do not have their growth inhibited by macrophages. Other cells or additional activation signals present in vivo might be needed to promote enhancement of growth inhibition of the fungus.

We also investigated mAbs to WI-1 in vivo. None of the 8 mAbs administered systemically into 3 different inbred strains of mice improved the course of experimental pulmonary blastomycosis. Six of the mAbs appeared not to influence the course of infection one way or another, whereas 2 of the mAbs (DD5-CB4 and DD5-AD11) appeared to enhance the infection. The fact that μ-deficient mice were more resistant to experimental blastomycosis than their intact littermates underscores the tendency of some antibodies raised during infection to exacerbate experimental blastomycosis.

There is evidence that the host can elicit both protective and nonprotective antibodies to a fungal pathogen. Nonprotective antibodies have been shown to block the activity of protective antibodies in some fungal [44] and bacterial [45–48] infections. Blocking activity may be due to direct competition for the binding of the same or closely related epitopes [45, 49], and enhancement of infection by nonprotective antibodies is postulated to occur through a variety of mechanisms [46, 50]. Because antibody responses elicited during infection are polyclonal and differ in isotype, epitope specificity, and protective efficacy, the relative amounts of protective and nonprotective antibodies might influence the outcome of infection. Therefore, one explanation for the higher resistance of μ-deficient mice to blastomycosis could be the absence of enhancing antibodies in these mice during infection. A corollary is that the poorer outcome of experimental infection in intact mice could reflect that the sum total of polyclonal antibodies in these mice might be shifted toward enhancement of the disease.

The better outcomes in μ-deficient mice might also have alternative explanations. μ-deficient mice show a reduced tendency toward generation of the Th2 subset during infection with Plasmodium chabaudi chabaudi [51]. In another study, splenocytes of Schistosoma mansoni–infected μ-deficient mice also displayed a predominant Th1-like profile compared with non-μ-deficient infected mice [52]. The authors concluded that B cells, either directly or through their secreted products, are involved in the in vivo generation and/or maximal expansion of Th2 lymphocytes during the course of murine S. mansoni infection. Brummer et al. [53] have shown that mice infected with B. dermatitidis yeasts develop features of a Th2 immune response during chronic infection and features of a Th1 response during the healing phase of infection after antifungal therapy. Therefore, it is conceivable that a favored Th1 phenotype in infected μ-deficient mice could explain the enhanced resistance of these mice.

Other compensatory mechanisms might also contribute to better outcomes in μ-deficient mice. P. chabaudi–infected μ-deficient mice showed a 50- to 100-fold increase in splenic γδ T cell number after suppression of parasitemia, compared with uninfected B cell–deficient controls [54]; the magnitude of this increase resulted in significantly greater numbers of splenic γδ T cells in the B cell–deficient mice than in infected B cell–intact controls (~10-fold). It is unknown whether γδ T cells are helpful in the context of murine blastomycosis, but the malaria study demonstrates that compensatory effects in μ-deficient mice do occur and could conceivably contribute to a higher resistance to B. dermatitidis infection.

To rule out any interference of nonprotective antibodies during the course of experimental B. dermatitidis infection, we passively transferred mAbs into μ-deficient mice. Passive transfer of 2 indifferent mAbs to WI-1 into an environment free of potentially exacerbating antibodies in μ-deficient mice did not modify the course of infection. We therefore conclude that the lack of protective efficacy of mAbs to WI-1 was not due to blocking activity of other interfering antibodies raised during infection. It is more likely that these antibodies were simply indifferent, with no effect in either direction.

Although mAbs to WI-1 were not protective in our study, we considered several caveats. First, our initial model of experimental infection, in which we used 10⁴ ATCC 60636 yeast, might have been too extreme. We addressed that possibility by lowering the inoculum and by using the less-virulent isolate ATCC 26199 for infection. Even under these adjusted circumstances, the mAbs continued to be either indifferent or enhancing.

A second possibility for the failure of mAbs to transfer protection is that the amount of mAbs in the alveoli was insufficient to promote phagocytosis and killing by leukocytes. However, the concentrations of mAbs we measured in the blood and in alveolar lavage fluids at 2 and 7 days postinjection are similar to the reported concentrations of a protective anti-GXM mAb 2H1 [37], which prolonged survival and reduced burden of in-
fection in experimental cryptococcosis. To minimize further any chance that our findings might result from inadequate mAb delivery to the alveolar space, we challenged mice with preopsonized yeast or repeatedly administered booster injections to ensure a constant supply of mAbs in the lungs. Even under those conditions, mAbs did not significantly improve the outcome of experimental blastomycosis.

Our data with mAbs to WI-1 are based on the use of 6 IgG2a and 2 IgG1 subclassantibodies. Antibody subclasses have been shown to differ in their ability to protect mice during experimental cryptococcosis [34, 55]. IgG2a [34] and IgG1 [55] subclasses have been the most protective. Although these IgG subclasses have been the most promising with C. neoformans, we cannot rule out that other subclasses or isotypes would be more protective against B. dermatitidis.

Lastly, we considered that mAbs to WI-1 might show greater protection if opsonophagocytic activity was eliminated or at least reduced. Removal of the Fc portion of mAb DDS-CB4 diminished WI-1–mediated binding and uptake of yeast by J774.16 cells in vitro. This reduction in binding of macrophages in vitro did not have a significant influence on the course of infection as assessed by the burden of infection. It is possible that a 50% reduction in binding of yeasts to macrophages may not have been enough to change the outcome of experimental infection. WI-1 also may exert additional effects on host cells or the immune system beyond its function in mediating adherence.

In summary, we studied 8 mAbs to WI-1 in vitro for opsonophagocytic activity and in vivo in passive transfer experiments designed to modify the course of experimental blastomycosis. Although most of the mAbs did not significantly influence the course of experimental infection one way or another, 2 antibodies appeared to exacerbate the infection. Our findings are therefore consistent with those in the literature, pointing to a biologically important role for antibodies in fungal disease, in this case experimental blastomycosis. However, the degree of biological impact is modest in our studies of mAbs to WI-1. We conclude that the host response to WI-1 apparently elicits a lot of useless or harmful antibody. Because immunization with WI-1 does evoke significant, albeit modest, protective immunity against experimental murine infection [22], our current findings would suggest that cellular immunity to WI-1 largely accounts for the protective effects of immunization. Nevertheless, we cannot rule out the existence of protective antibodies directed either against other WI-1 domains or other antigens of B. dermatitidis.

References