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In Vivo Role of Dendritic Cells in a Murine Model of Pulmonary Cryptococcosis

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Dendritic cells (DC) have been shown to phagocytose and kill *Cryptococcus neoformans* in vitro and are believed to be important for inducing protective immunity against this organism. Exposure to *C. neoformans* occurs mainly by inhalation, and in this study we examined the in vivo interactions of *C. neoformans* with DC in the lung. Fluorescently labeled live *C. neoformans* and heat-killed *C. neoformans* were administered intranasally to C57BL/6 mice. At specific times postinoculation, mice were sacrificed, and lungs were removed. Single-cell suspensions of lung cells were prepared, stained, and analyzed by microscopy and flow cytometry. Within 2 h postinoculation, fluorescently labeled *C. neoformans* had been internalized by DC, macrophages, and neutrophils in the mouse lung. Additionally, lung DC from mice infected for 7 days showed increased expression of the maturation markers CD80, CD86, and major histocompatibility complex class II. Finally, ex vivo incubation of lung DC from infected mice with *Cryptococcus*-specific T cells resulted in increased interleukin-2 production compared to the production by DC from naïve mice, suggesting that there was antigen-specific T-cell activation. This study demonstrated that DC in the lung are capable of phagocytosing *Cryptococcus* in vivo and presenting antigen to *C. neoformans*-specific T cells ex vivo, suggesting that these cells have roles in innate and adaptive pulmonary defenses against cryptococcosis.

Cryptococcus neoformans is an opportunistic fungal pathogen that predominantly causes disease in immunocompromised patients, including individuals with AIDS, transplant recipients, and individuals with lymphoid and hematological malignancies (43, 57, 70, 72). Initial exposure to *C. neoformans* normally occurs via inhalation, and it is thought that most infections are asymptomatic (1, 15, 26). This observation implies that in a normal host, a component(s) of the immune system in the lung plays a role in protection from infection. Therefore, examining interactions of *C. neoformans* with immune cells in the lung is essential for understanding the initial immune response to this organism.

Recent research on the host immune response to *C. neoformans* has focused on T-cell-mediated immunity (32, 34–36, 58, 60) and antibody-mediated immunity (68, 78, 82). Additional studies have examined cryptococcal uptake by macrophages (16, 17, 40, 46, 47) and neutrophils (14, 23, 40, 51), but little has been learned about the in vivo role of dendritic cells (DC) in the initial interactions with the organism in the lungs. DC are antigen-presenting cells (APC) that act as sentinels in the peripheral tissues, constantly sampling the antigens in their environment. They ultimately present foreign antigens to T cells in the lymphoid tissues, initiating an adaptive immune response against these antigens (3, 71, 77). An adaptive Th1-type immune response is required for protection against cryptococcal infection (32, 34–36, 58). Because DC are the most effective APC for inducing cell-mediated immune responses (18), it is important to investigate lung DC and their potential to initiate an adaptive anticryptococcal immune response. The respiratory tract, in particular, contains dense networks of DC specialized for antigen uptake (12, 33, 69).

Dendritic cells have previously been shown to phagocytose *C. neoformans* in vitro (38, 76) by a process which requires opsonization with either complement or antibody. Following phagocytosis, DC are capable of activity against *C. neoformans* (38). Additionally, studies using cryptococcal antigens have demonstrated the importance of DC for inducing an anticryptococcal immune response in vivo and in vitro (4, 5, 52). However, it is not known whether DC in the lung phagocytose *C. neoformans* and are capable of inducing protective immunity in an in vivo infection model. For other pulmonary pathogens, including *Mycobacterium tuberculosis* (28, 62, 64) and *Aspergillus fumigatus* (11), lung DC are important for phagocytosis and for initiating an adaptive immune response.

Alveolar macrophages, another type of APC in the lung, have long been regarded as the phagocytes that initially encounter inhaled *C. neoformans*. In vitro studies have demonstrated that alveolar macrophages efficiently phagocytose *C. neoformans* and have fungicidal activity (8–10, 44). Additionally, alveolar macrophages that have internalized *C. neoformans* have antigen-presenting activity and produce proinflammatory cytokines (30, 48, 79). In vivo, depletion of alveolar macrophages resulted in decreased resistance to cryptococcal infection (59). However, in these studies, no distinction was made between alveolar macrophages and pulmonary DC, and it is possible that some of the activity attributed to macrophages actually was mediated by DC. Neutrophils also phagocytose and kill *C. neoformans* in vitro (14, 40, 51, 56) and can modulate the immune response by the production of proinflammatory cytokines (67). In animal studies, neutrophil influx

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into tissues following cryptococcal challenge is associated with rapid partial clearance of organisms (24, 63) but is not necessarily associated with protective responses against a pulmonary cryptococcal infection (37, 55).

In the present study, we tried to determine the contribution of lung DC to innate and adaptive host defenses against cryptococcosis during the initial stages of an in vivo infection. Accordingly, we examined uptake of *C. neoformans* by pulmonary DC in vivo using a murine model of intranasal infection. The relative contributions of DC, alveolar macrophages, and neutrophils to *C. neoformans* uptake were then determined during the first 24 h following fungal challenge. Finally, we investigated the effect of in vivo exposure to *C. neoformans* on the expression of DC maturation markers, as well as the capacity of DC to present antigen to *Cryptococcus*-specific T cells following infection.

MATERIALS AND METHODS

Reagents. Unless indicated otherwise, chemical reagents that were the highest quality available were obtained from Sigma Chemical Co. (St. Louis, MO), tissue culture media were purchased from Gibco Life Technologies (Rockville, MD), and plastic ware was purchased from Fisher Scientific (Pittsburgh, PA). The medium used in bone marrow dendritic cell (BMDC) experiments was RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 100 U penicillin/ml, 100 μg of streptomycin/ml, and 50 mM 2-mercaptoethanol (complete medium). All cell cultures were incubated at 37°C in a humidified environment supplemented with 5% CO_2.

Staining of *Cryptococcus*. *C. neoformans* serotype A encapsulated strain 145 (= ATCC 62070; American Type Culture Collection, Manassas, VA) was cultured overnight in YPD containing 2% glucose. Yeast cells were heat killed by incubation at 56°C for 1 h. Cultures of both live and heat-killed *C. neoformans* were washed with sterile 0.1 M sodium bicarbonate buffer (pH 8.0) (staining buffer), and cells were counted and resuspended to obtain a concentration of 5×10^8 cells/ml. *C. neoformans* was incubated with 2 μg/ml Oregon green 488 (Molecular Probes, Eugene, OR) at room temperature in the dark for 1 h. The organisms were then washed three times with staining buffer, counted, and resuspended in staining buffer to obtain the concentration needed for each experiment.

Mice. C57BL/6 mice were purchased from the Jackson Laboratory (Bar Harbor, ME) and were housed in microisolator cages at the Boston University Medical Center Laboratory Animal Sciences Center. Mice were housed and handled according to institutionally recommended guidelines.

BMDC cultures. BMDC cultures were grown as described previously (38, 50). Briefly, bone marrow was flushed from femurs and tibiae of C57BL/6 mice. Cells were washed, counted, and plated at a concentration of 2×10^6 cells/plate in complete medium supplemented with 10% filter-sterilized supernatant from the J558L cell line (which constitutively produces granulocyte-macrophage colony-stimulating factor) (65). Cells were used on day 9 or 10 following plating. This method of culture yielded an average of 77% $CD11c^+$ cells (DC), as assessed by flow cytometry (data not shown).

BMDC phagocytosis of *Cryptococcus*. BMDC were harvested, counted, and incubated with Oregon green-labeled *Cryptococcus*. BMDC and *Cryptococcus* were added at a ratio of 1:1 and were incubated in the presence of 1 μg/ml of 3C2, an opsonizing anticapsular monoclonal antibody (74) (a gift from Thomas Kozel, University of Nevada, Reno) for 1 h at 37°C in 1.7-ml microcentrifuge tubes (Costar, Corning, NY). Opsonizing antibody was used so that the BMDC could phagocytose the encapsulated *C. neoformans* cells (38). Following incubation, mouse cells were examined for *Cryptococcus* uptake by flow cytometry and fluorescent microscopy. Phagocytosis was defined as the association of fluorescent *C. neoformans* cells with BMDC or lung cells as determined by flow cytometry or the presence of intracellular *C. neoformans* by microscopy.

Mouse infection. For intranasal infection, mice were anesthetized with halothane (Samuel Perkins, Quincy, MA) and subsequently inoculated intranasally with 1×10^7 Oregon green-stained *C. neoformans* cells in 50 μl of sterile staining buffer. At specific times following inoculation, mice were euthanized by CO_2 inhalation, and the pulmonary artery of each mouse was perfused with 1 ml heparin in phosphate-buffered saline (14.3 USP units/ml) to clear the lungs of peripheral blood. The lungs were then removed, minced, and incubated in digestion buffer consisting of 1 mg/ml collagenase type IV (Sigma, St. Louis, MO), 75 μg/ml bovine pancreatic DNase (Sigma), and 5% fetal bovine serum in RPMI 1640 (Gibco Life Technologies Rockville, MD) for 1.5 h at 37°C. Following digestion, lung tissue was passed through a sterile 70-μm filter in order to obtain single-cell suspensions, and red blood cells were lysed with red blood cell lysis buffer (eBioscience, San Diego, CA). Cells were then examined by fluorescent microscopy, confocal microscopy, or flow cytometry.

Isolation of lung DC. Lung DC were isolated from digested whole lungs by positive selection with CD11c-MACS magnetic beads (Miltenyi Biotec, Auburn, CA). Lung DC were defined by expression of intermediate levels of CD11c on the surface, as previously described (64, 75).

Cell surface marker analysis. Flow cytometry was performed with BMDC and single-cell suspensions of lung tissue. The antibodies used included CD11c (DC), F4/80 (macrophages), mouse neutrophil-specific antigen clone 7/4 (neutrophils), and CD80, CD86, and major histocompatibility complex class II (MHC-II) (DC maturation). Most antibodies and isotype controls were obtained from eBioscience; the exception was the 7/4 antibody, which was obtained from Serotec, Oxford, United Kingdom. Antibodies were diluted in FACS buffer (2% fetal bovine serum in phosphate-buffered saline) to obtain the concentration recommended by the manufacturer, and 100 μl of diluted antibody was added to 1×10^6 cells and incubated for 1 h on ice, protected from light. After three washes, cells were analyzed with a flow cytometer (BD FACScan, San Jose, CA). Data were analyzed using the WIN MDI software (Joseph Trotter, The Scripps Research Institute, La Jolla, CA).

Microscopy. BMDC or lung DC were incubated with Oregon green-labeled *C. neoformans* as described above and then visualized with an epifluorescence microscope (Nikon Diaphot 300; Nikon Instruments, Inc.) or a confocal microscope (Ultraview RS; Perkin-Elmer, Boston, MA). For the confocal studies, DC were stained with CD11c phycoerythrin antibody (eBioscience). The microscope used in this system was a Nikon TE2000-U inverted microscope with a Nikon 100× 1.4NA DIC lens and type A immersion oil (Nikon, Melville, NY). The camera used in these experiments was a Hamamatsu Orca ER camera (model C4742-95-12ERG). The Perkin-Elmer Ultraview ERS software was used for acquisition. Individual images were saved as tiff files and were processed using the Perkin-Elmer Ultraview software. Optimal contrast was obtained by adjusting the gray levels for the entire image.

T-cell activation. Lung DC isolated from naïve or infected mice were incubated overnight with *Cryptococcus*-specific hybridoma P1D6 T cells (45). P1D6 cells secrete interleukin-2 (IL-2) when they are stimulated with APC presenting the *C. neoformans* mannoprotein MP98. Supernatants were harvested and then incubated with the IL-2-dependent cell line CTLL-2 (25) for 24 h. AlamarBlue (TREK Diagnostic Systems, Cleveland, OH) was added during the final 8 h. Absorbance was determined with an automated plate reader, and data were compared to an IL-2 standard curve. The results were expressed as units of IL-2/ml.

Statistical analysis. For statistical comparisons we utilized the Student *t* test. *P* values of <0.05 were considered significant. Data were analyzed using the GraphPad Prism software (GraphPad Software, Inc., San Diego, CA).

RESULTS

BMDC phagocytosis of fluorescently labeled *Cryptococcus*. In order to establish that fluorescently labeled *C. neoformans* could be detected by flow cytometry and fluorescent microscopy, live and heat-killed organisms were labeled with Oregon green 488 and subsequently incubated with BMDC and opsonizing antibody for 1 h to examine the initial phagocytosis. Opsonization is required for BMDC to phagocytose encapsulated *C. neoformans* (38). The results showed that after 1 h of incubation at 37°C, $CD11c^+$ BMDC were able to bind and/or phagocytose both live and heat-killed *C. neoformans*, as determined by flow cytometry (Fig. 1A) and fluorescence microscopy (Fig. 1B).

In vivo uptake of *Cryptococcus* by lung phagocytic cells. To determine the population of cells in the lungs that phagocytose *C. neoformans* in vivo, live and heat-killed organisms were fluorescently labeled with Oregon green and then used to intranasally inoculate mice. The lung DC from naïve mice did not express CD8-α, as previously reported (75). Additionally,

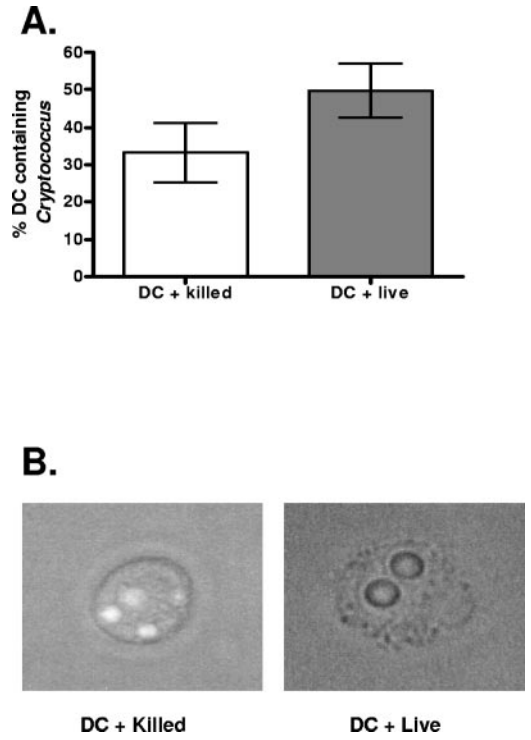

FIG. 1. Phagocytosis of live and heat-killed *C. neoformans* by BMDC in vitro. Live *C. neoformans* and heat-killed *C. neoformans* were stained with Oregon green and incubated with BMDC at a ratio of 1:1 in the presence of opsonizing antibody for 1 h at 37°C. (A) Percentage of BMDC containing one or more *C. neoformans* cells, as assessed by flow cytometry. The data are means ± standard errors of the means from five independent experiments. (B) Merged fluorescent and bright-field micrographs demonstrating that both killed *C. neoformans* and live *C. neoformans* are phagocytosed by BMDC.

lung CD11c$^+$ cells rarely expressed the macrophage marker F4/80 or the T-cell markers CD8 and CD3 (data not shown). In order to examine the early stages of *C. neoformans* uptake, the lung cells were examined soon after inoculation. At 2, 8, and 24 h postinoculation, mice were sacrificed, lungs were removed, and single-cell suspensions of lung tissue were prepared and stained for phagocyte cell surface markers. The percentages of DC, macrophages, and neutrophils present in the lungs during the initial stages of infection are shown in Table 1. Flow cytometric analysis demonstrated that both live and heat-killed organisms were phagocytosed in vivo by pulmonary DC (Fig. 2A). In order to verify that *C. neoformans*

TABLE 1. Percentages of lung cells during the initial stages of cryptococcal infection[a]

Cells	% of total lung cells in:			
	Naïve mice	Mice infected for 2 h	Mice infected for 8 h	Mice infected for 24 h
DC	6.6 ± 1.0	6.0 ± 0.6	5.5 ± 1.3	7.5 ± 0.7
Macrophages	6.0 ± 1.9	5.0 ± 1.2	4.6 ± 2.1	12.0 ± 2.4
Neutrophils	1.1 ± 0.2	3.8 ± 0.4	4.3 ± 1.6	12.2 ± 2.1

[a] Mice were not infected (naïve) or were infected intranasally with 1×10^7 *C. neoformans* cells for 2, 8, or 24 h. The data are the means ± standard errors of the means for cells from three to eight experiments.

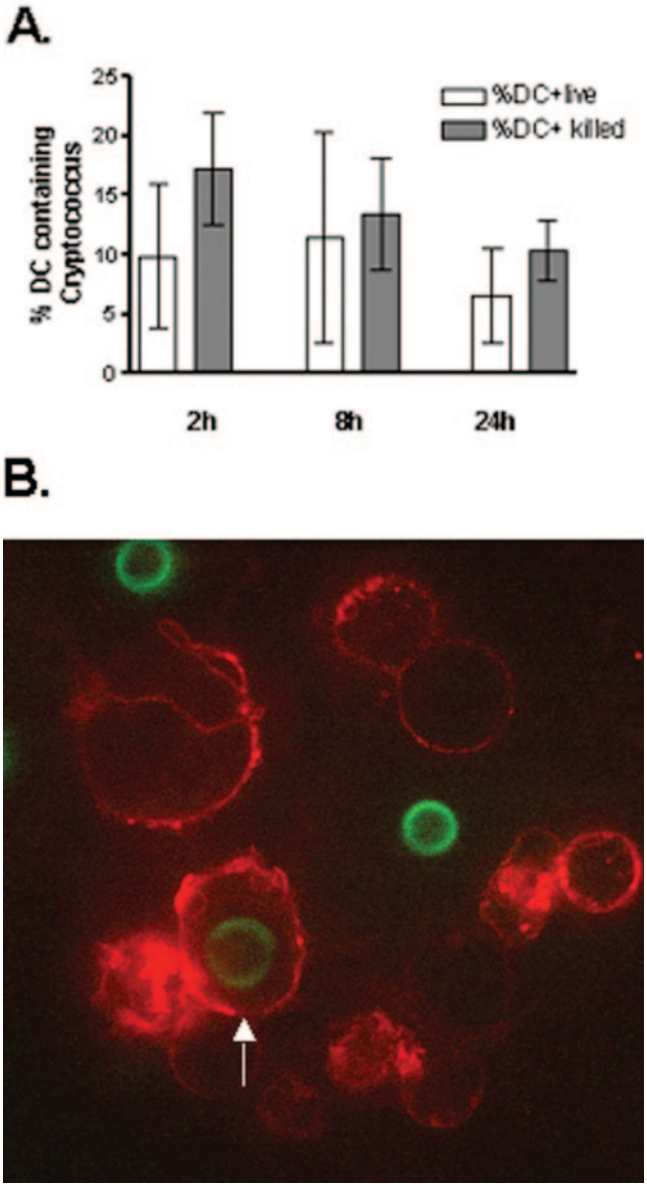

FIG. 2. Phagocytosis of *C. neoformans* by pulmonary DC in vivo. (A) Mice were intranasally inoculated with live or heat-killed *C. neoformans*. At 2, 8, and 24 h postinoculation, mice were sacrificed, and the percentages of pulmonary DC containing one or more *C. neoformans* cells were determined by flow cytometry. The data are means ± standard errors of the means for three to eight experiments. Lung cells from four mice per group were pooled for each experiment. (B) Confocal microscopy showing a live *C. neoformans* cell (green) internalized by a pulmonary DC stained with phycoerythrin-labeled antibody to CD11c (red) (arrow) at 24 h postinoculation. There are two other fungal cells, but we could not determine whether these cells were extracellular or internalized by other cell types.

cells were internalized by the DC, confocal microscopy was performed. Lung DC containing phagocytosed *C. neoformans* cells were observed by confocal microscopy (Fig. 2B). Analysis of Z-stack images confirmed the intracellular location of the fungus. We next examined the capacity of the two other major phagocytic cell populations in the lung, macrophages and neutrophils, to interact with *C. neoformans* (Fig. 3). Both live and

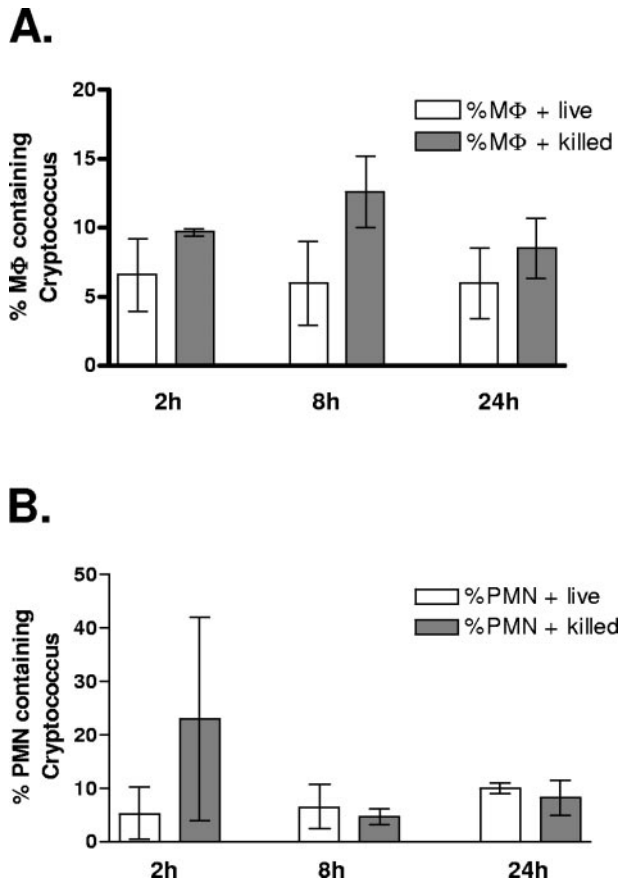

FIG. 3. Phagocytosis of *C. neoformans* by pulmonary macrophages and neutrophils in vivo. Mice were inoculated with live or heat-killed *C. neoformans*. At 2, 8, and 24 h postinoculation, mice were sacrificed and the percentages of pulmonary macrophages (MΦ) (A) and neutrophils (PMN) (B) containing one or more *C. neoformans* cells were determined by flow cytometry. The data are means ± standard errors of the means for three to seven experiments for macrophages and for two to six experiments for neutrophils. Lung cells from four mice per group were pooled for each experiment.

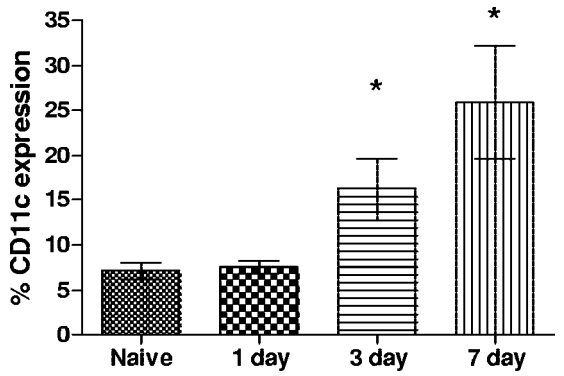

FIG. 4. Effect of infection with *C. neoformans* on the percentage of $CD11c^+$ DC in the lung over time. Mice were not challenged (naïve) or were inoculated with live *C. neoformans*. At 1, 3, and 7 days postinoculation, mice were sacrificed, and the percentages of lung cells expressing the DC marker CD11c were determined by flow cytometry. The data are means ± standard errors of the means from three independent experiments. Lung cells from four mice per group were pooled for each experiment. An asterisk indicates that there was a significant difference as determined by the Student *t* test for a comparison with naïve mice ($P = 0.002$ for day 3 and $P < 0.0001$ for day 7).

heat-killed organisms were phagocytosed in vivo by macrophages and neutrophils at 2, 8, and 24 h postinoculation.

DC expansion and expression of maturation markers during pulmonary infection. In order to further study the role of lung DC with *C. neoformans*, we examined lung DC at later times postinoculation. First, we monitored the percentage of lung cells expressing the dendritic cell marker CD11c to determine if infection caused DC expansion or infiltration. Lung cells examined at 3 and 7 days postinfection contained significantly higher percentages of $CD11c^+$ DC than lung cells from uninfected mice contained (Fig. 4). Upon maturation, DC upregulate MHC-II and the costimulatory molecules CD80 and CD86. Thus, we examined the levels of these surface molecules on $CD11c^+$ DC during the course of a cryptococcal infection (Fig. 5). The percentage of DC positive for CD80 (Fig. 5A) increased throughout infection and on day 7 postinoculation was significantly greater than the percentage of DC positive for CD80 in control animals. Additionally, the percentages of DC positive for CD86 (Fig. 5B) and MHC-II (Fig. 5C) also exhibited a trend, albeit not significant, toward increased expression on day 7.

***Cryptococcus*-specific T-cell activation by pulmonary DC.** In the final set of experiments we tried to determine whether lung DC from infected animals acquired the capacity to present cryptococcal antigen to T cells (Fig. 6). Lung DC were isolated on days 1, 3, and 7 postinfection and incubated with the *C. neoformans*-specific T-cell hybridoma P1D6. DC obtained from infected mice at all times were able to induce T-cell activation, as measured by IL-2 production, indicating that there was cryptococcal antigen presentation by lung DC.

DISCUSSION

Macrophages and neutrophils have long been thought to be the first phagocytes to encounter *Cryptococcus* following inhalation; however, the role of lung DC in phagocytosis and killing of *C. neoformans* during infection has not been studied. An immunization model using cryptococcal antigens showed that DC are important for induction of protective immunity against *C. neoformans* (4, 5), and work in our laboratory and by other researchers has shown that DC can phagocytose *C. neoformans* in vitro (38, 76). Therefore, we postulated that lung DC are important for phagocytosis of *C. neoformans*, presentation of cryptococcal antigens to T cells, and ultimately directing the immune response to the organism. Accordingly, in this study, we examined in vivo phagocytosis of *C. neoformans* by lung cells, lung DC maturation, and ex vivo T-cell activation following intranasal inoculation of mice.

This study demonstrated that lung DC can indeed phagocytose *C. neoformans* in vivo. Additionally, our studies confirmed previous findings which demonstrated that macrophages (8–10, 44) and neutrophils (14, 40, 51, 56) can also phagocytose *C. neoformans*. The relative importance of the three phagocytic cell types to host defenses cannot be gauged from our studies. Since DC are thought to be more important in the initial presentation of antigens to naïve T cells and can thus initiate

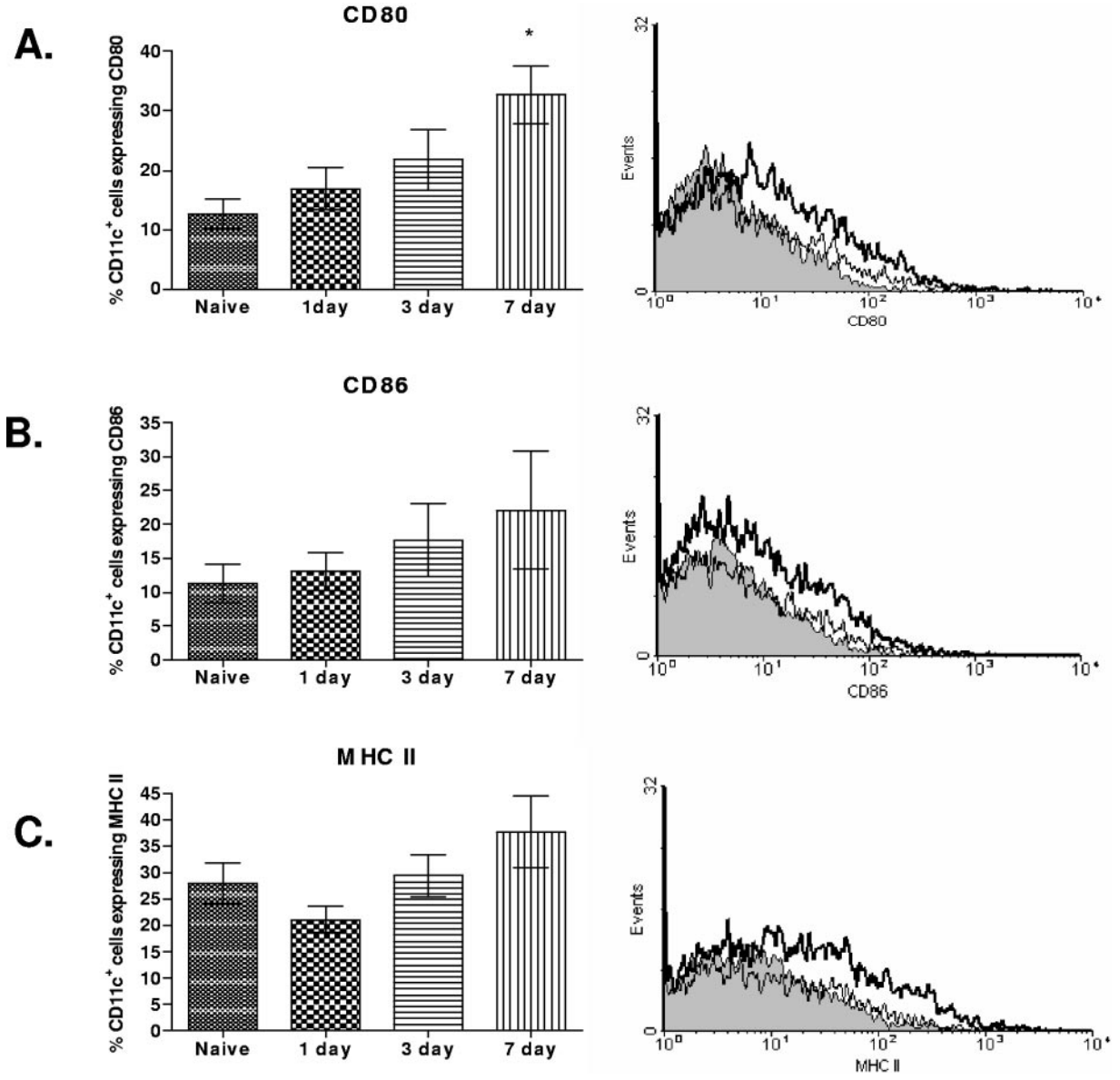

FIG. 5. Expression of costimulatory molecules on pulmonary DC following infection with *C. neoformans*. Mice were not challenged (naïve) or were inoculated with live *C. neoformans*. At 1, 3, and 7 days postinoculation, mice were sacrificed, and the percentages of $CD11c^+$ lung cells coexpressing CD80 (A), CD86 (B), and MHC-II (C) were determined by flow cytometry. The bar graphs show the means ± standard errors of the means from three independent experiments. Lung cells from four mice per group were pooled for each experiment. The asterisk indicates that there was a significant difference as determined by the Student *t* test for a comparison with naïve mice ($P = 0.006$). The histograms show the results of one representative experiment. Gray area, naïve mice; thin line, day 3 infected mice; thick line, day 7 infected mice.

an adaptive immune response, we focused on the role of DC in the uptake of *C. neoformans*. In order to verify that the DC were capable of internalizing *C. neoformans* rather than just binding extracellularly to the surface of the organism, we examined lung cells from infected mice by confocal microscopy. By examining confocal images, we determined that the *C. neoformans* cells were indeed inside the DC. In studies of other pathogens that infect the lung, such as *M. tuberculosis* (28, 62, 64, 66) and *A. fumigatus* (11), lung DC have been shown to phagocytose the organisms in vivo and are responsible for initiating an adaptive immune response. Moreover, we have shown previously that DC phagocytose *C. neoformans* in vitro when it is opsonized by complement or antibody (38). In our in vivo study, the mice were not previously exposed to *C. neoformans*, and thus it is unlikely that they had anticryptococcal antibodies. Therefore, we speculate that opsonization and phagocytosis of *C. neoformans* in vivo are probably the result of binding of complement components, such as iC3b, to the surface of the organism (40–42, 81).

Initially, we examined the uptake of both live and heat-killed *C. neoformans* following intranasal inoculation. We found that at several early times postinoculation, organisms were taken up by DC, macrophages, and neutrophils. We hypothesized that live and killed organisms would be phagocytosed at different

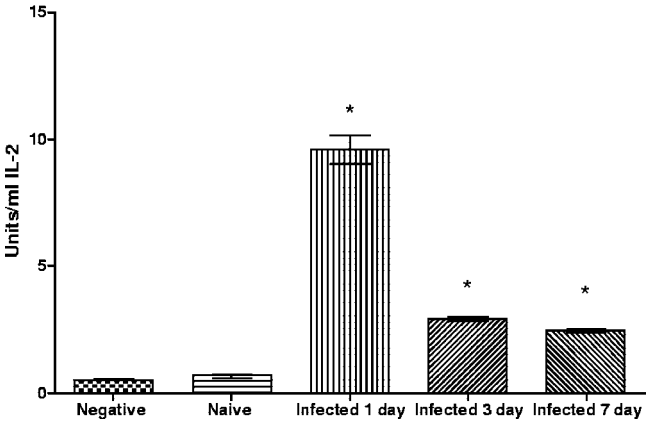

FIG. 6. Proliferation of *C. neoformans*-specific T cells in response to ex vivo stimulation by pulmonary DC from infected mice. Mice were not challenged (naïve) or were inoculated with live *C. neoformans*. At 1, 3, and 7 days postinoculation, mice were sacrificed, and pulmonary DC were purified and separated by cell sorting or by $CD11c^+$ magnetic bead positive selection. DC were then incubated with a T-cell hybridoma specific for *C. neoformans* mannoprotein (P1D6), and IL-2 production in the supernatant, a measure of T-cell activation, was determined. P1D6 cells alone (without DC) served as a negative control. Lung cells from four mice per group were pooled for each experiment. The data are representative of three experiments. An asterisk indicates that there was a significant difference as determined by the Student *t* test for a comparison with naïve mice ($P = 0.004$ for day 1; $P = 0.002$ for day 3; $P = 0.002$ for day 7).

rates because in vivo conditions stimulate growth and shedding of the antiphagocytic capsule, as well as secretion of chemotactic factors, each of which could have an impact on phagocytosis (20–22, 39, 49). However, the susceptibilities to phagocytosis were similar when live *C. neoformans* and heat-killed *C. neoformans* were compared.

In order to determine if cryptococcal infection could affect DC infiltration and maturation, we examined the percentage and maturation of lung DC after several days of infection. Our data showed that the percentage of $CD11c^+$ DC in the lungs increased throughout infection, suggesting that there was DC infiltration into the lungs during infection. DC infiltration into the lungs has also been reported with other proinflammatory stimuli in the lung, including gamma interferon (27), cigarette smoking (73), and allergen inhalation (53, 54), as well as during bacterial and viral infections (53, 54, 62). Also, the percentage of the lung DC that expressed the maturation marker CD80 significantly increased, and the levels of CD86 and MHC-II increased moderately throughout the course of the infection, suggesting that pulmonary cryptococcal infection leads to DC maturation. However, the increases were modest, and it is possible that many of the mature DC began to traffic to the regional lymph nodes. This is consistent with reports which indicated that after capturing antigens, DC express processed antigens in the context of MHC-II and upregulate costimulatory molecules, such as CD40, CD80, and CD86 (3). During these changes, DC migrate from the infected tissues into the T-cell area of the draining lymph nodes, where they encounter naïve antigen-specific $CD4^+$ T cells (29). If the mature DC indeed traffic to the regional lymph nodes, they could present cryptococcal antigens to T cells and thus initiate the adaptive immune response.

In agreement with other studies of pulmonary DC, we defined immature pulmonary DC by surface expression of intermediate levels of CD11c (64, 75), low to intermediate levels of MHC-II (31, 75), and undetectable levels of CD8-α (75). Recent studies have suggested that under some conditions, CD11c can be expressed on macrophages (64) and activated $CD8^+$ T cells (6). However, the $CD11c^+$ cells that we isolated from naïve and infected mice only rarely expressed the macrophage marker F4/80 and did not coexpress the T-cell markers CD3 and CD8. Thus, the vast majority of the $CD11c^+$ cells isolated from the lung in this study appeared to be DC. Additionally, two major subtypes of $CD11c^+$ DC have been described, plasmacytoid and myeloid DC. Myeloid DC are $CD11c^+$, $CD11b^+$, $CD8\alpha^-$, and $B220^-$ (2, 3, 19, 80), whereas plasmacytoid DC are $CD8\alpha^+$ and $B220^+$ and lack expression of costimulatory molecules (2, 3, 7, 13, 61, 80). In preliminary studies with both naïve and infected mice, less than 3% of lung DC expressed B220 (Wozniak and Levitz, unpublished observations), suggesting that the vast majority of these DC are myeloid DC.

Finally, we examined the potential for pulmonary DC from infected mice to present antigen ex vivo to *Cryptococcus*-specific T cells. We showed that the *Cryptococcus*-specific T cells were activated when they were incubated with $CD11c^+$ lung DC from infected mice but not when they were incubated with DC from uninfected mice. This suggests that lung DC from infected mice are capable of cryptococcal antigen presentation to T cells, which probably occurs once the DC traffic to the regional lymph nodes. Interestingly, the T cells stimulated by DC from mice infected for only 24 h produced more IL-2 than the T cells stimulated by DC from mice infected for 3 or 7 days. This is especially intriguing, as the highest percentage of DC was present in the lungs at 7 days postinfection. However, it is possible that by days 3 and 7 postinoculation, the mature DC that present cryptococcal antigen may already be trafficking out of the lung and into the regional lymph nodes, and the increase in DC in the lung may be due to immature DC trafficking into the lung.

In these experiments, we showed that lung DC are involved in cryptococcal phagocytosis in vivo and antigen presentation to *Cryptococcus*-specific T cells. This suggests not only that DC are important for initiating an adaptive immune response to the organism but also that DC may play a previously unappreciated role in innate defenses. We are performing studies to determine the interaction between *C. neoformans* and lung DC in development of protective immunity in the lung.

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