Dectin-1 Induces M1 Macrophages and Prominent Expansion of CD8\(^+\)IL-17\(^+\) Cells in Pulmonary Paracoccidioidomycosis

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Dectin-1, the innate immune receptor that recognizes \(\beta\)-glucan, plays an important role in immunity against fungal pathogens. *Paracoccidioides brasiliensis*, the etiological agent of paracoccidioidomycosis, has a sugar-rich cell wall mainly composed of mannans and glucans. This fact motivated us to use dectin-1–sufficient and –deficient mice to investigate the role of \(\beta\)-glucan recognition in the immunity against pulmonary paracoccidioidomycosis. Initially, we verified that *P. brasiliensis* infection reinforced the tendency of dectin-1–deficient macrophages to express an M2 phenotype. This prevalent anti-inflammatory activity of dectin-1–/– macrophages resulted in impaired fungicidal ability, low nitric oxide production, and elevated synthesis of interleukin 10 (IL-10). Compared with dectin-1–sufficient mice, the fungal infection of dectin-1–/– mice was more severe and resulted in enhanced tissue pathology and mortality rates. The absence of dectin-1 has also impaired the production of T-helper type 1 (Th1), Th2, and Th17 cytokines and the activation and migration of T cells to the site of infection. Remarkably, dectin-1 deficiency increased the expansion of regulatory T cells and reduced the differentiation of T cells to the IL-17\(^+\) phenotype, impairing the migration of IL-17\(^+\)CD8\(^+\) T cells and polymorphonuclear cells to infected tissues. In conclusion, dectin-1 exerts an important protective role in pulmonary paracoccidioidomycosis by controlling the innate and adaptive phases of antifungal immunity.

**Keywords.** *Paracoccidioides brasiliensis*; dectin-1 receptor; innate immunity; pulmonary pathology; adaptive immunity.

Among several families of pattern recognition receptors (PRRs), the family of C-type lectin receptors has emerged as a major sensor of pathogens by recognizing carbohydrate moieties on pathogens [1, 2]. The most studied C-type lectin receptor is dectin-1, which contains an extracellular C-type lectin domain and an intracellular immunoreceptor tyrosine-based activation motif (ITAM)–like motif that initiates cell signaling and activation following interaction with several fungal pathogens [3–5]. Dectin-1 recognizes \(\beta\)-1-3-glucans present on the cell wall of medically important fungi [3, 4]. Following ligation of \(\beta\)-glucans to dectin-1, a large number of cellular events, such as phagocytosis, activation of signaling pathways, generation of reaction oxygen species, and release of cytokines, occur [4, 6]. These events can directly affect the quality and quantity of the adaptive immune response. Several studies have shown that mouse and human dendritic cells that have taken up antigens via dectin-1 present antigenic peptides to both CD4\(^+\) and CD8\(^+\) T cells, resulting in potent antigen-specific CD4\(^+\) and CD8\(^+\) T-cell responses [7–11]. In addition, the cellular activation mediated by this receptor can drive host adaptive immunity to a prevalent T-helper type 17 (Th17) response [12–17].

Paracoccidioidomycosis (PCM) is a systemic granulomatous disease caused by the dimorphic fungus...
Paracoccidioides brasiliensis and constitutes the most prevalent deep mycosis in Latin America. In previous studies, our group showed that Toll-like receptor 2 (TLR2) deficiency leads to increased Th17 immunity, which was associated with diminished expansion of regulatory T cells (Tregs) and increased lung pathology due to unrestrained inflammatory reactions. In addition, a more severe P. brasiliensis infection associated with increased production of Th17 cytokines, enhanced proinflammatory immunity, and impaired expansion of regulatory T cells was shown to be influenced by TLR4 expression [18–21]. However, the role of dectin-1 in PCM was not investigated thoroughly. Nevertheless, a recent report indicated that dectin-1 was involved in the internalization of P. brasiliensis by human monocytes and neutrophils, suggesting that it has a function in immunity against P. brasiliensis infection [22]. Here, we report that dectin-1 receptor controls the innate and adaptive phases of the immune response in P. brasiliensis–infected mice. The absence of dectin-1 expression induces a preferential differentiation of macrophages to an antiinflammatory, M2-like phenotype that results in decreased fungicidal ability and nitric oxide (NO) production. Compared with wild-type (WT) mice, the absence of dectin-1 receptor controls the innate and adaptive phases of the immune response in P. brasiliensis–infected mice. The absence of dectin-1 expression induces a preferential differentiation of macrophages to an antiinflammatory, M2-like phenotype that results in decreased fungicidal ability and nitric oxide (NO) production. Compared with wild-type (WT) mice, the absence of dectin-1 expression induces a preferential differentiation of macrophages to an antiinflammatory, M2-like phenotype that results in decreased fungicidal ability and nitric oxide (NO) production. Compared with wild-type (WT) mice, the absence of dectin-1 expression induces a preferential differentiation of macrophages to an antiinflammatory, M2-like phenotype that results in decreased fungicidal ability and nitric oxide (NO) production.
medium (Sigma). Total lung leukocyte numbers were assessed in the presence of trypan blue, and viability was always >85%. For differential leukocyte counts, samples of lung cell suspensions were cytoospun (Shandon Cytospin) onto glass slides and stained by the Diff-Quik blood stain (Baxter Scientific). A total of 200–400 cells were counted from each sample. For flow cytometry, lung leukocytes were resuspended at 10^6 cells/mL in staining buffer. Anti-CD44, -CD25, -CD62L, -CD69, -CD4, and -CD8 monoclonal antibodies (eBiosciences) were used. Cells were fixed with 1% paraformaldehyde (Sigma) and analyzed in a FACSCanto flow cytometer (BD Pharmingen). For polymerase chain reaction (PCR) experiments, macrophages were isolated from lung leukocyte suspensions by positive selection, using anti-CD11b-coated magnetic beads (Miltenyi Biotec).

Quantitative Real-Time PCR
Total RNA from in vitro–infected macrophages and lung CD11b^+ cells obtained at week 2 after infection was extracted using Trizol (Invitrogen) reagent and reverse transcribed, and complementary DNA (cDNA) was amplified. Real-time PCR was performed using the TaqMan universal master mix. First-strand cDNAs were synthesized from 2 µg of RNA, using the High Capacity RNA-to-cDNA kit (Applied Biosystems). Real-time PCR was performed using the TaqMan real-time PCR assay (Applied) for the following molecules: TLR2, TLR4, suppressor of cytokine signaling-3 (SOCS3), suppressor of cytokine signaling-1 (SOCS1), arginase 1 (ARG1), NO-synthase 2 (NOS2), found in inflammatory zone protein (FIZZ1), and chitinase-3 like 3 (Chi3L3 or Ym1). Analysis was performed with the ABI Prism 7000 sequence detection system (Applied). All values were normalized to GAPDH, and the relative gene expression was calculated using the Pfaffl method [30].

Intracellular Staining
Lung leukocytes were stimulated for 6 hours in complete medium in the presence of 50 ng/mL phorbol 12-myristate 13-acetate, 500 ng/mL ionomycin (Sigma-Aldrich), and monensin (3 mM, eBioscience). After surface staining for CD4, CD8, CD25, and CD69, some cultures were fixed, permeabilized, and stained by anti-IFN-γ, anti-interleukin 4 (IL-4), and anti-interleukin 17 (IL-17) antibodies (eBioscience). The expression of cell surface markers, as well as the intracellular expression of IL-4, IFN-γ, and IL-17 in lung infiltrating leukocytes, were analyzed in a FACSCanto flow cytometer (BD Pharmingen) and with FlowJo software (Tree Star).

Statistical Analysis
Data are expressed as the mean ± SEM. Differences between groups were analyzed by Student t test or analysis of variance followed by the Tukey test. Differences between survival times were determined with the log-rank test. Data were analyzed using GraphPad Prism 5.01 software for Windows. Error bars represent ± SEM; P values of ≤0.05 were considered statistically significant.

RESULTS

Absence of Dectin-1 Leads to Decreased Phagocytic and Fungicidal Abilities and NO Production by P. brasiliensis–Infected Macrophages
Macrophages from dectin1^−/− and WT mice were infected with P. brasiliensis yeasts. Phagocytosis was determined by flow cytometry, using fluorescence-labeled yeasts and macrophages. As shown in Figure 1A, dectin-1^−/− macrophages showed a decreased frequency of ingested/adhered yeast cells. In addition, CFU assays demonstrated an increased number of viable yeasts recovered from dectin-1^−/− macrophages (Figure 1B). The levels of NO and cytokines were determined in cell supernatants of CFU assays, and diminished levels of NO (Figure 1C) and IL-6 (Figure 1D) were produced by dectin-1^−/− macrophages. Furthermore, deficient macrophages produced increased levels of monocyte chemoattractant protein-1 (MCP-1) and interleukin 10 (IL-10), an important macrophage-deactivating cytokine (Figure 1D).

Dectin-1 Controls the Expression of TLRs In Vitro
To further explore the influence of dectin-1 in the recognition of P. brasiliensis yeasts, the expression of other pathogen-recognition receptors (PRRs) previously known to be involved in the interaction of this fungal pathogen with macrophages [18–20] was also evaluated. As seen in Figure 2A and 2B, P. brasiliensis–infected dectin-1^−/− mice had a decreased percentage of TLR4^+ and a higher percentage of TLR2^+ macrophages, compared with WT mice. To confirm the opposite expression of TLR2 and TLR4 molecules in macrophages, the levels of messenger RNA (mRNA) to TLR2 and TLR4 were measured. As depicted in Figure 2C and 2D, the mRNA levels of TLR4 and TLR2 confirm the opposite expression of these receptors by WT and dectin-1^−/− macrophages.

Absence of Dectin-1 Determines a Prevalent Differentiation of Macrophages to an M2-like Phenotype
M1 macrophages are associated with NO production and enhanced microbicidal activity, whereas M2 macrophages promote healing and tissue repair but show impaired microbicidal activity [31]. The diverse behavior of dectin-1^−/− and WT macrophages led us to suppose that the expression of dectin-1 was inducing a prevalent inflammatory or M1-like differentiation in WT macrophages but that its absence was stimulating a predominant anti-inflammatory or M2-like differentiation. As can be seen in Figure 2E, macrophages from uninfected WT mice showed a prevalent expression of M1-polarized cells (SOCS3), whereas dectin-1^−/− macrophages expressed elevated levels of M2-associated markers (Ym1, ARG1, and SOCS1). P. brasiliensis infection induced enhanced levels of mRNA but was not able to alter the prevalent M1 and M2 behavior of dectin-1–sufficient and deficient macrophages, respectively. Importantly, P. brasiliensis infection enhanced the differences of M1 (NOS2 and SOCS3)
and M2 (YM1, ARG1, SOCS1, and FIZZ-1) markers between WT and dectin-1–deficient macrophages. Therefore, *P. brasiliensis* infection appears to intensify the differentiation of dectin-1−/− macrophages to an M2 phenotype.

**Absence of Dectin-1 Receptor Increases Mortality Rates Associated With Increased Fungal Loads and Tissue Pathology**

The severity of fungal infection was assessed at early and late periods of the disease. Pulmonary fungal burdens were increased in *P. brasiliensis*–infected WT and dectin-1−/− mice at 48 hours, as well as at 2 and 8 weeks after infection (Figure 3A–C). At week 8, CFU counts were also increased in the livers and spleens of the deficient mice (Figure 3C). In addition, an increased number of nonorganized lesions containing high numbers of fungal cells and intense tissue destruction were observed in dectin-1−/− mice (Figure 3D–K). Pulmonary lesions in dectin-1−/− mice replaced large part of normal tissue and were composed of confluent necrotic lesions containing many budding yeasts (Figure 3G), surrounded by inflammatory cells (Figure 3E). The lesions in the lungs of WT mice occupied a small area and were composed of organized granulomas of small sizes (Figure 3D and 3F). The hepatic lesions of WT mice were composed of scarce and isolated granulomas whereas in dectin-1−/− mice a high number of granulomas with elevated number of yeasts occupied a large area of the organ (Figure 3H–K). Compared with WT mice, the less organized and more frequent pulmonary and hepatic lesions of dectin-1−/− mice resulted in increased areas of damaged tissue (Figure 3L). To assess the influence of dectin-1...

**Figure 1.** Absence of dectin-1 impairs the phagocytic and fungicidal abilities of macrophages and alters nitric oxide and cytokines production. **A,** For phagocytic assays, interferon-γ (IFN-γ)–primed and unprimed peritoneal macrophages from dectin-1−/− and wild-type (WT) mice were infected with heat-inactivated fluorescein isothiocyanate–labeled *Paracoccidioides brasiliensis* yeasts at a macrophage-yeast ratio of 1:1 for 4 hours at 37°C in 5% CO2. Cell suspension were then obtained, macrophages were labeled with allophycocyanin anti-F4/80 antibodies, and fungi adhesion/ingestion was measured by flow cytometry. **B,** For fungicidal assay IFN-γ–primed and unprimed macrophages were infected with *P. brasiliensis* yeasts in a macrophage-to-yeast ratio of 25:1 for 2 hours. Infected macrophages were then cultivated for 48 hours at 37°C in 5% CO2. The monolayers were washed with distilled water to lyse macrophages, and 100 µL of cell homogenates were assayed for the presence of viable yeasts by a colony-forming units (CFU) assay. Supernatants obtained from fungicidal assays were used to determine the levels of nitrite (C) and cytokines (D). Data are the means ± standard errors of the mean of quintuplicate samples from 1 experiment representative of 3 independent determinations. *P < .05. Abbreviations: IL-6, interleukin 6; IL-10, interleukin 10; IL-12, interleukin 12; ND, not done; Pb, *P. braziliensis*.
Figure 2. Dectin-1 controls the expression of Toll-like receptors (TLRs) and determines the differentiation of *Paracoccidioides brasiliensis*-infected macrophages to an “M1-like” phenotype. Normal and interferon-γ (IFN-γ)–primed macrophages from wild-type (WT) and dectin-1−/− mice were infected with *P. brasiliensis* yeasts in a macrophage-to-yeast ratio of 25:1 and cocultivated for 48 hours. The expression of TLR4 (A) and TLR2 (B) was then assayed by flow cytometry. The acquisition and analysis gates were restricted to the F4/80+-labeled macrophage population. For quantitative polymerase chain reaction (PCR) analysis of TLR4 (C) and TLR2 (D) messenger RNA (mRNA) expression, macrophages from dectin-1−/− and WT mice were infected by viable *P. brasiliensis* yeasts (fungus-to-macrophage ratio, 1:25) for 2 hours. After 12 hours at 37°C in 5% CO2, the total RNA from macrophage cultures was obtained and reverse transcribed, and complementary DNA (cDNA) was amplified. Real-time PCR was performed using TaqMan universal master mix. Amplified
deficiency in the disease outcome, mortality of infected mice was registered daily. As shown in Figure 3M, at day 137 of infection all dectin-1−/− mice were dead. In the same period, 5 of 12 WT mice were still alive and apparently healthy.

**Absence of Dectin-1 Results in Reduced Levels of Cytokines**

By eight weeks after infection, reduced levels of Th1 (interleukin 12 [IL-12], TNF-α, and IFN-γ), Th2 (IL-4 and IL-10), Th17 cytokines (TGF-β, IL-6, IL-17, and interleukin 23 [IL-23]) and MCP-1 chemokine were detected in the lungs of dectin-1−/− mice (Figure 4B). In addition, reduced levels of IL-1β, the cytokine produced upon inflammasome activation, were also detected in the lungs of dectin-1−/− mice. No major differences were found in the liver, but IL-12 was found in reduced levels in dectin-1−/− mice. When splenic cytokines were measured, decreased levels of IFN-γ, IL-10, TGF-β, IL-17, and IL-1β were detected in dectin-1−/− mice (Supplementary Figure 1).

**Dectin-1 Controls the Recruitment of Polymorphonuclear Cells (PMNs) to the Lungs**

To better characterize the influence of dectin-1 expression in the inflammatory reaction induced by *P. brasiliensis* infection, leukocyte recruitment to the lungs of *P. brasiliensis*-infected dectin-1−/− and WT mice was studied. A reduced number of total leukocytes and a decreased frequency and numbers of PMNs were observed in the lungs of dectin-1−/− mice. No important differences were noted in the numbers of macrophages and lymphocytes (Figure 4A and 4B).

**Dectin-1 Signaling Increases the Differentiation of CD4+ and CD8+ T Cells**

To determine the role of dectin-1 receptor in the acquired phase of immunity against *P. brasiliensis*, the phenotype and activation of lung inflammatory cells were analyzed. When lymphocytes were phenotyped (Figure 5A), a reduced number of activated-effector CD4+CD25− and CD4+CD44highCD62Llow T cells were observed in the lungs of dectin-1−/− mice at week 2 of infection (Figure 5B). By week 8 after infection, only CD4+CD25− T cells appeared in reduced numbers in the lungs of dectin-1−/− mice (Figure 5C). A significantly reduced recruitment of total, naive, and activated-effector (CD8+, CD8+CD44lowCD62Lhigh, and CD8+CD44highCD62Llow, respectively) T cells was observed in the lungs of dectin-1−/− mice at week 2 of infection (Figure 5D). However, at week 8 after infection only CD8+CD44highCD62Llow appeared in reduced numbers in the lungs of dectin-1−/− mice (Figure 5E).

**Absence of Dectin-1 Promotes Downregulation of IL-17-Producing CD8+ T Cells Associated With Regulatory T-Cell Expansion**

To better clarify the importance of dectin-1 in the polarization of T-cell responses, the phenotype of IL-17−, IFN-γ−, and IL-4−producing cells was defined in the inflammatory infiltrates of lungs (Figure 6A). As shown in Figure 6B and 6C, significantly diminished numbers of CD8+IL-17+ T cells were detected in the lungs of dectin-1−/− mice, but no differences in the numbers of CD4+IL-17+ T cells were detected. Similar numbers of IL-4− and IFN-γ−producing cells were also observed. These findings indicate that the absence of dectin-1 receptor induces an impaired migration of IL-17+CD8+ T cells, also known as Tc17 cells, to the lungs of dectin-1−/− mice. Additionally, increased frequency and numbers of CD4+CD25+FoxP3+ Tregs were observed in the lung inflammatory exudates of dectin-1−/− mice (Figure 6D and 6E).

**DISCUSSION**

In response to fungal cell wall components, dectin-1 induces intracellular signaling that promote the activation of transcription factors (NFκB and NFAT), which control the production of cytokines, and chemokines, as well as the release of reactive oxygen intermediates and eicosanoids [32–35].

In this study, we verified that dectin-1−/− macrophages ingested decreased numbers of yeasts but allowed increased growth of *P. brasiliensis*, compared with WT cells. Dectin-1−/− macrophages also showed impaired NO production, suggesting that dectin-1 receptor participates in the recognition of *P. brasiliensis* and in the induction of cellular mechanisms that control fungal growth. The higher levels of IL-10 produced by dectin-1−/− macrophages may have contributed to the ineffective activation of these cells. Indeed, WT cells expressed elevated levels of SOCS3, indicating a tendency to an inflammatory “M1-like” profile, whereas dectin-1−/− macrophages showed elevated levels of Ym1, ARG1, FIZZ1, and SOCS1, typical markers of alternatively activated macrophages, suggesting a prevalent “M2-like” differentiation. Importantly, *P. brasiliensis* infection reinforced the expression of M2 markers by dectin-1−/− macrophages, possibly influencing the ineffective immunity developed by...
Figure 3. *Paracoccidioides brasiliensis*–infected *dectin-1*−/− mice have increased mortality associated with increased fungal loads and tissue pathology. A–C, Colony-forming unit (CFU) counts from organs were determined 48 hours (A), 2 weeks (B), and 8 weeks (C) after *P. brasiliensis* infection of wild-type (WT) and *dectin-1*−/− mice. The bars represent means ± standard errors of the mean (SEM) of log10 CFU counts obtained from groups of 5–6 mice. D–K, Photomicrographs of lesions of WT mice (D–G) and *dectin-1*−/− mice (H–K) at week 8 of infection with 1 × 10⁶ *P. brasiliensis* yeasts. Compared with *dectin-1*−/− mice (E and G), the pulmonary lesions of WT mice (D) were smaller and composed of organized granulomas containing lower numbers of yeasts (F). The pulmonary lesions of *dectin-1*−/− mice were composed of confluent and unorganized granulomas of various sizes (E) containing an elevated number of fungal cells (G). The livers of WT (H and J) and *dectin-1*−/− (I and K) mice presented organized granulomas, which, however, appeared in lower numbers and contained fewer yeasts (J) in WT mice than in *dectin-1*−/− mice (K). Staining of lesions was performed with hematoxylin-eosin (D, F, H, and J) and Grocott (E, G, I and K; original magnification, 100×). L, Total area of lesions in the lungs and livers of mice (*n* = 6) at week 8 after infection. M, Survival times of *dectin-1*−/− and WT mice after intratracheal infection with 1 × 10⁶ *P. brasiliensis* yeast cells was determined in a period of 137 days. The results are representative of 2 experiments with equivalent results. Data represent the means ± SEM of at least 5 mice/group and are representative of 3 independent experiments. *P* < .05.
dectin-1−/− mice. However, a recent report showed an association between M2 markers and dectin-1 expression, indicating that not all fungal ligands behave similarly [36].

Although the expression of TLR4 and TLR2 (protein and mRNA) was not affected by *P. brasiliensis* infection of WT macrophages, dectin-1−/− cells expressed reduced levels of TLR4...
Figure 5. Absence of dectin-1 determines decreased numbers of activated T lymphocytes in the lungs. A, Flow cytometry gating strategy to detect effector/memory and naive CD4+ T cells in lung infiltrating leukocytes (LILs). LIL lymphocytes were identified on forward-scatter (FSC) and side-scatter (SSC) analysis. Gated cells were measured for CD4 expression followed by CD44 expression, and cells expressing high and low levels of this molecule were gated. Gated CD44^{high} cells were then measured for expression of low levels of CD62L identifying the effector/memory CD4^{+}CD44^{high}CD62L^{low} subpopulation. Gated CD44^{low} cells were then measured for expression of high levels of CD62L identifying the naive CD4^{+}CD44^{low}CD62L^{high} subpopulation. B–E, Characterization of CD4+ T cells (B and C) and CD8+ T cells (D and E) by flow cytometry in the LILs from dectin-1−/− and WT mice inoculated intratracheally with 1 × 10^6 Paracoccidioides brasiliensis yeast cells. At weeks 2 (B and D) and 8 (C and E) after infection, lungs of both mouse strains (n = 5–6) were excised and digested enzymatically. Cell suspensions were obtained and stained as described in Materials and Methods. The stained cells were analyzed immediately on FACS Canto II equipment with gating of lymphocytes, as judged from FSC and SSC findings. One hundred thousands cells were counted and the data expressed as absolute number of positive cells. Data are expressed as means ± standard errors of the mean and are representative of three independent experiments. *P < .05.
Absence of dectin-1 promotes downregulation of interleukin 17 (IL-17)–producing CD8+ T (Tc17) cells associated with regulatory T-cell expansion.

**A**, Flow cytometry gating strategy to detect cytokine positive CD4+ T cells in lung infiltrating leukocytes (LILs). LIL lymphocytes were identified on forward-scatter (FSC) and side-scatter (SSC) analysis. Gated cells were measured for CD4 or CD8 expression. Gated cells were then measured for IL-17 expression. The same procedure was used to identify interleukin 4 (IL-4)–positive and interferon γ (IFN-γ)–positive CD4+ and CD8+ T cells. **B** and **C**, The presence of IL-17+, IFN-γ+, and IL-4+ CD4+ and CD8+ T cells in the LILs was assessed by intracellular cytokine staining by flow cytometry at weeks 2 (**B**) and 8 (**C**) after infection. Lung cells were restimulated in vitro with PMA/ionomycin for 6 hours and subjected to intracellular staining for IL-17, IL-4, and IFN-γ. The lymphocyte population was gated by FSC/SSC analysis. **D**, Flow cytometry gating strategy to detect T-regulatory cells (Tregs) in LILs. LIL lymphocytes were identified on FSC and SSC analysis. Gated cells were measured for CD4 expression and then for CD25 expression. Gated CD4+CD25+ cells were measured for Foxp3 expression, identifying Tregs (**E**). Results are from 1 experiment and are representative of 3 independent experiments. Data are expressed as means ± standard errors of the mean. *P < .05.
and increased levels of TLR2. Interestingly, our previous studies have shown the inhibitory and stimulatory effects of TLR2 and TLR4, respectively, on Th17 immunity against *P. brasiliensis* infection [18, 20, 21]. Thus, it is possible that the inhibited Th17 response of dectin-1−/− mice was influenced by the increased expression of TLR2 and the lower expression of TLR4 detected in dectin-1−/− macrophages.

Consistent with the in vitro data, the results of in vivo CFU assays showed a more severe infection in dectin-1−/− mice than in WT mice. Importantly, at the chronic phase, the increased fungal loads were concomitant with reduced levels of IL-1β, Th1, Th2, and Th17 cytokines, indicating a major role for dectin-1 in the differentiation of all T-cell subsets.

Some studies have described the involvement of dectin-1 in the cleavage of pro–IL-1β and pro–IL-18 into their active forms by caspase-1 or other inflammatory or pathogen-derived proteases. As an example, Hise et al. [37] demonstrated that the interaction of *Candida albicans* with TLR2 and dectin-1 regulates the production of IL-1β via the NLPR3 inflammasome caspase-1–dependent pathway. Using MyD88−/− mice, we have previously demonstrated an association between disease severity and reduced Th17 response and IL-1β production [21]. Thus, the decreased levels of IL-1β here observed could have contributed to the suppressed T-cell immunity developed by dectin-1−/− mice.

The diminished synthesis of IL-1β, TGF-β, IL-6, and IL-23 was linked to the defective CD8+17+ T-cell responses developed by dectin-1−/− mice. Recent studies have described the involvement of dectin-1 in the induction of Th17 immune response [38, 39]. Besides, the proliferation of antigen-specific CD8+ T cells and the in vivo cross-priming of cytotoxic T lymphocytes were reported to be mediated by dectin-1 signaling [9]. Similar responses were observed in mice and humans infected by fungal pathogens [40] and were here confirmed and expanded in pulmonary paracoccidioidomycosis.

Th17 immunity is generally associated with enhanced synthesis of CXC chemokines and the induction of neutrophil chemotaxis to inflammatory sites [41, 42]. Here, the reduced neutrophil influx into the lungs of infected dectin-1−/− mice was concurrent with decreased Th17 cytokine production. This finding is consistent with our previous report showing that Th17 polarization in pulmonary PCM is associated with PMN-rich inflammatory reactions [18, 20, 21].

The less organized and more severe lesions observed in the histopathology study, the elevated fungal burdens, and the increased fungal dissemination to several organs appear to have contributed to the increased mortality rates of dectin-1−/− mice. Importantly, this profile was associated with impaired activation of effector/memory CD4+ and CD8+ T cells concomitantly with increased number and frequency of CD4+CD25−FoxP3− Tregs at the site of infection. Thus, in pulmonary PCM, dectin-1 seems to be involved in the modulation of adaptive immunity, and its expression contributes to the development of efficient T-cell immunity modulated by moderate expansion of Tregs.

Our laboratory characterized the role of CD4+ and CD8+ T cells in murine PCM and demonstrated that, whatever the pattern of host susceptibility, in pulmonary PCM the fungal loads are primarily controlled by CD8+ T cells [43, 44]. Several studies have demonstrated the importance of dectin-1 in the activation of CD8+ T lymphocytes, as well as in their cytokinetic activity [9–11]. Human dendritic cells activated with curdlan act as efficient antigen-presenting cells and induce prevalent Th17 and CD8+ T-cell responses [10, 11]. Our data on intracellular cytokines confirmed the prominent involvement of dectin-1 in the development of CD8+ T cells and their polarization toward IL-17 production. A reduced number of infiltrating CD8+IL-17+ T cells was detected at weeks 2 and 8 after infection of dectin-1−/− mice, indicating that in pulmonary PCM dectin-1 expression has a more critical influence in the differentiation and migration of IL-17+CD8+ T cells than CD4+ T cells.

In conclusion, the absence of dectin-1 appears to impair inflammatory innate immunity, as evidenced by the M2-like profile of macrophages, which present impaired fungicidal ability. Moreover, dectin-1 deficiency suppresses the development of protective adaptive immunity, as shown by the decreased production of Th1, Th2, and Th17 cytokines, and diminished activation and migration of CD4+ and CD8+ T cells to the site of infection. This defective innate and adaptive immunity of dectin-1−/− mice, which was concomitant with increased Treg expansion, resulted in uncontrolled growth and dissemination of the fungal cells, which consistently compromises the survival of *P. brasiliensis*–infected hosts.

**Supplementary Data**

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

**Acknowledgments.** We thank Paulo Albee for invaluable technical assistance.

**Financial support.** This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (grant VLG 2010/52275-5; postdoctoral fellowships to F. V. L. and S. B. B.) and Coordenadoria de Aperfeiçoamento de Pessoal de Ensino Superior (postdoctoral fellowship to C. F.).

**Potential conflicts of interest.** All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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