Dectin-2 Is a C-Type Lectin Receptor that Recognizes *Pneumocystis* and Participates in Innate Immune Responses

Theodore J. Kottom¹, Deanne M. Hebrink¹, Paige E. Jenson¹, Paige L. Marsolek¹, Marcel Wüthrich², Huafeng Wang², Bruce Klein^{2,3,4}, Sho Yamasaki⁵, and Andrew H. Limper¹

¹Thoracic Diseases Research Unit, Departments of Medicine and Biochemistry, Mayo Clinic College of Medicine, Rochester, Minnesota; ²Department of Pediatrics, ³Department of Internal Medicine, and ⁴Department of Medical Microbiology and Immunology, University of Wisconsin Medical School, University of Wisconsin Hospital and Clinics, Madison, Wisconsin; and ⁵Division of Molecular Immunology, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan

Abstract

Pneumocystis is an important fungal pathogen that causes life-threatening pneumonia in patients with AIDS and malignancy. Lung fungal pathogens are recognized by C-type lectin receptors (CLRs), which bind specific ligands and stimulate innate immune responses. The CLR Dectin-1 was previously shown to mediate immune responses to *Pneumocystis* spp. For this reason, we investigated a potential role for Dectin-2. Rats with *Pneumocystis* pneumonia (PCP) exhibited elevated *Dectin-2* mRNA levels. Soluble Dectin-2 carbohydrate-recognition domain fusion protein showed binding to intact *Pneumocystis carinii* (Pc) and to native *Pneumocystis* major surface glycoprotein/glycoprotein A (Msg/gpA). RAW macrophage cells expressing V5-tagged Dectin-2 displayed enhanced binding to Pc and increased protein tyrosine phosphorylation. Furthermore, the binding of Pc to Dectin-2 resulted in Fc receptor- γ -mediated intracellular signaling. Alveolar macrophages from Dectin-2-deficient mice (Dectin-2^{-/-}) showed significant decreases in phospho-Syk activation after challenge with Pc cell wall components. Stimulation of Dectin-2^{-/-} alveolar macrophages with Pc components showed significant decreases in the proinflammatory cytokines IL-6 and TNF- α . Finally, during infection with *Pneumocystis murina*, Dectin-2^{-/-} mice displayed

downregulated mRNA expression profiles of other CLRs implicated in fungal immunity. Although Dectin-2^{-/-} alveolar macrophages had reduced proinflammatory cytokine release *in vitro*, Dectin-2^{-/-} deficiency did not reduce the overall resistance of these mice in the PCP model, and organism burdens were statistically similar in the long-term immunocompromised and short-term immunocompetent PCP models. These results suggest that Dectin-2 participates in the initial innate immune signaling response to *Pneumocystis*, but its deficiency does not impair resistance to the organism.

Keywords: Dectin-2; inflammation; innate immunity; *Pneumocystis* pneumonia

Clinical Relevance

The opportunistic fungal pathogen *Pneumocystis* interacts with a novel C-type lectin receptor named Dectin-2. Although this interaction triggers the host cell inflammatory response, the absence of the Dectin-2 receptor does not alter clearance of *Pneumocystis* infection.

(Received in original form October 18, 2016; accepted in final form August 17, 2017)

This work was supported by the Mayo Foundation, the Walter and Leonore Annenberg Foundation, and National Institutes of Health grant R01-HL62150 (A.H.L.).

Author Contributions: T.J.K. conceived and performed experiments, interpreted data, and drafted the manuscript. D.M.H., P.E.J., and P.L.M. performed and interpreted critical experiments. M.W., H.W., B.K., and S.Y. provided cellular reagents, designed experiments, and provided critical editing for the manuscript. A.H.L. designed experiments, interpreted data, and wrote and edited the manuscript.

Correspondence and requests for reprints should be addressed to Andrew H. Limper, M.D., 8-24 Stabile, Mayo Clinic, Rochester, MN 55905. E-mail: limper.andrew@mayo.edu.

This article has a data supplement, which is accessible from this issue's table of contents at www.atsjournals.org.

Am J Respir Cell Mol Biol Vol 58, Iss 2, pp 232–240, Feb 2018

Copyright © 2018 by the American Thoracic Society

Originally Published in Press as DOI: 10.1165/rcmb.2016-0335OC on September 8, 2017

Internet address: www.atsjournals.org

Pneumocystis pneumonia (PCP) is an opportunistic infection caused by *Pneumocystis jirovecii* (Pj) in humans. Overall, PCP is one of the top 10 severe fungal infections worldwide (1). *Pneumocystis* is a member of the phylum Ascomycota (2). *Pneumocystis* organisms have two predominant life-cycle forms: a cyst life form (or ascus) rich in β -glucans, and smaller trophic forms that bind the host airway epithelium and contain abundant major surface glycoproteins, termed Msg or gpA (Msg/gpA), that are believed to be important for organism binding to the lung and avoidance of host recognition (3, 4). Organism-induced lung inflammation contributes to respiratory impairment during PCP (5–7). A number of studies have supported a significant role for cell wall components of fungi in stimulating these robust host inflammatory responses. For example, the C-type lectin recognition receptor (CLR) Dectin-1 has been extensively shown to be important for binding fungal β -glucans, and is required for innate immune signaling and resolution of various fungal infections (8–11). Other fungal cell components, including mannoproteins such as α -mannan and its binding to the CLR Mincle, have recently been implicated in immune responses to *Candida albicans* and *Malassezia* spp. (12–14). The CLRs that recognize various cell wall ligands of *Pneumocystis* spp. are considerably less well defined compared with the culturable yeast pathogen *C. albicans*. For example, CLRs reported to be involved in the *C. albicans* host immune response include C-type lectin domain family 4, member K (CLEC4K), dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), Dectin-1, Dectin-2, macrophage C-type lectin (MCL), Mincle, and mannose receptor (MR) (15–21), whereas in CLR/*Pneumocystis* interactions, only Dectin-1, MR, and, more recently, Mincle have been noted (22–27). Given this paucity of information about CLR/*Pneumocystis* biology and the importance of CLRs for fungal host immunity, we investigated the potential role of CLR Dectin-2 in the host response to *Pneumocystis* spp.

Methods

All animal experiments were conducted in accordance with the guidelines of the Mayo Institutional Animal Care and Use Committee.

Strains and Reagents

Unless specified otherwise, all reagents were obtained from Sigma-Aldrich (St. Louis, MO). *Pneumocystis carinii* (Pc) and *Pneumocystis murina* (Pm) organisms were derived from the American Type Culture Collection (Manassas, VA) (28). Pc organisms were propagated for 10 weeks in immunosuppressed, corticosteroid-treated rats as previously described (29, 30). Whole populations of Pc organisms containing both cystic and trophic forms were purified from chronically infected rat lungs by homogenization and filtration through 10- μ m filters. For experiments requiring isolated Pc cystic and trophic forms, previously described methods were employed (31, 32). To exclude the presence of other infectious organisms in the Pc and Pm isolates, the preparations were stained (Diff Quick Modified Wright-Giemsa stain; Dade Diagnostics), and examined to exclude concurrent infection with bacteria or fungi. Isolates with contamination of other microorganisms were discarded (33).

Experimental Procedures

Details regarding the experimental procedures used in this work can be found in the data supplement, including generation of Pc cell wall components (30, 34, 35), carbohydrate analysis of purified Pc Msg/gpA, sources and specifics of the mouse strains used (19), *Pneumocystis* infection models in immunocompetent and CD4-depleted mice (36), immunofluorescence microscopy analysis (32, 37), quantitative PCR (qPCR) analysis of mRNA levels of Pc- and Pm-infected rodents, Dectin-2-Fc fusion protein plate-binding assays, construction of the mouse Dectin-2 lentiviral expression vector (38), Pc binding assays to RAW cell transfectants that overexpress Dectin-2 (39), tyrosine phosphorylation analysis of cells stimulated with Pc (38), Dectin-2 reporter cell stimulation assays (40), analysis of Syk and cytokine responses in Dectin-2^{-/-} and wild-type alveolar macrophages (41), ELISA for the Pm antibody response, Western blotting for Dectin-2 carbohydrate-recognition domain (CRD) Fc fusion binding to *Pneumocystis* spp., cytokine assays, and qPCR analysis of the Pm burden in Pm-infected mouse lungs (42). In addition, the specific PCR primers used in these studies are listed in Table E1 in the data supplement.

Statistical Analysis

Data are shown as the mean $\pm$ SEM from multiple experimental runs. Statistical differences between various data groups were first assessed by ANOVA and then by Student's *t* tests as indicated. Nonparametric statistics were utilized when data were distributed in a non-Gaussian manner. Statistical testing was performed using GraphPad Prism version 5.0b software, and statistical differences were considered significant if *P* < 0.05.

Results

Dectin-2 Interacts with Pc Organisms and Is Upregulated during Infection

For an initial evaluation of the potential interactions of Dectin-2 with *Pneumocystis* spp., we incubated Pc life forms with the Dectin-2 Fc fusion protein or Fc fragment alone and analyzed binding by immunofluorescence (Figure 1). The Dectin-2 Fc fusion protein demonstrated strong binding to Pc, with punctate staining noted on the cell wall of numerous organisms (Figures 1B and 1D). No appreciable staining was observed using the Fc fusion fragment alone (Figure 1F). These findings support the ability of Dectin-2 receptors to bind to intact Pc organisms.

In addition, prior studies have indicated that *Dectin-2* mRNA is constitutively expressed in the lung (43) and upregulated during pulmonary aspergillosis (44). Accordingly, we sought to determine whether Dectin-2 was similarly upregulated during PCP in rats (Figure 1G). Steady-state *Dectin-2* mRNA expression was significantly increased during *Pneumocystis* infection compared with normal uninfected lung mRNA (Figure 1G).

Dectin-2 Protein Recognizes Pc Surface Components, Including Msg/gpA

To further determine whether the Dectin-2 CRD-containing fusion protein could bind isolated Pc organism components, we utilized a plate-binding assay in which Pc components were coated onto microtiter plates and the Fc Dectin-2 fusion protein or the Fc fragment alone was allowed to interact with the coated surfaces (Figure 2). Both total Pc homogenates and purified native Msg/gpA demonstrated significantly stronger binding of the Dectin-2/Fc fusion protein compared with binding of the Fc fragment alone. Lastly, the mouse Dectin-2 CRD Fc fusion protein

Figure 1. Soluble Dectin-2 Fc binds *Pneumocystis carinii* (Pc) and is induced during Pc pneumonia. (A, C, and E) Phase-microscopy image of Pc life forms. (B and D) Pc organisms were stained with the Dectin-2 Fc and slides were examined for binding at $\times 40$ or $\times 60$, respectively. (F) Pc organisms were stained with the Fc fragment alone, followed by staining and viewing with FITC-conjugated anti-human Fc antibody at $\times 40$ magnification. (G) Pc infection induces rat *Dectin-2* expression in the lungs. *Pneumocystis* pneumonia was induced in rats as described in the text. After 10 weeks of infection, total lung *Dectin-2* expression was determined by semiquantitative PCR, with uninfected rats matched for age used as controls. * $P < 0.05$ compared with uninfected control rat lung. Shown are the data from three determinations. Scale bars for panels A, B, E, and F: 20 μm , and scale bars for panels C and D: 10 μm .

we used in this study binds equally well to both Pc and Pm cell wall components (most likely Msg/gpA based on the molecular weight of the glycoprotein and various isoforms) (27), as well as to both Pc and Pm protein lysates (Figure E1). These results indicate that the Dectin-2 CLR can recognize *Pneumocystis* surface components in a manner consistent with the observed immunofluorescence studies.

Macrophage Overexpression of Full-Length Dectin-2 Increases Binding of Pc to the Host Cell

To determine whether enhanced macrophage expression of Dectin-2 would augment interactions between the organisms and host cells, RAW264.7 macrophages were transfected with the Dectin-2 cDNA in an expression vector with a C-terminal V5 epitope tag. It should be noted that parental RAW cells express considerably less *Dectin-2* mRNA than bone marrow–derived dendritic cells (45, 46). Western blotting with an anti-V5 antibody confirmed the expression of the molecule in transfected RAW cells (Figure 3A), and the surface expression was further confirmed by FACS analysis (data not shown). To assess organism binding, ^{51}Cr -labeled Pc life forms were incubated with the transfected RAW cells and washed to remove nonadherent Pc life forms. Bound Pc life forms were then enumerated by scintillation counting (Figure 3B). In parallel with previous observations in *C. albicans* yeast forms (38), more Pc organisms bound to the Dectin-2V5–expressing RAW cells than to untransfected RAW cells expressing only native Dectin-2. Of note, ^{51}Cr -labeled Pc exhibited minimal binding to the microtiter plate (Figure 3B). These results indicate that Dectin-2 receptors expressed on the macrophage cell surface facilitate binding of Pc organisms.

Pc Binding to Dectin-2 Leads to Protein Tyrosine Phosphorylation in Macrophages

We next sought to determine whether challenging the RAW cells overexpressing Dectin-2V5 receptors with Pc organisms would result in overall activation of intracellular signaling. After applying Pc life forms to this cell line or the parent cell line alone, we performed Western blotting using a pan-specific antiphosphotyrosine antibody (Figure E2). Compared with parental untransfected RAW cells alone, enhanced tyrosine phosphorylation was observed as early as 5 minutes after the Pc

Figure 2. Dectin-2 Fc protein can bind *Pc* homogenates (A) and purified native Msg/gpA (B). Total Dectin-2 Fc fusion protein (circles) or Fc fragment alone (squares) was applied to increasing concentrations of the respective ligands and binding assays were performed. The data are expressed as the mean $\pm$ SEM of triplicate determinations. * $P < 0.05$ comparing Dectin-2 Fc fusion protein binding with control Fc fragment alone. Shown are the data from three experimental determinations. O.D. = optical density.

organisms were applied to the Dectin-2V5-expressing RAW cells, further indicating that the binding of *Pc* to Dectin-2 results in cellular signaling (Figure E2).

Ligation of Dectin-2 Induces Fc Receptor- γ -Mediated Cellular Signaling after Interactions with Whole *Pc* Organisms and *Pc* Cell Wall Components

Upon application of total *Pc* homogenate or whole *Pc* organisms to T cell hybridoma cells expressing both Dectin-2 and Fc receptor- γ (FcR γ) or the FcR γ alone, both whole organisms and *Pc* components induced significant cell signaling, as evidenced by

increased lacZ reporter activity compared with the T cells expressing the FcR γ receptor alone (Figure 4). These results indicate that not only can Dectin-2 bind *Pc*, but this binding leads to FcR γ -mediated intracellular signaling. Of note, stimulation of these reporter cells with *Pc* β -glucan alone did not induce significant signaling such as that seen with either whole *Pc* organisms or *Pc* homogenate.

Dectin-2 Mediates Syk Activation after Interactions with *Pc* Cell Wall β -Glucans

To further characterize whether interactions of *Pc* with Dectin-2 lead to downstream

Syk activation and innate immune signaling, we isolated alveolar macrophages from age-matched wild-type and Dectin-2^{-/-} mice. After stimulating macrophages with isolated *Pc* cell wall β -glucans for 30 minutes, which was previously shown to initiate robust signaling (37, 47–51), we observed a pronounced enhancement of phospho-Syk (p-Syk) in the wild-type mouse alveolar macrophages that was greatly diminished in the Dectin-2^{-/-} macrophages (Figure 5A). Some p-Syk was still observed in the Dectin-2^{-/-} alveolar macrophages after stimulation with *Pc* cell wall β -glucans, most likely related to Dectin-1 and/or other pattern recognition receptor signaling. These results suggest that *Pc* β -glucans or host components embedded in the *Pc* β -glucan preparation are capable of activating Dectin-2/FcR γ Syk-coupled receptor complexes.

Downstream Proinflammatory Cytokine Production Is Decreased in Dectin-2^{-/-} Alveolar Macrophages Treated with *Pc* Organism Cell Wall Components

After observing that Dectin-2 Syk-coupled receptor signaling induced by *Pc* cell wall components was impaired in the Dectin-2^{-/-} alveolar macrophages, and knowing that these events lead to altered proinflammatory cytokine production in other fungal infections (40, 52), we further examined the TNF- α and IL-6 responses in Dectin-2^{-/-} alveolar macrophages stimulated with *Pc* cell wall components. These inflammatory cytokines were significantly decreased in the Dectin-2^{-/-} mice alveolar macrophages compared with the wild-type alveolar macrophages after stimulation with the *Pc* cell wall components (Figures 5B and 5C). This finding further suggests a role for Dectin-2 CLR in innate immune responses during *Pneumocystis* infection.

Lung Organism Burdens in CD4-Depleted Dectin-2^{-/-} Mice Are Similar to Those in CD4-depleted Wild-Type Mice during PCP

To determine whether Dectin-2 CLR is important for the clearance of *Pneumocystis* infection during pneumonia, we infected CD4-depleted Dectin-2^{-/-} mice and 10 weeks later measured the total viable organism burden in the lung by qPCR (Figure 6). Notably, there were no significant differences between Dectin-2^{-/-} mice and wild-type controls with regard to

Figure 3. Overexpression of Dectin-2 by RAW macrophages increases *Pc* organism binding. (A) Protein expression of Dectin-2 in transfected RAW cells as measured by V5 Western blotting. (B) ⁵¹Cr-labeled *Pc* organisms were cultured for 1 hour with alveolar macrophages, nonattached organisms were removed, and organism binding was determined. Shown are means $\pm$ SEM for three separate determinations. * $P < 0.05$ comparing *Pc* binding to parental RAW cells with *Pc* binding to RAW cells overexpressing Dectin-2. CPM = counts per minute.

Figure 4. Reporter cells expressing Dectin-2 with FcR γ or FcR γ alone were stimulated with *Pc* components and whole *Pc* organisms. Cells expressing either Dectin-2 with FcR γ (open bars) or only FcR γ (solid bars) were stimulated with the respective *Pc* components *Pc* β -glucan (200 μ g/ml) or *Pc* homogenate (10 μ g/ml) or whole *Pc* organisms at the indicated *Pc*/host cell ratios. After 18 hours, cell signaling was determined by measuring lacZ activity using a colorimetric assay read at the OD 560/620 wavelength ratio. * $P < 0.05$ comparing signaling in cells expressing Dectin-2 with FcR γ and expressing only FcR γ , for a given stimulus. Shown are the data from four experimental determinations.

the quantity of *Pm* ribosomal RNA detected in total lung tissues. Therefore, these results suggest that although the Dectin-2 CLR recognizes *Pneumocystis* organisms that lead to early innate immune signaling events and inflammatory cytokine production, as noted above, the receptor's absence during PCP is not predominantly important for clearance of infection.

Cytokine and CLR Expression during PCP in CD4-depleted Dectin-2^{-/-} Mice

After observing that Dectin-2^{-/-} and wild-type mice exhibited similar *Pm* organism burdens during infection, we further investigated whether markers of inflammation in the whole lung were altered during infection (Table 1). Although IL-6 and TNF- α mRNA expression levels were

lower or relatively unchanged, respectively, in the Dectin-2^{-/-} mice, these differences were not statistically significant. In addition, because other researchers have shown the importance of Dectin-2 for T helper type 2 (Th2) responses to fungi (41, 53), we analyzed Th2 responses via mRNA levels for IL-5 and IL-13. In addition, we evaluated IL-17 expression. Again, the expression levels of IL-5, IL-13, and IL-17 were not significantly different between the wild-type mice with *Pm* pneumonia (PmP) and the Dectin-2^{-/-} mice with PmP at the 10-week time point tested (Table 1).

We further hypothesized that other CLRs and host cell receptors implicated in *Pneumocystis* clearance (23, 54, 55) and host responses to fungi (8, 9, 12, 14, 20, 30) might be upregulated in Dectin-2^{-/-} mice during infection, which could account for the similar organism burden levels observed during PmP in the Dectin-2^{-/-} mice and controls. Interestingly, though, fungal immune receptors such as Dectin-1, Mincle, Toll-like receptor 2 (TLR2), and complement receptor 3 (CR3) were significantly downregulated in the Dectin-2^{-/-} *Pm*-infected mice compared with the control *Pm*-infected mice (Table 1). MR expression levels were not different between the two groups. In addition, TLR7, a receptor for single-strand RNA, was used as a negative control transcript, and also did not differ significantly between Dectin-2^{-/-}

Figure 5. Macrophage activation by *Pc* cell wall components is impaired in Dectin-2-deficient macrophages. (A) Dectin-2^{-/-} and wild-type alveolar macrophages were stimulated with *Pc* β -glucans (200 μ g/ml) and then Syk activation was assessed. The phospho-Syk (p-Syk) levels in the Dectin-2^{-/-} cells were largely suppressed. (B) Alveolar macrophages were treated with the respective *Pc* cell wall components (either *Pc* β -glucans [200 μ g/ml] or msg/gpA [10 μ g/ml]) in concentrations that were determined to yield optimal responses in preliminary experiments. After overnight incubation, TNF- α release into the media was determined by ELISA. (C) In a similar fashion, IL-6 release by the macrophages was determined after overnight culture with the stimulants. The Dectin-2^{-/-} macrophages exhibited suppressed release of TNF- α and IL-6 in response to the *Pc* cell wall components. The open bars indicate wild-type alveolar macrophages and the solid bars indicate Dectin-2^{-/-} macrophages. * $P < 0.05$ comparing the Dectin-2^{-/-} macrophage responses with the corresponding wild-type cytokine responses for each stimulant. Shown are the data from three experimental determinations.

Figure 6. Dectin-2 deficiency does not significantly impair *Pneumocystis* clearance. Mice in the respective groups were immune suppressed by CD4 depletion with GK 1.5 and inoculated with *Pneumocystis murina* (Pm). After 10 weeks of infection, the Pm organism burdens were assayed by 16S mitochondrial RNA determination. Over large numbers of assayed mice ($n = 12$ mice), the Dectin-2^{-/-} and wild-type mice had statistically similar burdens ($n = 11$; $*P = 0.26$, not significantly different comparing Pm organism burdens in Dectin-2^{-/-} Pm-infected mice and control Pm-infected mice).

Pm-infected mice and wild-type control Pm-infected mice (Table 1). Therefore, the observed lack of difference in organism burdens during infection in the absence of Dectin-2 cannot be explained on the basis of upregulation of other CLR and receptors implicated in the host defense against fungal infection.

The *Pneumocystis* organism burdens in immunocompetent Dectin-2^{-/-} mice were

similar to those observed in wild-type mice after *Pneumocystis* challenge. However, Dectin-2^{-/-} mice failed to generate an augmented antibody response. We tested a short-term immunocompetent *Pneumocystis* challenge model for up to 20 days to assess whether the absence of Dectin-2 would result in an altered Pm burden in the lung (Figure E3). Wild-type and Dectin-2^{-/-} immunocompetent mice were inoculated with Pm as described above. Notably, the overall organism burden determined by qPCR in immunocompetent Dectin-2^{-/-} mice was not significantly different between the immunocompetent wild-type mice and the Dectin-2^{-/-} mice after *Pneumocystis* challenge. In addition, the immunocompetent Dectin-2^{-/-} failed to significantly augment anti-*Pneumocystis* IgG over 20 days after infection compared with wild-type mice (Figure E4). As anticipated, the wild-type mice generated a significantly augmented antibody response after the Pm exposure. In contrast, although the Dectin-2^{-/-} immunocompetent mice exhibited somewhat higher basal anti-Pm IgG, they did not exhibit a significant stimulation of anti-Pm IgG after Pm exposure.

Discussion

Dectin-2 is a member of the group II CLR family, which consists of one or more CRDs that recognize various pathogen epitopes that lead to innate immune signaling, either by using their own cytoplasmic domains or by associating with and utilizing the immunoreceptor tyrosine-based activation

motifs present in the FcR γ chain (56). Compared with Dectin-1, the role of the Dectin-2 CLR is less well defined and its importance for resistance in fungal infections is more variable. For instance, in *C. albicans* and *C. glabrata*, as well as in *Aspergillus fumigatus*, *Blastomyces dermatitidis*, *Fonsecaea pedrosoi*, and *Malassezia* spp., Dectin-2 has been implicated in mediating host innate and adaptive immune responses, and clearing fungal organisms from the host (12, 19, 57–59). In other pathogenic fungi, such as *Cryptococcus neoformans* and *Coccidioides immitis*, the absence of Dectin-2 leads to aberrant cytokine responses in host immunity, but these differences result in no difference in clearance of the organism from the host and therefore have no influence on the outcome of infection (41, 52). Thus, we believed it was important to examine the potential role of Dectin-2 in host defense and infection with *Pneumocystis*.

The current investigation specifically demonstrates that Dectin-2 mRNA levels are increased significantly during *Pneumocystis* infection, and that Dectin-2 can bind Pc organisms and components exposed on the cell surface, such as Msg/gpA and β -glucans. Furthermore, we demonstrated that Pc organisms bind Dectin-2 on macrophages, leading to tyrosine protein phosphorylation and initiation of Dectin-2/FcR γ -mediated signaling. Dectin-2^{-/-} alveolar macrophages exhibited impaired host p-Syk activation and downstream proinflammatory cytokine after challenge

Table 1. Cytokine and Receptor Expression during *Pneumocystis* Pneumonia in Dectin-2^{-/-} Mice

Target Measured (Fold Expression)	Wild-Type No Infection	Wild-Type PmP Infection	Dectin-2 ^{-/-} No Infection	Dectin-2 ^{-/-} PmP Infection	P Value Comparing Wild-Type with PmP and Dectin-2 ^{-/-} with PmP
IL-6 mRNA	0.04331 $\pm$ 0.009054	0.4966 $\pm$ 0.08508	0.1102 $\pm$ 0.03563	0.3947 $\pm$ 0.08912	0.4193
TNF- α mRNA	0.1671 $\pm$ 0.02158	2.310 $\pm$ 0.4908	0.1594 $\pm$ 0.05293	1.727 $\pm$ 0.3093	0.3032
IL-5 mRNA	5.862 $\pm$ 1.238	2.256 $\pm$ 0.1886	6.393 $\pm$ 1.509	2.516 $\pm$ 0.8116	0.7678
IL-13 mRNA	0.09574 $\pm$ 0.04222	3.373 $\pm$ 0.4927	0.5517 $\pm$ 0.4651	2.444 $\pm$ 0.3873, $n = 12$	0.1494
IL-17 mRNA	0.02642 $\pm$ 0.01555	0.04019 $\pm$ 0.005059	0.1433 $\pm$ 0.04688	0.1225 $\pm$ 0.04968, $n = 12$	0.1300
Dectin-1 mRNA	0.1614 $\pm$ 0.04012	3.489 $\pm$ 0.4884	0.03343 $\pm$ 0.01712	1.486 $\pm$ 0.3228	0.0022*
Mincle mRNA	0.03362 $\pm$ 0.01093	2.214 $\pm$ 0.3638	0.03871 $\pm$ 0.01438	1.125 $\pm$ 0.2336	0.0181*
TLR2 mRNA	0.2660 $\pm$ 0.04795	2.147 $\pm$ 0.1395	0.3005 $\pm$ 0.08105	1.571 $\pm$ 0.2291	0.0477*
CR3 mRNA	0.1761 $\pm$ 0.03917	1.787 $\pm$ 0.1678	0.1889 $\pm$ 0.1101	1.187 $\pm$ 0.1900	0.0288*
Mannose receptor mRNA	1.104 $\pm$ 0.1696	1.279 $\pm$ 0.2466	0.6971 $\pm$ 0.1152	0.9398 $\pm$ 0.1510	0.2455
TLR7 mRNA	0.1829 $\pm$ 0.03029	0.9226 $\pm$ 0.1985	0.1932 $\pm$ 0.05355	0.6244 $\pm$ 0.05085	0.1448

Definition of abbreviations: CR3 = complement receptor 3; PmP = Pm pneumonia; TLR = Toll-like receptor.

* $P < 0.05$, comparing expression of wild-type and Dectin-2^{-/-} mice with PmP.

with *Pc* cell wall components. However, Th2 mRNA transcriptional responses and the clearance of *Pneumocystis* organisms and proinflammatory cytokines were similar between the *Pm*-infected Dectin-2^{-/-} mice and wild-type mice. Furthermore, other CLRs implicated in fungal clearance were significantly downregulated in the Dectin-2^{-/-} mice compared with the wild-type *Pm*-infected mice. Our results indicate that although Dectin-2 likely participates in the initiation of host innate immune signaling, overall, the Dectin-2 CLR alone is not necessary for the control of infection.

Our studies indicate that the Dectin-2 CLR recognizes both whole *Pc* organisms and *Pc* cell wall components such as Msg/gpA and *Pc* β -glucans. Currently, the exact structures of ligands that interact with Dectin-2 are not fully characterized. Glycan arrays demonstrated that the CLR has the greatest affinity for the high-mannose structure Man₉GlcNac₂ (60). Dectin-2 has been shown to interact with other *bona fide* fungal cell wall components, including glycoproteins containing O-linked α -1,2-mannobiose residues from *Malassezia* spp. (12) and α -mannans from *C. albicans* (19, 21). Furthermore, zymosan, a glucan with repeating glucose units connected by β -1,3-glycosidic linkages, has also been shown to activate Dectin-2, leading to increased proinflammatory production (40). Therefore, the fungal cell wall repertoire implicated in Dectin-2 binding and signaling is likely broad and involves multiple fungal components. Our studies suggest that whole *Pc* organisms and abundant *Pc* cell wall surface components are able to activate Dectin-2 signaling. Previous studies and an examination of the completed *Pneumocystis* genomes in *Pc*, *Pj*, and *Pm* have demonstrated the presence of β -glucans (both β -1,3 and β -1,6 linkages) and related synthetic genes (31, 37), as well as genetic pathways for mannoprotein production across the genus (61, 62). These

observations further support the notion that *Pneumocystis* spp. contains fungal cell wall components homologous to the known fungal Dectin-2 ligands. Interestingly, the Syk responses to β -glucans in Dectin-2^{-/-} macrophages were suppressed, despite the presence of Dectin-1 and other CLR molecules that are known to activate Syk. Although the mechanisms of this overall suppression of Syk activation in the absence of Dectin-2 are not yet clear, it may indicate crosstalk between Dectin-2 and other CLR molecules.

Of note, we observed no significant differences in the proinflammatory cytokine mRNA (Table 1) and protein levels of IL-6 and TNF- α (data not shown) in *Pneumocystis*-infected Dectin-2^{-/-} mice compared with wild-type controls. This was surprising in light of the significant differences in cytokines, as well as in T cell Dectin-2/FcR γ -coupled signal initiation, that we observed when we tested isolated alveolar macrophages from these mice. The reason for this discrepancy is not fully understood, but it is entirely in accord with previous findings in other infection models (41, 52). It may very well be that cytokine responses in whole-lung protein lysates are insufficiently sensitive to detect differences that are magnified (and greatly simplified) in examinations of isolated cell types. Indeed, prior studies suggested a linkage between Th2 (IL-5, IL-13, and IL-17) responses and Dectin-2 in some infections (41).

Finally, we demonstrated that the absence of Dectin-2 in the lung led to no apparent deficiency in the burden of *Pneumocystis* organisms from the lung, in either the CD4-depleted PCP model or the immunocompetent animals. Similarly, in other fungal infections, the absence of Dectin-2 can lead to profound decreases in cell-specific proinflammatory cytokine production, but this has little effect on fungal organism clearance (40, 41, 52). We used standard time points to assess infection intensity in the *Pm* infection model to study well-established infection (36).

Based on our observations, we examined other mammalian fungal receptors that were previously implicated in the immune response to *Pneumocystis* and other fungi, including Dectin-1, Mincle, TLR2, CR3, and MR (14, 20, 23, 55, 63). Interestingly, although Dectin-1, Mincle, TLR2, and CR3 mRNAs were upregulated in Dectin-2^{-/-} mice during active PCP infection, they were expressed at significantly lower levels in Dectin-2^{-/-} *Pm*-infected mice compared with the control *Pm*-infected mice. To our knowledge, this is the first observation of other CLRs being downregulated during a fungal infection in the absence of Dectin-2^{-/-}, which merits further exploration. Furthermore, although these fungal cell innate immune receptors were reduced in the Dectin-2^{-/-} mice, the levels were still adequate to control *Pm* organism burdens in the lung. Thus, the redundancy of these multiple CLRs benefits the host in the overall control of fungal infection (64–66).

In summary, we have shown that the Dectin-2 CLR recognizes and responds to *Pneumocystis* organisms, initiating cellular responses *in vitro*. However, the lack of Dectin-2 receptors in Dectin-2^{-/-} mice did not make the animals susceptible to more severe PCP infections. Therefore, although we believe that Dectin-2 receptors participate in host innate immune responses to organisms, the presence of redundant (and perhaps more potent) CLRs and other receptors, such as Dectin-1, indicates that Dectin-2 does not by itself play a predominant role in affecting the clearance of *Pneumocystis* organisms during infection. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Acknowledgments: The authors thank Dr. Yasuhiro Ikeda (Mayo Clinic, Rochester, MN) for generating the Dectin-2 lentiviral constructs for the RAW cell experiments.

References

1. Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC. Hidden killers: human fungal infections. *Sci Transl Med* 2012;4:165rv13.
2. Sugiyama J, Hosaka K, Suh SO. Early diverging Ascomycota: phylogenetic divergence and related evolutionary enigmas. *Mycologia* 2006;98:996–1005.
3. Skalski JH, Kottom TJ, Limper AH. Pathobiology of *Pneumocystis* pneumonia: life cycle, cell wall and cell signal transduction. *FEMS Yeast Res* 2015;15:fov046.
4. Thomas CF Jr, Limper AH. *Pneumocystis* pneumonia. *N Engl J Med* 2004;350:2487–2498.
5. Bang D, Emborg J, Elkjaer J, Lundgren JD, Benfield TL. Independent risk of mechanical ventilation for AIDS-related *Pneumocystis carinii* pneumonia associated with bronchoalveolar lavage neutrophilia. *Respir Med* 2001;95:661–665.
6. Benfield TL, Vestbo J, Junge J, Nielsen TL, Jensen AB, Lundgren JD. Prognostic value of interleukin-8 in AIDS-associated *Pneumocystis carinii* pneumonia. *Am J Respir Crit Care Med* 1995; 151:1058–1062.

7. Limper AH, Offord KP, Smith TF, Martin WJ II. *Pneumocystis carinii* pneumonia. Differences in lung parasite number and inflammation in patients with and without AIDS. *Am Rev Respir Dis* 1989;140:1204–1209.
8. Kimberg M, Brown GD. Dectin-1 and its role in antifungal immunity. *Med Mycol* 2008;46:631–636.
9. Liu ZC, Wang M, Sun WK, Xia D, Tan MM, Ding Y, Qian Q, Su X, Shi Y. Up-regulation of Dectin-1 in airway epithelial cells promotes mice defense against invasive pulmonary aspergillosis. *Int J Clin Exp Med* 2015;8:17489–17497.
10. Nieto-Patlán A, Campillo-Navarro M, Rodríguez-Cortés O, Muñoz-Cruz S, Wong-Baeza I, Estrada-Parra S, Estrada-García I, Serafín-López J, Chacón-Salinas R. Recognition of *Candida albicans* by Dectin-1 induces mast cell activation. *Immunobiology* 2015;220:1093–1100.
11. Viriyakosol S, Jimenez MdelP, Gurney MA, Ashbaugh ME, Fierer J. Dectin-1 is required for resistance to coccidioidomycosis in mice. *MBio* 2013;4:e00597–12.
12. Ishikawa T, Itoh F, Yoshida S, Saijo S, Matsuzawa T, Gono T, Saito T, Okawa Y, Shibata N, Miyamoto T, et al. Identification of distinct ligands for the C-type lectin receptors Mincle and Dectin-2 in the pathogenic fungus *Malassezia*. *Cell Host Microbe* 2013;13:477–488.
13. Vijayan D, Radford KJ, Beckhouse AG, Ashman RB, Wells CA. Mincle polarizes human monocyte and neutrophil responses to *Candida albicans*. *Immunol Cell Biol* 2012;90:889–895.
14. Yamasaki S, Matsumoto M, Takeuchi O, Matsuzawa T, Ishikawa E, Sakuma M, Tateno H, Uno J, Hirabayashi J, Mikami Y, et al. C-type lectin Mincle is an activating receptor for pathogenic fungus, *Malassezia*. *Proc Natl Acad Sci USA* 2009;106:1897–1902.
15. Cambi A, Gijzen K, de Vries J, Torensma R, Joosten B, Adema GJ, Netea MG, Kullberg BJ, Romani L, Figdor CG. The C-type lectin DC-SIGN (CD209) is an antigen-uptake receptor for *Candida albicans* on dendritic cells. *Eur J Immunol* 2003;33:532–538.
16. Castro M, Ralston NV, Morgenthaler TI, Rohrbach MS, Limper AH. *Candida albicans* stimulates arachidonic acid liberation from alveolar macrophages through α -mannan and β -glucan cell wall components. *Infect Immun* 1994;62:3138–3145.
17. de Jong MA, Vriend LE, Theelen B, Taylor ME, Fluitsma D, Boekhout T, Geijtenbeek TB. C-type lectin Langerin is a β -glucan receptor on human Langerhans cells that recognizes opportunistic and pathogenic fungi. *Mol Immunol* 2010;47:1216–1225.
18. Gow NA, Netea MG, Munro CA, Ferwerda G, Bates S, Mora-Montes HM, Walker L, Jansen T, Jacobs L, Tsoni V, et al. Immune recognition of *Candida albicans* β -glucan by dectin-1. *J Infect Dis* 2007;196:1565–1571.
19. Saijo S, Ikeda S, Yamabe K, Kakuta S, Ishigame H, Akitsu A, Fujikado N, Kusaka T, Kubo S, Chung SH, et al. Dectin-2 recognition of α -mannans and induction of Th17 cell differentiation is essential for host defense against *Candida albicans*. *Immunity* 2010;32:681–691.
20. Wells CA, Salvage-Jones JA, Li X, Hitchens K, Butcher S, Murray RZ, Beckhouse AG, Lo YL, Manzanero S, Cobbold C, et al. The macrophage-inducible C-type lectin, mincle, is an essential component of the innate immune response to *Candida albicans*. *J Immunol* 2008;180:7404–7413.
21. Zhu LL, Zhao XQ, Jiang C, You Y, Chen XP, Jiang YY, Jia XM, Lin X. C-type lectin receptors Dectin-3 and Dectin-2 form a heterodimeric pattern-recognition receptor for host defense against fungal infection. *Immunity* 2013;39:324–334.
22. Castro M, Morgenthaler TI, Hoffman OA, Standing JE, Rohrbach MS, Limper AH. *Pneumocystis carinii* induces the release of arachidonic acid and its metabolites from alveolar macrophages. *Am J Respir Cell Mol Biol* 1993;9:73–81.
23. O'Riordan DM, Standing JE, Limper AH. *Pneumocystis carinii* glycoprotein A binds macrophage mannose receptors. *Infect Immun* 1995;63:779–784.
24. Steele C, Marrero L, Swain S, Harmsen AG, Zheng M, Brown GD, Gordon S, Shellito JE, Kolls JK. Alveolar macrophage-mediated killing of *Pneumocystis carinii* f. sp. muris involves molecular recognition by the Dectin-1 β -glucan receptor. *J Exp Med* 2003;198:1677–1688.
25. Zhang C, Wang SH, Liao CP, Shao S, Lasbury ME, Durant PJ, Lee CH. Downregulation of PU.1 leads to decreased expression of Dectin-1 in alveolar macrophages during *Pneumocystis* pneumonia. *Infect Immun* 2010;78:1058–1065.
26. Ricks DM, Chen K, Zheng M, Steele C, Kolls JK. Dectin immunoadhesins and *Pneumocystis* pneumonia. *Infect Immun* 2013;81:3451–3462.
27. Kottom TJ, Hebrink DM, Jenson PE, Nandakumar V, Wüthrich M, Wang H, Klein B, Yamasaki S, Lepenies B, Limper AH. The interaction of *Pneumocystis* with the c-type lectin receptor mincle exerts a significant role in host defense against infection. *J Immunol* 2017;198:3515–3525.
28. Krajicek BJ, Kottom TJ, Villegas L, Limper AH. Characterization of the PcCdc42 small G protein from *Pneumocystis carinii*, which interacts with the PcSte20 life cycle regulatory kinase. *Am J Physiol Lung Cell Mol Physiol* 2010;298:L252–L260.
29. Kottom TJ, Thomas CF Jr, Mubarak KK, Leof EB, Limper AH. *Pneumocystis carinii* uses a functional cdc13 B-type cyclin complex during its life cycle. *Am J Respir Cell Mol Biol* 2000;22:722–731.
30. Hahn PY, Evans SE, Kottom TJ, Standing JE, Pagano RE, Limper AH. *Pneumocystis carinii* cell wall β -glucan induces release of macrophage inflammatory protein-2 from alveolar epithelial cells via a lactosylceramide-mediated mechanism. *J Biol Chem* 2003;278:2043–2050.
31. Kottom TJ, Limper AH. Cell wall assembly by *Pneumocystis carinii*. Evidence for a unique gsc-1 subunit mediating β -1,3-glucan deposition. *J Biol Chem* 2000;275:40628–40634.
32. Kottom TJ, Kennedy CC, Limper AH. *Pneumocystis* PCINT1, a molecule with integrin-like features that mediates organism adhesion to fibronectin. *Mol Microbiol* 2008;67:747–761.
33. Vassallo R, Standing JE, Limper AH. Isolated *Pneumocystis carinii* cell wall glucan provokes lower respiratory tract inflammatory responses. *J Immunol* 2000;164:3755–3763.
34. Coligan JE, Dunn BM, Ploegh HL, Speicher DW, Wingfield PT, editors. Current protocols in protein science. New York: John Wiley and Sons; 1996.
35. Wozniak KL, Levitz SM. Isolation and purification of antigenic components of *Cryptococcus*. *Methods Mol Biol* 2009;470:71–83.
36. Vuk-Pavlovic Z, Mo EK, Icenhour CR, Standing JE, Fisher JH, Limper AH. Surfactant protein D enhances *Pneumocystis* infection in immune-suppressed mice. *Am J Physiol Lung Cell Mol Physiol* 2006;290:L442–L449.
37. Kottom TJ, Hebrink DM, Jenson PE, Gudmundsson G, Limper AH. Evidence for proinflammatory β -1,6 glucans in the *Pneumocystis carinii* cell wall. *Infect Immun* 2015;83:2816–2826.
38. Sato K, Yang XL, Yudate T, Chung JS, Wu J, Ruby-Phelps K, Kimberly RP, Underhill D, Cruz PD Jr, Ariizumi K. Dectin-2 is a pattern recognition receptor for fungi that couples with the Fc receptor γ chain to induce innate immune responses. *J Biol Chem* 2006;281:38854–38866.
39. Yong SJ, Vuk-Pavlovic Z, Standing JE, Crouch EC, Limper AH. Surfactant protein D-mediated aggregation of *Pneumocystis carinii* impairs phagocytosis by alveolar macrophages. *Infect Immun* 2003;71:1662–1671.
40. Robinson MJ, Osorio F, Rosas M, Freitas RP, Schweighoffer E, Gross O, Verbeek JS, Ruland J, Tybulewicz V, Brown GD, et al. Dectin-2 is a Syk-coupled pattern recognition receptor crucial for Th17 responses to fungal infection. *J Exp Med* 2009;206:2037–2051.
41. Nakamura Y, Sato K, Yamamoto H, Matsumura K, Matsumoto I, Nomura T, Miyasaka T, Ishii K, Kanno E, Tachi M, et al. Dectin-2 deficiency promotes Th2 response and mucin production in the lungs after pulmonary infection with *Cryptococcus neoformans*. *Infect Immun* 2015;83:671–681.
42. Wakefield AE, Pixley FJ, Banerji S, Sinclair K, Miller RF, Moxon ER, Hopkin JM. Detection of *Pneumocystis carinii* with DNA amplification. *Lancet* 1990;336:451–453.
43. Kanazawa N, Tashiro K, Inaba K, Lutz MB, Miyachi Y. Molecular cloning of human dectin-2. *J Invest Dermatol* 2004;122:1522–1524.
44. Sun H, Xu XY, Shao HT, Su X, Wu XD, Wang Q, Shi Y. Dectin-2 is predominantly macrophage restricted and exhibits conspicuous expression during *Aspergillus fumigatus* invasion in human lung. *Cell Immunol* 2013;284:60–67.
45. Ariizumi K, Shen GL, Shikano S, Ritter R III, Zukas P, Edelbaum D, Morita A, Takashima A. Cloning of a second dendritic cell-associated C-type lectin (dectin-2) and its alternatively spliced isoforms. *J Biol Chem* 2000;275:11957–11963.

46. Ariizumi K, Shen GL, Shikano S, Xu S, Ritter R III, Kumamoto T, Edelbaum D, Morita A, Bergstresser PR, Takashima A. Identification of a novel, dendritic cell-associated molecule, dectin-1, by subtractive cDNA cloning. *J Biol Chem* 2000;275: 20157–20167.
47. Carmona EM, Kottom TJ, Hebrink DM, Moua T, Singh RD, Pagano RE, Limper AH. Glycosphingolipids mediate *Pneumocystis* cell wall β -glucan activation of the IL-23/IL-17 axis in human dendritic cells. *Am J Respir Cell Mol Biol* 2012;47:50–59.
48. Carmona EM, Lamont JD, Xue A, Wylam M, Limper AH. *Pneumocystis* cell wall β -glucan stimulates calcium-dependent signaling of IL-8 secretion by human airway epithelial cells. *Respir Res* 2010;11:95.
49. Carmona EM, Vassallo R, Vuk-Pavlovic Z, Standing JE, Kottom TJ, Limper AH. *Pneumocystis* cell wall β -glucans induce dendritic cell costimulatory molecule expression and inflammatory activation through a Fas-Fas ligand mechanism. *J Immunol* 2006;177: 459–467.
50. Evans SE, Hahn PY, McCann F, Kottom TJ, Pavlovic' ZV, Limper AH. *Pneumocystis* cell wall β -glucans stimulate alveolar epithelial cell chemokine generation through nuclear factor- κ B-dependent mechanisms. *Am J Respir Cell Mol Biol* 2005;32: 490–497.
51. Prigge JR, Hoyt TR, Dobrinen E, Capeocchi MR, Schmidt EE, Meissner N. Type I IFNs act upon hematopoietic progenitors to protect and maintain hematopoiesis during *Pneumocystis* lung infection in mice. *J Immunol* 2015;195:5347–5357.
52. Viriyakosol S, Jimenez MdelP, Saijo S, Fierer J. Neither dectin-2 nor the mannose receptor is required for resistance to *Coccidioides immitis* in mice. *Infect Immun* 2014;82:1147–1156.
53. Tjota MY, Hrusch CL, Blaine KM, Williams JW, Barrett NA, Sperling AI. Signaling through FcR γ -associated receptors on dendritic cells drives IL-33-dependent TH2-type responses. *J Allergy Clin Immunol* 2014;134:706–713.e8.
54. Saijo S, Fujikado N, Furuta T, Chung SH, Kotaki H, Seki K, Sudo K, Akira S, Adachi Y, Ohno N, et al. Dectin-1 is required for host defense against *Pneumocystis carinii* but not against *Candida albicans*. *Nat Immunol* 2007;8:39–46.
55. Zhang C, Wang SH, Lasbury ME, Tschang D, Liao CP, Durant PJ, Lee CH. Toll-like receptor 2 mediates alveolar macrophage response to *Pneumocystis murina*. *Infect Immun* 2006;74:1857–1864.
56. Geijtenbeek TB, Gringhuis SI. Signalling through C-type lectin receptors: shaping immune responses. *Nat Rev Immunol* 2009;9: 465–479.
57. Loures FV, Röhm M, Lee CK, Santos E, Wang JP, Specht CA, Calich VL, Urban CF, Levitz SM. Recognition of *Aspergillus fumigatus* hyphae by human plasmacytoid dendritic cells is mediated by dectin-2 and results in formation of extracellular traps. *PLoS Pathog* 2015;11:e1004643.
58. Wüthrich M, Wang H, Li M, Lerksuthirath T, Hardison SE, Brown GD, Klein B. *Fonsecaea pedrosoi*-induced Th17-cell differentiation in mice is fostered by Dectin-2 and suppressed by Mincle recognition. *Eur J Immunol* 2015;45:2542–2552.
59. Wang H, LeBert V, Hung CY, Galles K, Saijo S, Lin X, Cole GT, Klein BS, Wüthrich M. C-type lectin receptors differentially induce Th17 cells and vaccine immunity to the endemic mycosis of North America. *J Immunol* 2014;192:1107–1119.
60. McGreal EP, Rosas M, Brown GD, Zamze S, Wong SY, Gordon S, Martinez-Pomares L, Taylor PR. The carbohydrate-recognition domain of Dectin-2 is a C-type lectin with specificity for high mannose. *Glycobiology* 2006;16:422–430.
61. Cissé OH, Pagni M, Hauser PM. De novo assembly of the *Pneumocystis jirovecii* genome from a single bronchoalveolar lavage fluid specimen from a patient. *MBio* 2012;4:e00428–12.
62. Slaven BE, Meller J, Porollo A, Sesterhenn T, Smulian AG, Cushion MT. Draft assembly and annotation of the *Pneumocystis carinii* genome. *J Eukaryot Microbiol* 2006;53:S89–S91.
63. Ross GD, Cain JA, Myones BL, Newman SL, Lachmann PJ. Specificity of membrane complement receptor type three (CR3) for β -glucans. *Complement* 1987;4:61–74.
64. Court N, Vasseur V, Vacher R, Frémond C, Shebzukhov Y, Yermeev VV, Maillet I, Nedospasov SA, Gordon S, Fallon PG, et al. Partial redundancy of the pattern recognition receptors, scavenger receptors, and C-type lectins for the long-term control of *Mycobacterium tuberculosis* infection. *J Immunol* 2010;184: 7057–7070.
65. Rothfuchs AG, Bafica A, Feng CG, Egen JG, Williams DL, Brown GD, Sher A. Dectin-1 interaction with *Mycobacterium tuberculosis* leads to enhanced IL-12p40 production by splenic dendritic cells. *J Immunol* 2007;179:3463–3471.
66. Hardison SE, Brown GD. C-type lectin receptors orchestrate antifungal immunity. *Nat Immunol* 2012;13:817–822.