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*J Immunol* 2006; 176:3717-3724; doi: 10.4049/jimmunol.176.6.3717

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Dectin-1 and TLRs Permit Macrophages to Distinguish between Different Aspergillus fumigatus Cellular States

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Aspergillus fumigatus is a common cause of invasive and allergic pulmonary disease. Resting conidia of the filamentous fungus are constantly inhaled, but cause infection only after initiating hyphal growth. In this study, we have explored whether macrophages can distinguish between resting spores and the maturing, potentially invasive form of the fungus. Although macrophages bind and ingest A. fumigatus resting conidia efficiently, there is little inflammatory response; NF-κB is not activated, inflammatory cytokines are not induced, and reactive oxygen species are not produced. However, maturing A. fumigatus conidia and germ tubes stimulate NF-κB, secretion of proinflammatory cytokines and production of reactive oxygen by human monocyte-derived macrophages and murine macrophages from multiple anatomical sites. These responses are in part mediated by dectin-1, which binds cell wall β-glucan that is not present on the surface of dormant conidia, but is present after cellular swelling and loss of the hydrophobic proteinaceous cell wall. Dectin-1 binding to germ tubes augments, but is not required for, TLR2-mediated inflammatory cytokine and chemokines and playing a role in Ag presentation (4, 5).

Pulmonary infections caused by Aspergillus fumigatus are a concern in immunosuppressed individuals, and this organism is now a leading cause of infection-related morbidity in patients who receive cytotoxic chemotherapy and allogeneic hematopoietic stem cell transplantation (1). Aspergillus spores (resting conidia) are ubiquitous in the environment, are inhaled frequently, and are normally cleared without pathology in immunocompetent people. Alveolar macrophages constitute a primary line of innate cellular defense, because they bind, ingest, and kill conidia (2, 3). Organisms that escape macrophage killing are able to mature into germ tubes and hyphae, which can invade vessels and disseminate hematogenously; invasive pneumonia and disseminated infection is particularly common in people who lack effective hyphal defense by polymorphonuclear phagocytes (2).

Recently, animal studies have demonstrated a protective role of Th1-type CD4+ T cells; macrophages and pulmonary dendritic cells (DC) mediate secondary responses by secreting cytokines and chemokines and playing a role in Ag presentation (4, 5).

Multiple studies have been performed to determine the mechanisms by which macrophages recognize A. fumigatus for both organism phagocytosis and secretion of inflammatory cytokines. Lectin-binding receptors (mannose receptor and DC-specific ICAM-3 grabbing nonintegrin) play a role in A. fumigatus binding to phagocytic cells, including macrophages and DC (5, 6). Human and murine macrophage secretion of proinflammatory cytokines in response to live and dead A. fumigatus cells involves TLR2 and -4 (7–10). Specific conidial and hyphal components recognized by each receptor have not been determined, and no molecular mechanisms to discriminate between different morphological states has been described.

We hypothesized that macrophages could tailor proinflammatory responses toward the more mature forms of the organism (germ tubes and hyphae) and that this may be mediated by recognition of cell surface components exposed after activation (swelling) of resting conidia. During maturation, conidia undergo a morphologic change that involves loss of a thin proteinaceous hydrophobic cell wall before development of hyphal forms, the cell walls of which are composed primarily of β(1,3)-glucan, chitin, and galactomannan (11, 12). We specifically examined the role of dectin-1, a type II transmembrane receptor that triggers phagocytosis of β-glucan-containing particles (13) and collaborates with TLR2 to coordinate inflammatory responses, including cytokine secretion and production of reactive oxygen species (ROS) (14, 15). Our recent studies have demonstrated that dectin-1 recognizes β-glucan exposed on bud scars of Candida albicans yeasts, but not hyphae, providing a mechanism by which macrophages differentiate between morphological forms of this pathogenic fungus (16). In this study, we have shown that dectin-1, TLR2, and TLR4 recognize the hyphal form of A. fumigatus, but not conidia. This recognition leads to secretion of proinflammatory cytokines and production of antimicrobial ROS. These results provide a mechanism by which pulmonary macrophages can differentiate between resting fungal spores, which are constantly inhaled, and potentially invasive (maturing) forms of the organism.
Materials and Methods

Reagents

Escherichia coli LPS, lipoteichoic acid and soluble β-glucan from Laminaria digitata (laminarin) were obtained from Sigma-Aldrich.

Human and mouse macrophages

PBMC were harvested from healthy donor blood (obtained after consent using a protocol approved by the Fred Hutchinson Cancer Research Center institutional review board) by centrifugation over a Ficoll-Hypaque density gradient. Monocytes were isolated by adherence, washed with PBS/EDTA, and resuspended in IMDM (Invitrogen Life Technologies) supplemented with 0.3 mg/ml l-glutamine, 200 U/ml penicillin/streptomycin, 10% autologous human serum, and 40 ng/ml rhM-CSF (R&D Systems). Cultures were maintained in a humidified atmosphere (5% CO₂) at 37°C for 7 days, and medium was replaced on days 2 and 4. Differentiated cells were >95% CD14⁺, with a viability >90%, as determined by trypan blue exclusion. TLR2-, TLR4-, and MyD88-deficient mice on a C57BL/6J background were provided by S. Akira (Osaka University, Osaka, Japan). F4 or F4 generation mice were used at 8–11 wk of age and compared with littermate controls. Mouse studies were performed under institutional animal care guidelines. Murine peritoneal macrophages were collected 5 days after peritoneal injection of 1.5 ml of Brewer-modified thioglycolate broth (5%; BD Biosciences). Murine alveolar macrophages were harvested from the lungs of killed animals by lavage. Bone marrow-derived macrophages were prepared by 7 days of culture in RPMI 1640 containing 20% L929 cell-conditioned medium.

A. fumigatus cellular products

Well-characterized clinical A. fumigatus isolates B5233 and AF293 were used in these studies. Conidia were prepared from mature colonies grown on potato dextrose agar by flushing with PBS containing 0.05% Tween 80. Conidia were killed by heat (65°C, 30 min) or exposure to 1% paraformaldehyde (4°C, overnight). Germ tubes were prepared by incubating conidia (10⁹/ml) in RPMI 1640 medium (with 10% FCS) for 8 h at 37°C. Cells were killed as described above. Typically, >80% of conidia were visibly swollen after 5 h.

Staining with soluble dectin-1 and β-glucan

A soluble hemagglutinin (HA) epitope-tagged form of the extracellular region of murine dectin-1 (sDectin; aa 69–244) was produced in HEK293 cells as previously described (16). A. fumigatus resting conidia were incubated in DMEM containing 0.025% Tween 80 at 37°C for 0, 5, 10, or 20 h as indicated. Where indicated, cells were pelleted and resuspended in HEK293 cell supernatant containing sDectin on ice for 1 h, and sDectin binding was detected with an FITC-coupled Ab to the HA epitope (HA.11-FITC; Covalink). Images were acquired with a confocal microscope (Leica) using identical gain settings. Binding specificity was confirmed using control cell supernatants without sDectin and by blocking with soluble β-glucan data (not shown). In some experiments, germ tubes were pretreated with β-1,3-, β-1,6-glucanase from Helix pomatia (Fluka; 10 U/ml) or α-mannosidase (Sigma-Aldrich; 10 U/ml) at 1 h at 37°C. Germ tubes were washed with 1 ml of PBS (plus 10% calf serum) with centrifugation, resuspended in PBS (plus 2.5% formalin), and analyzed by flow cytometry (FACSCalibur; BD Biosciences).

Live A. fumigatus were stained with a mouse mAb to linear β-(1,3)-glucan (Biospecs). For colocalization studies, the cell wall was also stained with sDectin-1, and this binding was detected using the rabbit anti-dectin-1 polyclonal Ab (16).

Functional assays

All reagents were confirmed to be endotoxin free (<0.01 endotoxin unit/ml) with the Limulus amebocyte assay (QCL-1000; BioWhittaker). Human CD14⁺ or murine macrophages were exposed to LPS, killed A. fumigatus germ tubes, or killed conidia for 24 h. Culture supernatants were analyzed for TNF-α (BD Biosciences), IL-6 (BD Biosciences), or IL-18 (Diaclone) as indicated. Where indicated, cells were pretreated with rabbit preimmune serum or anti-dectin-1 rabbit antisera (5%) for 30 min before stimulation (16).

ROS production was detected by Luminol-ECL as previously described (15, 16). Mouse bone marrow-derived macrophages were plated (100,000/well) in a 96-well plate with 50 U/ml IFN-γ (PeproTech) 24 h before the assay. Where indicated, cells were pretreated with laminarin (0.5 mg/ml) for 1 h to block β-glucan recognition. Where indicated, cells were pre-treated with control rabbit serum or anti-dectin-1 rabbit antisera (5%) for 30 min.

NF-κB activation was measured in RAW264 cells, in which the firefly luciferase gene directed by the NF-κB-dependent endothelial leukocyte adhesion molecule-luciferase was stably integrated into the genome (17). Cells exposed to stimuli for 16 h were harvested and analyzed for firefly luciferase activity using the Promega Steady-Glo luciferase assay system according to the manufacturer’s instructions.

To measure organism phagocytosis, mouse peritoneal macrophages were exposed to paraformaldehyde-fixed, FITC (0.1 mg/ml; Sigma-Aldrich)-labeled conidia or germ tubes for 30 min at 37°C (5% CO₂). Calcein white (25 μM; Molecular Probes) was added as a counterstain to distinguish between ingested and adherent conidia or germ tubes. Cells were washed at 4°C and visualized by phase contrast and fluorescent microscopy using a Nikon Eclipse TE300 microscope equipped with a CoolSNAP digital camera (Meridian Instrument). Phagocytic function was identified by counting internalized conidia or germ tubes in 300 macrophages from at least 10 random fields. The phagocytic index was calculated according to the following formula: phagocytic index = (total number of engulfed early germ tubes/total number of counted macrophages) × (number of macrophages containing engulfed early germ tubes/total number of counted macrophages) × 100.

Results

A. fumigatus germ tubes, but not conidia, activate proinflammatory responses in human and murine macrophages

We determined whether killed conidia and germ tubes stimulate proinflammatory cytokine secretion by human monocyte-derived macrophages (MDM) and murine macrophages harvested from different anatomical sites (peritoneal and alveolar). Cytokines, TNF-α, IL-6, and IL-18 were produced by MDM in large quantities in a dose-dependent fashion in response to germ tube exposure (Fig. 1). However, cytokines were not detectable after exposure to conidia, even at very high doses (100:1, conidia:MDM). Similarly, murine peritoneal and alveolar macrophages secreted TNF-α in response to germ tube forms of the organism only (Fig. 2a). NF-κB activation is crucial for macrophage production of many inflammatory cytokines and chemokines, so we measured the induction of NF-κB by germ tubes and conidia. Using RAW264 macrophages stably transfected with an NF-κB reporter, we observed that germ tubes stimulated NF-κB to a greater degree than conidia (Fig. 2b).

In addition to inflammatory cytokine production, initial host defense to A. fumigatus involves macrophage production of ROS (18). We examined the relative abilities of resting conidia and germ tubes to induce ROS production by murine bone marrow-derived macrophages. Macrophages produced ROS after exposure to modest doses of germ tubes (Fig. 2c), and this activation was independent of whether live, heat-killed, or paraformaldehyde-fixed fungi were used (data not shown). However, resting conidia did not trigger ROS production, even at much higher doses (Fig. 2c). Despite the failure of conidia to trigger ROS production or stimulate cytokine secretion, macrophages bound and ingested conidia efficiently (Fig. 2d). Collectively, these data demonstrate that macrophages have an innate ability to distinguish between A. fumigatus conidia and germ tubes.

Dectin-1 binds to maturing conidia and hyphal forms only

We have previously demonstrated that dectin-1 is important for ROS production by macrophages in response to zymosan and C. albicans, and that the receptor collaborates with TLRs for induction of inflammatory responses (15, 16). We therefore examined the ability of dectin-1 to recognize A. fumigatus at different stages of maturation. During activation and maturation into germ tubes, conidia swell, in the process losing a cell wall that consists largely of hydrophobic proteins (rodlets) and pigments, both of which confer resistance to macrophage killing (11, 19). We produced sDectin in HEK293 cells (16) and used it to identify dectin-1 binding sites on A. fumigatus resting conidia, swollen conidia, germ tubes, and...
hyphae. Confocal microscopy revealed that dectin-1 cannot bind dormant conidia; however, as A. fumigatus matures in culture, dectin-1 binding sites are exposed (Fig. 3a). The soluble β-glucan, laminarin, completely blocked binding of dectin-1 (data not shown).

Germ tube binding of sDectin was measured by flow cytometry, before and after blocking with laminarin. Laminarin blocks sDectin binding to zymosan (Fig. 3b) and germ tubes (Fig. 3c), suggesting that the β-glucan binding site on dectin-1 is required for recognition of A. fumigatus. To provide additional evidence that dectin-1 recognizes β-glucan on germ tubes, cells were pre-digested with β-(1,3)-d-glucanase or α-mannosidase (as a control) before sDectin exposure (Fig. 3d). Soluble dectin binding to germ tubes is inhibited by pretreatment with β-(1,3)-d-glucanase, but not with α-mannosidase, consistent with the mammalian receptor specificity for β-(1,3)-glucan.

These data suggest that β-glucan is exposed after conidial loss of the outer cell wall during cellular swelling and maturation and remains exposed on the surface of more mature germ tubes and hyphae. To confirm this, β-glucan exposure on A. fumigatus morphotypes was assessed directly using an mAb specific to linear β-glucan. The β-glucan Ab binds to swollen conidia and hyphal forms (Fig. 4a). Colocalization studies were performed; live germ tubes were stained with the Ab to β-(1,3)-glucan (green) and sDectin-1 (red), and the overlay demonstrated identical staining patterns (Fig. 4b). Binding of β-glucan Ab was abrogated after exposure to soluble β-glucan (laminarin; Fig. 4c). We also attempted to block binding of β-glucan Ab with sDectin-1 (13, 14, 16); however, the soluble receptor did not block Ab binding despite multiple experimental conditions (data not shown). It is possible that the shear abundance of cellular β-glucan binding sites makes efficient blocking difficult; alternatively, dectin-1 and the β-glucan mAb may recognize different specific epitopes.

**Germ tubes activate macrophages through dectin-1**

Dectin-1 mediates macrophage internalization of and activation by glucan-containing particles, including zymosan, C. albicans yeasts, Pneumocystis carinii, and Coccioides posadasii spherules (15, 16, 20, 21). We determined the role of dectin-1 in mediating murine macrophage responses to Aspergillus using laminarin (a soluble β-glucan) or anti-dectin-1 polyclonal Ab, which specifically blocks the receptor (13, 14, 16). Unlike fully mature hyphae, germ tubes are small enough to be completely phagocytosed by macrophages. Consistent with a role for dectin-1 in macrophage recognition of germ tubes, we observed that laminarin and blocking Ab pretreatment, but not rabbit serum alone, resulted in decreased germ tube internalization by murine peritoneal macrophages (Fig. 5a).

Dectin-1 activation is necessary for macrophage production of ROS in response to exposure to C. albicans yeasts and zymosan. Laminarin pretreatment resulted in complete blocking of ROS production after exposure to zymosan (Fig. 5b). In contrast, laminarin, and dectin-1 Ab only partially blocked ROS production in response to germ tubes (Fig. 5, c and d). Pretreatment with anti-dectin serum had no effect on the induction of ROS by Staphylococcus aureus, which does not contain β-glucan (16). Finally, macrophage pre-exposure to dectin-1 Ab, but not serum, decreased macrophage release of TNF-α in response to germ tubes (Fig. 5e). As expected, blocking dectin-1 did not abrogate TNF-α production in response to the TLR4 agonist control, LPS.

**Role of dectin-1 in TLR2-mediated TNF-α production**

As a central modulator of pulmonary inflammation, TNF-α serves a pivotal function in protecting against invasive pulmonary disease caused by A. fumigatus (22). Previous studies have shown that dectin-1 activation enhances TLR2 stimulation of TNF-α in response to β-glucan-containing zymosan particles (14, 15); it is also known that TLR2 and TLR4 mediate macrophage production of TNF-α in response to challenge with live and killed A. fumigatus (7–10). Blocking experiments were performed to determine whether dectin-1 activation enhances TLR2 stimulation of TNF-α production in response to A. fumigatus germ tubes. As expected, peritoneal macrophages harvested from TLR2- and MyD88-null mice exhibited deficient production of TNF-α in response to germ tubes (Fig. 6, wild type (WT) vs TLR2−/− and WT vs MyD88−/−, p < 0.0001). Blocking with both the anti-dectin-1 Ab and laminarin decreased germ tube-stimulated TNF-α production from WT, TLR2−/−, and MyD88−/− macrophages. Dectin-1 blockade in the setting of TLR2 deficiency led to an ~90% decrease in TNF-α production compared with WT cells exposed to germ tubes; similarly, the combination of dectin-1 blockade and MyD88 deficiency abrogated TNF-α production by ~90%. As expected, neither dectin-1-blocking agent abrogated TNF-α secretion in response to the pure TLR2 agonist, lipoteichoic acid (data not shown).

**Discussion**

Collectively, these data demonstrate that dectin-1 is a key receptor for macrophage activation by and phagocytosis of A. fumigatus germ tubes. Resting (nonswollen) conidia of the organism are not recognized by dectin-1, whereas germ tubes are, as indicated by
the ability of germ tubes to activate dectin-1 for ROS production. These results provide a mechanism by which macrophages distinguish between inhaled resting forms of the organism and maturing cells; because the latter present a threat for invasive pneumonia, selective secretion of inflammatory mediators probably minimizes lung damage that would be incurred with constant inhalation of ubiquitous fungal spores.

Our studies show that dectin-1 is one key receptor for macrophage phagocytosis of and activation by *A. fumigatus* germ tubes. These data do not rule out the participation of additional receptors. For example, the observation that laminarin completely blocks zymosan-induced ROS production, but only partially inhibits *Aspergillus* germ tube-induced ROS production, suggests that additional receptors that specifically recognize germ tubes and transmit stimulatory signals are yet to be identified.

Soluble dectin-1 and the β-glucan Ab bind to swollen conidia, germ tubes, and hyphae, but do not bind resting conidia. Colocalization studies support the contention that the dectin-1 receptor binds to β-glucan exposed during conidial maturation. Soluble dectin-1 does not block β-glucan Ab binding (and vice versa). The lack of blocking may be explained by the high number of β-glucan binding sites on cell walls. It is also possible that dectin-1 recognizes an alternative epitope compared with the β-glucan Ab, which is specific to linear β-(1,3)-glucan polymers. This is supported by the recent finding that dectin-1 binds exclusively to long (10- to 11-mer) 1,3-linked glucose oligomers in clustered form (23).

The cell wall of resting conidia is known to have a different structure than that of hyphae, but the polysaccharide composition of conidial cell walls is not well described (24). An outer conidial cell wall, which consists largely of hydrophobic proteins and pigments, is lost in conidial development into filaments; the cell wall of mycelia, which has been more extensively characterized, consists of branched β-(1,3)-glucan covalently bound to chitin, β-(1,3)- and β-(1,4)-glucans, and galactomannan (12). In *A. fumigatus*, β-(1,3)-glucans are synthesized by an enzyme complex bound to the plasma membrane; this complex, which is found in highest concentrations at hyphal tips, extrudes β-(1,3)-glucan chains into mycelial periplasmic space (24, 25). The results of this study suggest that macrophages differentiate between morphotypes of *A. fumigatus* using the dectin-1 receptor, which recognizes β-glucans that are exposed only on swollen conidia and hyphae. The mechanism by which β-glucans are differentially presented on *A. fumigatus* morphotypes is not clear; however, given the differences in cell wall structure, it is likely that the dense pigmented outer layer of conidia serves to mask β-glucans that may be produced and/or exposed during filamentous cellular growth.

The studies presented demonstrate that swollen conidia and germ tubes, but not resting conidia, activate macrophages to produce inflammatory mediators. Previous studies demonstrated that functional signaling through TLR2 is necessary for optimal responses to *A. fumigatus* hyphal products (7–10). We have now established that dectin-1 recognition of *A. fumigatus* β-glucan is an
important cofactor in triggering inflammatory responses through TLR2. Furthermore, we have demonstrated that this pair of receptors accounts for the bulk of the TNF-α produced in response to germ tubes. Dectin-1 augmentation of TLR2-mediated TNF-α secretion was reported previously for zymosan (15). Zymosan-stimulated TNF-α secretion is dependent on functional TLR2 signaling through MyD88. However, the results of this study suggest that recognition of germ tubes also triggers a small amount of TNF-α secretion.
production in the absence of both TLR2 and MyD88 signaling. The identity of the receptor(s) responsible for this remaining TNF-α production is not known.

Previous studies reported that conidia stimulate low amounts of TNF-α production by murine peritoneal macrophages, a response variously abrogated by blocking TLR4 (9, 10). In our experiments, exposure to killed (heat-killed or paraformaldehyde-fixed) A. fumigatus conidia did not stimulate the production of ROS or cytokines. Our findings are consistent with those of two recent groups, who also report the role of dectin-1 in recognizing β-glucan exposed with conidial germination (26, 27). The different findings may be explained by differences in inoculum preparation, because incomplete (or delayed) killing of conidia could result in exposure to swollen conidia or early germ tubes, as opposed to resting cells in the previous study. It is also of interest that previously reported responses to inactivated conidia have generally been weak and variable depending on the method of macrophage priming (9, 10). Alternative explanations for the different observations could involve the site from which macrophages are harvested or the methods of maturation, because published studies have reported responses of macrophages variously prepared from the peritoneum or lung or bone marrow-derived cells matured by incubation in M-CSF- or L929-conditioned medium (7–10, 28). Our data clearly show that resting conidia induce little or no TNF response from multiple types of macrophages in vitro (peritoneal, alveolar, and bone marrow derived); additional examination of the roles and activities of macrophage subtypes in vivo is warranted.

These studies demonstrate that dectin-1 recognition of β-glucan permits the innate immune system to discriminate between inactive resting Aspergillus and the maturing, potentially invasive forms of the organism. This finding may play a role in mediating adaptive responses to the fungus as well. Recently, Rivera et al. (29) found that mice exposed to live A. fumigatus conidia develop Th1-biased CD4+ T cell responses, whereas mice exposed to inactivated conidia demonstrate CD4+ T cell responses that are more Th2 biased. The data suggest that differential recognition of conidia and hyphae can skew downstream adaptive immune responses. It is likely that dectin-1 recognition of β-glucan exposed on the metabolically active cells is one mechanism by which the innate immune system confers specificity to both innate and adaptive responses to inhaled conidia.

Resting conidia are efficiently phagocytosed, which must occur via a receptor other than dectin-1. This observation is consistent with previous reports suggesting that DC-specific ICAM-3 grabbing non-integrin is involved in recognizing conidia for phagocytosis (5). This
receptor may also participate in coordinating downstream adaptive responses.

The finding that resting conidia do not elicit robust production of ROS has implications regarding the mechanisms of macrophage killing of different Aspergillus morphotypes. Our data are consistent with those of a previous study that showed that intracellular conidial swelling is necessary to elicit production ROS by alveolar macrophages (18). In the absence of dectin-1-mediated ROS production, other, potentially less inflammatory, antimicrobial killing mechanisms may play a role in macrophage recognition and killing of inhaled, resting conidia. One likely mechanism involves the soluble pattern recognition molecule, pentraxin-3, which binds to A. fumigatus conidia (but not hyphae), mediating innate and adaptive immune responses in vivo (30).

**FIGURE 5.** Germ tubes activate macrophages through dectin-1. *a*, Phagocytosis (phagocytic index) of germ tubes by murine peritoneal macrophages after exposure to control rabbit serum (5%), laminarin, and anti-dectin-1 Ab. Phagocytosis by unexposed macrophages (−) is shown as a control. The results are means from triplicate measurements (±SD) from one experiment representative of two. *b–d*, Production of ROS by bone marrow-derived macrophages was measured by Luminol-based chemiluminescence. Macrophages were stimulated with zymosan (Zym; *b*) or germ tubes (GT; *c*) with or without pretreatment with laminarin (Lam). *d*, Macrophages were pretreated with anti-dectin 1 Ab or preimmune rabbit serum. *e*, TNF-α measured by ELISA in LPS- (10 µg/ml) and GT-stimulated supernatants of peritoneal macrophages pretreated with medium (−), preimmune serum, and serum containing dectin-1 Ab. The results shown are the mean ± SEM from two experiments. *, p < 0.001, by Student’s *t* test.

**FIGURE 6.** Germ tube-induced TNF-α secretion is dependent on both TLR2 and TLR4. Peritoneal macrophages harvested from WT C57BL/6, TLR2−/−, and MyD88−/− mice were exposed to germ tubes (GT; 10:1), with and without anti-dectin-1 Ab (Ab) or laminarin (lam; 0.5 mg/ml), and TNF-α was measured by ELISA. The results shown are means of triplicate measurements from three to six experiments. *, Student’s *t* test *p* values compared with GT-exposed, nonblocked controls < 0.001. Results did not differ in experiments using bone marrow-derived macrophages (data not shown).
The data emphasize the importance of dectin-1 in mediating inflammatory responses to inhaled fungal pathogens, because this receptor is now implicated in recognition of the filamentous fungus, *A. fumigatus*, as well as *P. carinii* and *C. posadasii* spherules (15, 16, 20, 21). Inhalation of resting *Aspergillus* spores is common and constant, and a robust inflammatory response in the absence of a real threat could be disruptive to the airway. Recognition of glucan exposed only during maturation of the organism allows the tailoring of the inflammatory response to potentially invasive forms of the dimorphic fungus. This probably represents one mechanism by which the host avoids unnecessary pulmonary tissue damage.

**Disclosures**

The authors have no financial conflict of interest.

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