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# The Induction of Inflammation by Dectin-1 In Vivo Is Dependent on Myeloid Cell Programming and the Progression of Phagocytosis<sup>1</sup>

Marcela Rosas,<sup>2\*</sup> Kate Liddiard,<sup>2\*</sup> Matti Kimberg,<sup>†</sup> Inês Faro-Trindade,<sup>†</sup> Jacqueline U. McDonald,<sup>\*</sup> David L. Williams,<sup>‡</sup> Gordon D. Brown,<sup>†</sup> and Philip R. Taylor<sup>3\*</sup>

**Dectin-1 is the archetypal signaling, non-Toll-like pattern recognition receptor that plays a protective role in immune defense to *Candida albicans* as the major leukocyte receptor for  $\beta$ -glucans. Dectin-1-deficiency is associated with impaired recruitment of inflammatory leukocytes and inflammatory mediator production at the site of infection. In this study, we have used mice to define the mechanisms that regulate the dectin-1-mediated inflammatory responses. Myeloid cell activation by dectin-1 is controlled by inherent cellular programming, with distinct macrophage and dendritic cell populations responding differentially to the engagement of this receptor. The inflammatory response is further modulated by the progression of the phagocytosis, with “frustrated phagocytosis” resulting in dramatically augmented inflammatory responses. These studies demonstrate that dectin-1 in isolation is sufficient to drive a potent inflammatory response in a context-dependent manner. This has implications for the mechanism by which myeloid cells are activated during fungal infections and the processes involved in the therapeutic manipulation of the immune system via exogenous dectin-1 stimulation or blockade. *The Journal of Immunology*, 2008, 181: 3549–3557.**

Infections with opportunistic fungal pathogens such as the *Candida* and *Aspergillus* species are an increasing problem and are associated with high incidence of mortality and morbidity (1). The relatively new appreciation of this problem has highlighted deficiencies in current antifungal therapies and the knowledge of the underlying mechanisms by which normal immune responses control fungal infections. Neutrophils and macrophages (M $\phi$ )<sup>4</sup> are thought to be crucial in host defense to fungi via phagocytosis, killing of the invading organism, and the induction of a Th1-like immune response, which further aids fungal killing by inducing effectors such as the respiratory burst (1). Despite their suspected importance, the mechanism by which these cells recognize the fungal organism is only recently becoming clearer (2).

The cell walls of fungi predominantly consist of carbohydrates, including mannose-based structures,  $\beta$ -glucans, and chitin.  $\beta$ -Glucans compose up to 50% of the cell wall, can be expressed on the fungal cell surface (3, 4), and are biologically active agents that are

used therapeutically to modify immune responses. Experimentally, these carbohydrates can confer protection against a variety of challenges including tumor development and infection (fungal, bacterial, viral, and protozoal) (5–7).

Dectin-1 is the major leukocyte receptor for  $\beta$ -(1,3)-glucans. It is a type II transmembrane with a C-type lectin-like domain and a ITAM-like motif in its cytoplasmic tail (8–10). Dectin-1 recognizes a variety of fungal organisms (4, 11–16) and has been shown to mediate both TLR-dependent and -independent responses, including the production of cytokines and chemokines (9, 11, 14, 17–19), respiratory burst (17, 20), phagocytosis (20, 21), and the regulation of phospholipase A<sub>2</sub> activation and cyclooxygenase 2 expression (22). The ability of  $\beta$ -glucan particles to directly induce cytokine production by myeloid cells is unclear, with the limited purity and the structural characterization of the reagents used often rendering interpretation difficult. Tentative conclusions from recent studies may suggest that bone marrow-derived dendritic cells (BMDC) are able to mediate dectin-1-dependent but Myd88-independent cytokine production (19, 23, 24). This is compared with M $\phi$ , where cytokine production is not directly evident but where a synergy of response with TLR agonists for cytokine production has been described (22, 25, 26). On the surface, this appears to identify a fundamental difference in the abilities of M $\phi$  and dendritic cells (DC) to regulate immunity via dectin-1 activation.

We recently reported that *dectin-1*<sup>-/-</sup> mice are more susceptible to *Candida albicans* infection (27). The *dectin-1*<sup>-/-</sup> mice exhibited impaired production of inflammatory mediators and inflammatory cell recruitment in response to the initial infection (27). However, although this study confirmed the importance of  $\beta$ -glucan recognition by dectin-1 in the induction of the inflammatory response, it did not reveal the mechanism via which this occurs. In this report, we aim to define the mechanism by which dectin-1 engagement leads to an inflammatory response. A contextual understanding of how dectin-1 induces an inflammatory response is important not only for determining the mechanism of how  $\beta$ -glucans influence immune activation, but also as a model for how the

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<sup>4</sup> Abbreviations used in this paper: M $\phi$ , macrophage; BMM $\phi$ , bone marrow-derived M $\phi$ ; BMDC, bone marrow-derived dendritic cell; Curd-mp, curdlan microparticle; DC, dendritic cell; Glu-mp, glucan microparticle; M $\phi$ P, M $\phi$  progenitor.

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activation of non-Toll-like pattern recognition receptors in general can modulate inflammation.

## Materials and Methods

### Mice

129S6/SvEv, 129S6/SvEv.*dectin-1*<sup>-/-</sup> (27), and C57BL/6 mice were obtained from our own colonies or commercial suppliers (e.g., B&K International). Age- and sex-matched animals (6–12 wk) were used for all experiments strictly in accordance with institutional guidelines.

### Glucans, Abs, and ELISA reagents

Structurally characterized glucan microparticles were prepared from *Saccharomyces cerevisiae* as previously described (28). Curdlan, a  $\beta$ -1,3-glucan preparation from *Alcaligenes faecalis* that is known to be able to hydrate and form gels, was purchased from Wako Chemicals. Curdlan was sonicated into microparticulate form using a Vibra-Cell VCX 500 processor with 13-mm standard probe set to 50% amplitude. Thirty milliliters of 5 mg/ml curdlan was sonicated four times for 6 min each (in pulses of 48 s on and 12 s off) with cooling on ice in between sonications in a similar way to that previously described (29). Destruction of the curdlan aggregates was confirmed by microscopy. Sonication of glucans could also result in some polymer shearing and other structural alterations of the particles.

The following Abs were used in this study: anti-Ly-6G-PE (clone 1A8), anti-mouse TNF-allophycocyanin, biotinylated anti-CD117, and the control Ab (all from BD Biosciences); anti-F4/80-PE or anti-F4/80-allophycocyanin, anti-7/4-FITC, anti-CD11b-FITC or anti-CD11b-PE-Cy5, and anti-dectin-1-biotin (all either produced in house or from AbD Serotec). Cytokine production by the myeloid cells was characterized by detection of TNF, IL-2, IL-6, IL-10, and IL-12p40, measured with ELISA kits (BD Biosciences).

### Isolation and culture of primary mouse M $\phi$ and DC

Thioglycollate-elicited and resident peritoneal cells were isolated as previously described (30). Bone marrow-derived M $\phi$  (BMM $\phi$ ) and BMDC were produced by culture of bone marrow cells from the femur and tibia of mice in the presence of M-CSF or GM-CSF (both Peprotec), respectively, for 7–10 days as previously described (31, 32).

### Conditional immortalization of M $\phi$ progenitors

The Moloney murine leukemia virus-derived retroviral vector pMXs-IP, a gift from Prof. T. Kitamura (33), was used as a backbone for the conditional-immortalization of M $\phi$  progenitors (M $\phi$ P) as described previously (34). A FLAG-tagged human estrogen receptor binding domain-Hoxb8 fusion cDNA was generated and cloned into the backbone vector generating pMXs-IP:FL-ER-Hoxb8. The human estrogen receptor binding domain was cloned into the *EcoRI* site of pMXs-IP by RT-PCR from MCF-7 cells using the *EcoRI*-containing (indicated in boldface) primers: 5'-AAGGGAATTCGCCACCATGGACTACAAGGACGACGATGACAAGGAACGCGTTCTGTGCTGGAGACATGAGAGCTG-3' and 5'-AAGGGAATTCCTCAGGACTGTGGCAGGAAACCCCTC-3', which introduced a FLAG tag (italicized) at the 5'-end and a *XhoI* site (underlined) at the 3'-end for introduction of transcription factors, such as Hoxb8. The sequence obtained corresponded to the wild-type sequence and was therefore first mutated using the mutagenic primers 5'-GGAGCACCCAGTGAAGCTACTGTT-3' and 5'-AACAGTAGCTTCACTGGGTGCTCC-3' to generate the Gly400Val mutation with altered estrogen-binding sensitivity (35). Hoxb8 was amplified by RT-PCR from 10.5-day-postcoitum embryonic somite cDNA using the primers 5'-AAGGCTCGAGGGAAGCTCTTATTCGTC AACTCAC-3' and 5'-AAGGCTCGAGCTACTTCTTGTCACCCTTCTGCG-3' and cloned by *XhoI* (underlined sites) digest into the *XhoI* site immediately downstream of the estrogen receptor binding domain.

Packaged retrovirus was produced using the Phoenix cell line as previously reported (36) and used to infect bone marrow cells in a similar way to that previously described (34) with slight modification. In short, CD117<sup>+</sup> cells were enriched using biotin-labeled anti-CD117 followed by anti-biotin MACS beads (Miltenyi Biotec) as per the manufacturer's instructions. CD117-enriched cell preparations were prestimulated for 2 days in IL-3 (10 ng/ml), IL-6 (20 ng/ml), and stem cell factor (25 ng/ml) (Peprotec) in IMDM medium (Invitrogen) supplemented with 15% heat-inactivated FCS as described (34) before transduction by spin infection with packaged FLAG-tagged estrogen receptor binding domain-Hoxb8 fusion gene-modified pMXs-IP (pMXs-IP:FL-ER-Hoxb8)-containing supernatants. The cells were selected in puromycin (1.5  $\mu$ g/ml; Sigma-Aldrich) for 10–14 days in the presence of GM-CSF (10 ng/ml; Peprotec) and estradiol (1  $\mu$ M; Sigma), after which polyclonal, conditionally immortalized M $\phi$ P

were stable in culture. To differentiate M $\phi$ P they were washed three times with RPMI 1640 medium containing 10% heat-inactivated FCS (R10) to remove the estradiol and GM-CSF before differentiation in R10 containing M-CSF or GM-CSF (20 ng/ml; Peprotec) for 3 to 4 days.

For reconstitution of *dectin-1*<sup>-/-</sup> M $\phi$ P, a modified form of pMXs-IP (named pMXs-IZ) was created by replacing the puromycin resistance cassette with the zeocin resistance cassette of pSecTag2 (Invitrogen). The puromycin resistance cassette was removed by *NcoI* and *Sall* digestion and the zeocin resistance cassette was amplified by PCR using the primers 5'-AACCCATGGCCAAGTTGACCAGTG-3' and 5'-AACGTCGACTCAGTCTGCTCCTCGGCCAC-3' and cloning with *NcoI* and *Sall* (sites indicated in boldface). pMXs-IZ was used as a backbone vector to transduce the M $\phi$ P with *dectin-1* isoforms (37). pMXs-IZ-transduced M $\phi$ P were selected for 5 days with 50  $\mu$ g/ml zeocin (Invitrogen).

### In vitro cell stimulation assays

Thioglycollate M $\phi$  were plated in R10 in 48 well plates (0.2  $\times$  10<sup>6</sup> M $\phi$  per well) and washed free of non-adherent cells after 3 h, after which the medium was replaced with the addition of growth factors (20 ng/ml) as required. The following day the cultures were stimulated as indicated with the various glucans for the indicated times (usually 6 h). Supernatants were aliquoted and stored at -80°C and analyzed for cytokine production by ELISA.

BMM $\phi$  and BMDC, generated as described above, were plated in 48-well plates (2  $\times$  10<sup>5</sup> cells per well). The BMM $\phi$  were plated the day before the experiment to ensure a healthy adherent culture, and the non-adherent BMDC were plated at the time of assay. The cultures were stimulated as indicated for 6–16 h and supernatants were analyzed as described above.

M $\phi$ P were plated in 48-well plates after washing three times with R10 medium and resuspension in R10 with either GM-CSF (3  $\times$  10<sup>4</sup> cells per well) or M-CSF (8  $\times$  10<sup>4</sup> cells per well) at 20 ng/ml. Fewer cells were plated when differentiating with GM-CSF because of the additional potent proliferative effect of GM-CSF during this period. Medium was replenished on day 3 and experiments were conducted on monolayers of M $\phi$  on day 4. Stimulations were performed as described above.

For experiments with fresh resident peritoneal cells, cells were washed with R10 medium and cultured immediately in the presence of Golgi block (BD Biosciences) and the indicated concentrations of glucan particles in a final volume of 200  $\mu$ l with 5  $\times$  10<sup>5</sup> cells per well of a 96-well V-bottom plate. After 3 h the cells were fixed, permeabilized, and stained as previously described (36). Resident peritoneal M $\phi$  and DC populations were identified by F4/80 and CD11b profiles as previously described (38).

When cytochalasin D was used, cells were pretreated for 1 h with 2  $\mu$ M cytochalasin D or vehicle before the addition of stimulants.

### Flow cytometry

Flow cytometry was essentially performed as previously described (30, 36, 39) on a FACSCalibur flow cytometer (BD Biosciences). We developed a rapid, single-step, differential analysis protocol for inflammatory leukocytes. In brief,  $\sim$ 0.5–1  $\times$  10<sup>5</sup> cells were directly added to FACS block (30). After 10 min, an equal volume of Ab mix was added and after a further 15 min this was diluted in a 10-fold excess of 1% formaldehyde in PBS for flow cytometric analysis. Intracellular cytokine production was represented as a "stimulation index" in arbitrary units and defined as follows: stimulation index = percentage of cells producing cytokine  $\times$  mean fluorescence intensity of producing cells.

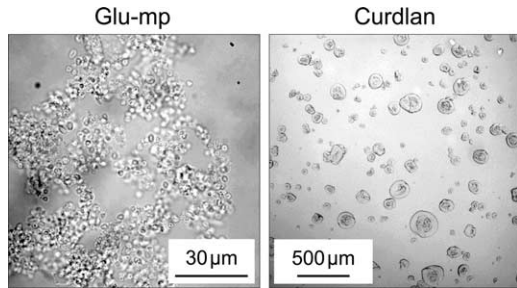
### Microscopic imaging

Microscopic images were recorded either using a Leica DM LB microscope with DFC490 digital camera (Leica) and QWin software (Leica) or a Zeiss Axiovert 40. Images were processed using Adobe Photoshop version 6.0.

### In vivo inflammatory studies

Mice were injected i.p. with the indicated doses of stimulants and left for 4 h for the progression of the inflammatory response as previously described (27). Peritoneal inflammatory cells were harvested after this time by i.p. injection of 1 ml of PBS with 5 mM EDTA, followed by extensive massaging of the abdomen and recovery of the exudate with a fresh syringe. Inflammatory cell populations were determined by flow cytometry as previously reported (27, 30, 39). In brief, neutrophils were defined as Ly-6G<sup>+</sup>7/4<sup>+</sup>, inflammatory monocytes as Ly-6G<sup>-</sup>7/4<sup>+</sup>, and resident peritoneal M $\phi$  as CD11b<sup>high</sup>F4/80<sup>high</sup>. Cell-cleared lavage was stored immediately at -80°C for future cytokine analysis (see above). Because the mechanism of inflammation is complex, multiple inflammatory parameters were assessed, namely the recruitment of neutrophils and inflammatory





**FIGURE 1.** Particulate  $\beta$ -glucan particles. Glu-mp and curdlan are physically distinct preparations of  $\beta$ -1,3-glucan particles. Scale bars are as indicated.

monocytes, the activation and “disappearance” of tissue-resident M $\phi$ , and cytokine production within the lesion.

#### Statistical analysis

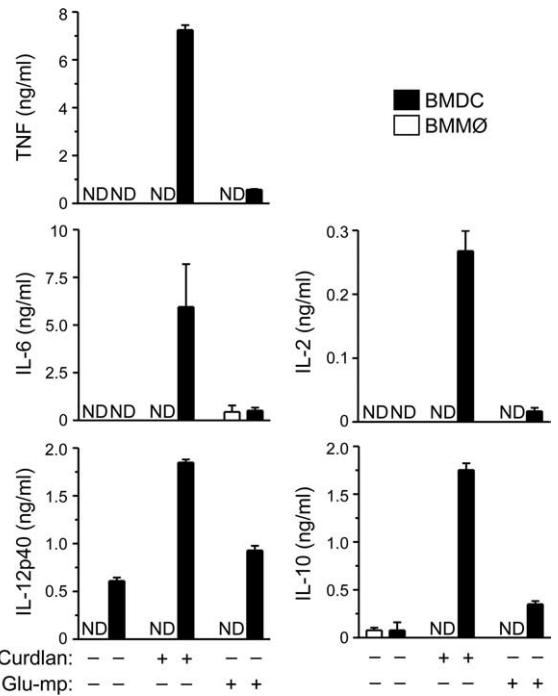
Data were analyzed using GraphPad Prism with the appropriate tests as indicated in the text. *p* values are represented generically as follows: \*, *p* < 0.05; \*\*, *p* < 0.01; and \*\*\*, *p* < 0.001.

## Results

### Particulate $\beta$ -glucans and the induction of cytokines in M $\phi$ and DC

$\beta$ -glucans exhibit apparent differences in their ability to activate M $\phi$  and DC for cytokine responses (19, 22–26), and several factors may be contributing to these differences: 1) the dectin-1 specificity of the response; 2) the purity of the glucans used; 3) the structural and physical nature of the particulate glucans used; 4) the activation state of the cells under study; and 5) the type of cell under study (e.g., DC or M $\phi$ ). Indeed, the studies reported in the literature used different  $\beta$ -glucan preparations, such as glucan microparticles (Glu-mp) derived from *S. cerevisiae* and curdlan derived from *A. faecalis* (19, 22, 24, 25). We have previously used Glu-mp to specifically evaluate the role of dectin-1 in the signaling responses of M $\phi$  (22, 25), whereas curdlan has received significant attention for its ability to activate DC via dectin-1 without a requirement for the MyD88 pathways (19, 24). Both of these glucans are primarily  $\beta$ -1,3-linked. We found that Glu-mp were  $\sim 3 \mu\text{m}$  in diameter in contrast to curdlan, which were significantly larger with some particles in excess of 0.2 mm in diameter (Fig. 1). We were interested in determining how these physical differences would impact their ability to activate M $\phi$  and DC.

To directly compare the ability of both glucan preparations to stimulate cytokine production, BMM $\phi$  and BMDC were cocultured with glucan particles for up to 16 h and analyzed for the production of TNF, IL-2, IL-6, IL-10, and IL-12p40 (Fig. 2). As previously reported (19), curdlan induced robust cytokine responses from the BMDC. We noted that Glu-mp also induced cytokine responses by BMDC, albeit at reduced levels when compared with the curdlan. The large size of curdlan means that the particle:cell ratio used in this assays is very low (10  $\mu\text{g}$  of curdlan is  $\sim 550$  curdlan particles). In contrast, the Glu-mp particle:cell ratio in these experiments is substantially higher (10  $\mu\text{g}$  of Glu-mp is  $> 10^6$  particles). This fundamental difference makes it difficult to directly compare the two agents but does clearly indicate, since fewer cells are involved in the response to curdlan than in the response to Glu-mp, that curdlan is a substantially more potent inducer of cytokine production (see also Figs. 3 and 5 below). Consistent with our previous observations, BMM $\phi$  did not produce cytokines in response to Glu-mp, nor did these cells make a significant response to curdlan (Fig. 2). Thus, it appears that DC



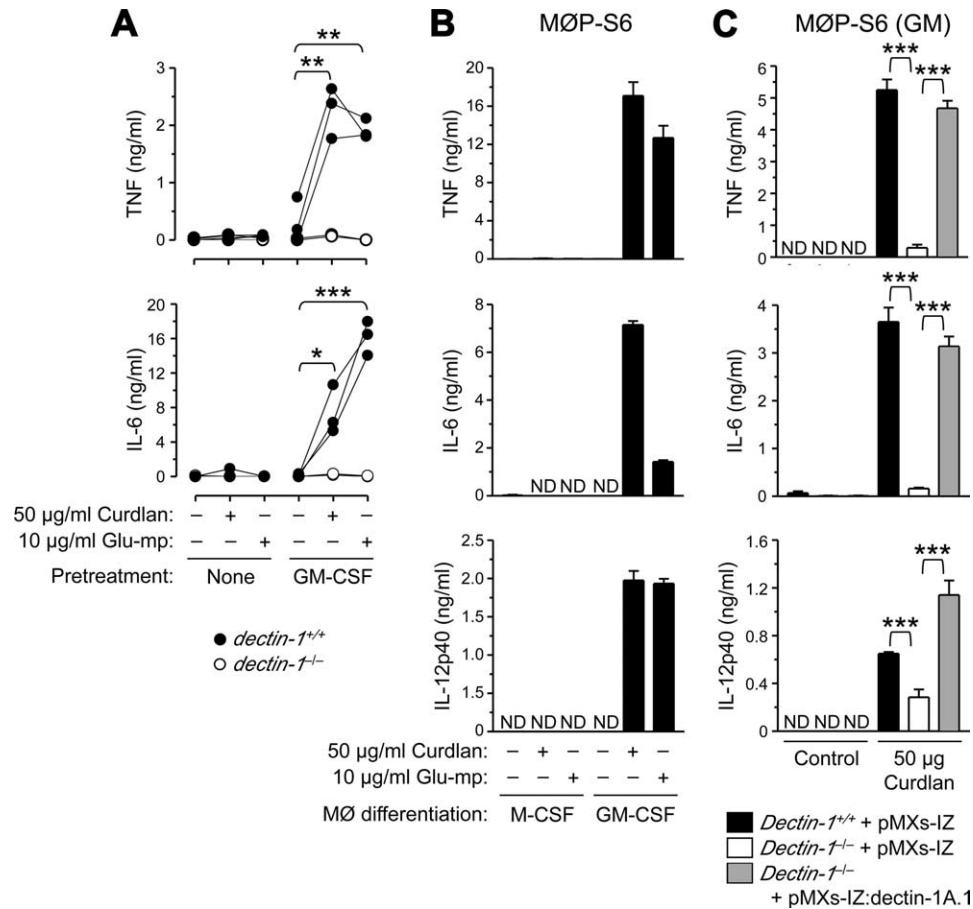
**FIGURE 2.** BMDC but not M $\phi$  respond to  $\beta$ -glucan particles. BMDC (filled bars) and BMM $\phi$  (open bars) were stimulated *in vitro* with glucan particles for 6 (TNF, IL-6, and IL-12p40) or 16 (IL-2 and IL-10) h and the cytokines produced were assessed.  $\beta$ -Glucan particles induced reproducible and strong cytokine responses in BMDC, but BMM $\phi$  in comparison had almost no cytokine response to the glucan particles. The response of the BMDC to curdlan was substantially stronger than that to Glu-mp. Data represent one of two experiments and are consistent with previous reports (19, 25). ND denotes not detectable.

and M $\phi$  may have a differing capacity to respond to particulate  $\beta$ -glucans.

### The role of GM-CSF in cellular programming of a proinflammatory dectin-1 signaling response in M $\phi$

A principal difference between the culture of BMDC and BMM $\phi$  (and other M $\phi$  populations we have studied) is the inclusion of GM-CSF in the culture medium, which may be especially important as we have previously reported that GM-CSF affects M $\phi$  dectin-1 expression and function (40). To determine whether GM-CSF in the BMDC culture system was dictating the responsiveness of these cells to  $\beta$ -glucan stimulation and whether M $\phi$  could be similarly responsive, we cultured thioglycollate-elicited peritoneal M $\phi$  in GM-CSF before particulate  $\beta$ -glucan stimulation (Fig. 3A). Thioglycollate-elicited M $\phi$  are normally unable to mount a significant cytokine response to  $\beta$ -glucan particles (25); however, pretreatment with GM-CSF markedly altered this (Fig. 3A). Importantly, the cytokine response of GM-CSF-“primed,” thioglycollate-elicited M $\phi$  remained dectin-1 dependent (Fig. 3A).

To directly compare the effect of M-CSF and GM-CSF on the cellular programming of M $\phi$  in a similar way to that achieved with the use of bone marrow cultures, we established conditionally immortalized M $\phi$  from 129S6/SvEv (M $\phi$ P-S6) mice (34). These cells are immortalized in the presence of estrogen, but after estrogen withdrawal the progenitors can be differentiated into M $\phi$  in the presence of either GM-CSF or M-CSF. We examined the ability of M $\phi$  generated in this way to respond to particulate glucans. When differentiated in GM-CSF, the M $\phi$  responded to particulate  $\beta$ -glucan stimulation with potent TNF, IL-6, and IL-12p40 production (Fig. 3B). By analogy to BMM $\phi$  cultures, the M $\phi$  derived



**FIGURE 3.** Cellular programming regulates the myeloid cell response to  $\beta$ -glucans. **A**, Thioglycollate-elicited mouse MØ were cultured with or without GM-CSF before stimulation with  $\beta$ -glucan particles. MØ from individual 129S6/SvEv mice (●,  $n = 3$ ) consistently mounted a robust cytokine response only after pretreatment with GM-CSF. Data shown are representative of two independent experiments. The *dectin-1*<sup>-/-</sup> mice (○,  $n = 2$ ) were included in this experiment to demonstrate the requirement of dectin-1 for this response. Data were analyzed by paired one-way ANOVA with Bonferroni post-tests to determine significant differences between glucan-stimulated and control cells. Connecting lines identify the cells from individual mice. **B**, MØ-committed progenitors from 129S6/SvEv mice (MØP-S6) were differentiated in either M-CSF or GM-CSF. Only cells differentiated in GM-CSF were able to mount robust cytokine responses to  $\beta$ -glucan particles. Data represent mean  $\pm$  SEM of triplicates from one of three independent experiments. **C**, Use of *dectin-1*<sup>-/-</sup> MØ-committed progenitors (open bars) differentiated in GM-CSF (MØP-S6 (GM)) confirmed the specificity of this response for dectin-1 when compared with wild-type cells (filled bars). Reconstitution of the deficient cells with dectin-1 restored the response (gray bars), confirming this specificity. Data represent mean  $\pm$  SEM of triplicates from one of two independent experiments. Significant differences between the three groups were determined by one-way ANOVA with Bonferroni posttests. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ . ND denotes not detectable.

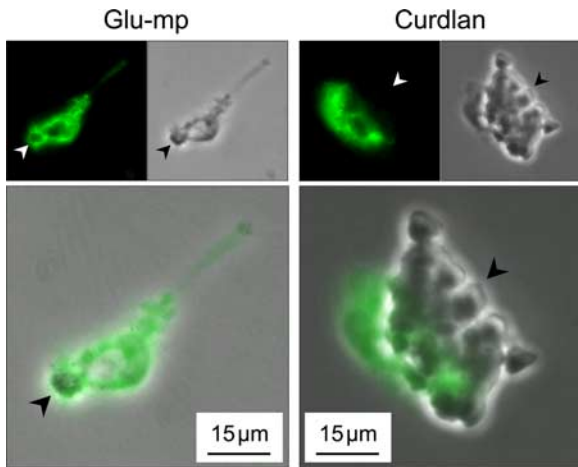
from MØP differentiated in M-CSF failed to make these cytokines after stimulation with particulate  $\beta$ -glucans (Fig. 3B).

To further facilitate the study of the involvement of Dectin-1 in this response, MØP were generated at the same time from 129S6/SvEv.*dectin-1*<sup>-/-</sup> mice. MØP from *dectin-1*<sup>-/-</sup> mice differentiated in GM-CSF were found to be deficient in  $\beta$ -glucan-induced cytokine production (Fig. 3C). Our previous observation of an up-regulation of dectin-1 expression by GM-CSF (40) led us to examine dectin-1 expression by MØ derived from progenitors differentiated in GM-CSF and M-CSF. MØP differentiated in GM-CSF have significantly higher dectin-1 expression (data not shown). Retroviral reconstitution of *dectin-1*<sup>-/-</sup> MØP with a high level of dectin-1 (data not shown), which bypasses the normal regulation of dectin-1 expression, allowed the generation of GM-CSF- and M-CSF-differentiated dectin-1 high-expressing MØ. The  $\beta$ -glucan-induced cytokine production was rescued by retroviral transduction of the GM-CSF-differentiated *dectin-1*<sup>-/-</sup> MØ with the different dectin-1 isoforms, confirming the dependence of this production on dectin-1 (Fig. 3C and data not shown) (37). The dectin-1-reconstituted M-CSF-differentiated cells remained unable to respond with cytokine production after  $\beta$ -glucan stimulation

(data not shown). Taken together, these data confirmed that GM-CSF-mediated cell programming is required for cytokine responses to dectin-1 ligation and that differences in the expression level of dectin-1 was not a fundamental factor to explain these observations. Thus, in vitro "priming" of MØ with GM-CSF is sufficient to allow them to respond to  $\beta$ -glucan particles with cytokine production in a similar way to that of BMDC (cells that have been also been cultured in GM-CSF). This suggests that primary MØ, which are very heterogeneous, may be preprogrammed in vivo depending on location and function in a manner that is either permissive or not permissive of a cytokine response to particulate  $\beta$ -glucan stimulation.

#### *The dectin-1-mediated signaling response is enhanced by frustrated phagocytosis*

The large size of the curdlan particles raised the possibility that it may not be possible for them to be engulfed by phagocytes, so we examined the phagocytic process using thioglycollate-elicited peritoneal MØ (Fig. 4). Although the Glu-mp were readily phagocytosed, MØ were unable to phagocytose the larger curdlan particles (Fig. 4), indicating that they may be experiencing "frustrated



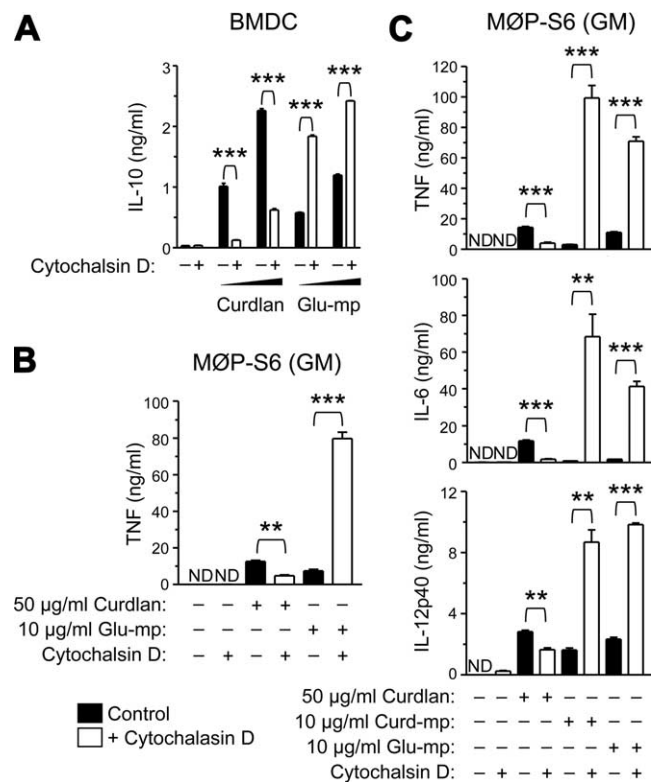
**FIGURE 4.** Curdlan and frustrated phagocytosis. Thioglycollate-elicited mouse peritoneal MØ, labeled with CFSE, were able to phagocytose Glu-mp (left panel), but not curdlan particles (right panel). CFSE imaging and phase contrast are shown in the upper left and right insets, respectively, of the merged images. Arrowheads indicate glucan particles.

phagocytosis” (41), although this does not exclude the possibility that any sufficiently small curdlan particles present may have been internalized.

We have previously shown that cytochalasin D can enhance the inflammatory response to fungal particles (11). Having established that GM-CSF is one factor important in vitro for permitting a cytokine response to  $\beta$ -glucan particles, we next examined the possibility that enhanced responses to curdlan may be a direct consequence of “frustrated phagocytosis.” We used both nonadherent BMDC (Fig. 5A) and in vitro programmed MØ (Fig. 5B). To model frustrated phagocytosis, the cells were treated with cytochalasin D before stimulation with particulate glucans. The relatively weak BMDC response to Glu-mp (which are normally phagocytosed) was markedly enhanced by cytochalasin D treatment. This enhanced response to Glu-mp after treatment with cytochalasin D is consistent with the hypothesis that prevention of phagocytosis would enhance the cytokine response (Fig. 5A). Similar results were obtained when examining IL-6, TNF, IL-12p40, and IL-2 production by BMDC (data not shown). These responses were absent in *dectin-1*<sup>-/-</sup> BMDC (data not shown). Similar results were obtained with GM-CSF-differentiated MØP. Glu-mp stimulated a greatly enhanced cytokine response when uptake was inhibited by cytochalasin D (Fig. 5B). Cytochalasin D alone did not induce cytokines from any of the tested cell types.

Interestingly, the robust BMDC response to curdlan was reduced by cytochalasin D treatment (Fig. 5A). Similarly, MØP differentiated in GM-CSF made a cytokine response to curdlan that was reduced in the presence of cytochalasin D (Fig. 5B). In these monolayer experiments it was clear that in the presence of cytochalasin D the MØ were prevented from migrating into contact with the curdlan particles and that this was the most likely explanation for the reduced cytokine responsiveness observed (not shown).

To confirm that frustrated phagocytosis was the main cause of the difference in magnitude of the cytokine response to the two particulate  $\beta$ -glucans we generated curdlan microparticles (Curd-mp) by high intensity sonication (see *Materials and Methods* and Ref. 29). It was anticipated that converting curdlan to a microparticulate form would reduce its cytokine-inducing potential and render it susceptible to the enhancing effects of cytochalasin D, as was seen with Glu-mp. If true, this would also confirm that the differ-



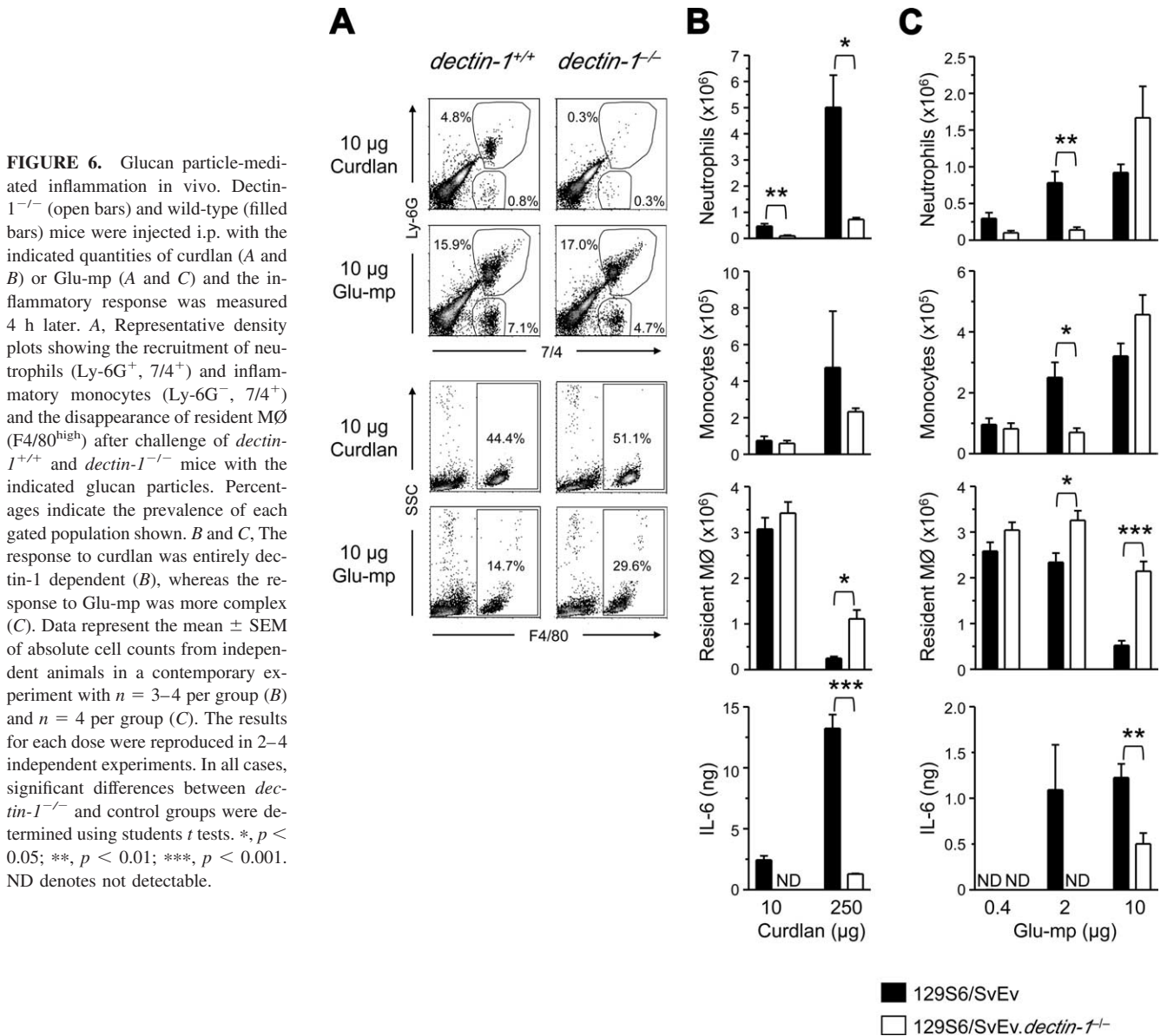
**FIGURE 5.** Frustrated phagocytosis regulates the dectin-1-mediated response. *A*, BMDC cultured in cytochalasin D exhibited enhanced IL-10 responses to the normally phagocytosed Glu-mp compared with untreated cells. The production of IL-10 in response to curdlan was inhibited by cytochalasin D. Data show stimulation experiments with two doses of each glucan particle (10 and 100  $\mu$ g/ml). Data represent mean  $\pm$  SEM of triplicates from one of two independent experiments. *B*, In vitro GM-CSF-programmed MØ (MØP-S6 (GM)) exhibited enhanced cytokine responses to Glu-mp and reduced responses to curdlan when pretreated with cytochalasin D. Data represent mean  $\pm$  SEM of triplicates from one of three independent experiments. *C*, To test the role of phagocytosis in the response to curdlan further, Curd-mp was generated by sonication and tested on GM-CSF-differentiated MØP (MØP-S6 (GM)). Curd-mp were found to behave exactly as Glu-mp and no longer like the aggregated nonphagocytosable curdlan material. Data represent mean  $\pm$  SEM of triplicates from one of two independent experiments. In all cases, significant differences between cytochalasin D-treated (open bars) and control groups (filled bars) were determined using Student's *t* test. \*\*,  $p < 0.01$ ; and \*\*\*,  $p < 0.001$ . ND denotes not detectable.

ences in cytokine induction were not due to chemical structure or reagent contamination differences between the two glucan preparations. We found that Curd-mp behaved exactly as Glu-mp in the cell stimulation assays, indicating that it was the difference in the cellular capacity to phagocytose the particles that dictated the magnitude of the cytokine response rather than differences between the two glucans in purity or chemical structure (Fig. 5C). Similar results were obtained with BMDC (data not shown).

#### *Dectin-1*-mediated inflammation in vivo

In vitro, both curdlan and Glu-mp exhibit high specificity for dectin-1. We used both particles to examine the role of dectin-1 in the initiation and regulation of inflammation in vivo. To achieve this, we measured several parameters of inflammation in a well-established mouse model of peritoneal inflammation. Both particles induced inflammation characterized by the recruitment of both neutrophils and inflammatory monocytes to the inflammatory lesion,





the proinflammatory cytokine response (as typified by IL-6 production), and the activation of resident peritoneal MØ by evaluation of the MØ “disappearance reaction” (39, 42, 43). A dose-dependent study of both Glu-mp and curdlan in 129S6/SvEv wild-type and *dectin-1*<sup>-/-</sup> mice revealed that each particle induced inflammation with its own distinct mechanism (Fig. 6).

After the injection of curdlan the recruitment of neutrophils and production of IL-6 within the peritoneal cavity was evident, and this was found to be fully dependent on the presence of dectin-1, even when very high doses of curdlan were used (Fig. 6, A and B). At higher doses of curdlan the disappearance of MØ from the peritoneal cavity became apparent, and this too was impaired in the absence of dectin-1 (Fig. 6B). At low doses of Glu-mp ( $\leq 2$  µg), inflammation was largely abrogated in the *dectin-1*<sup>-/-</sup> mice, indicating that inflammation was being driven by a similar mechanism (dectin-1-mediated cellular activation) (Fig. 6, A and C). However, injection of 10 µg of Glu-mp resulted in a marked inflammatory response in the *dectin-1*<sup>-/-</sup> mice (Fig. 6, A and C).

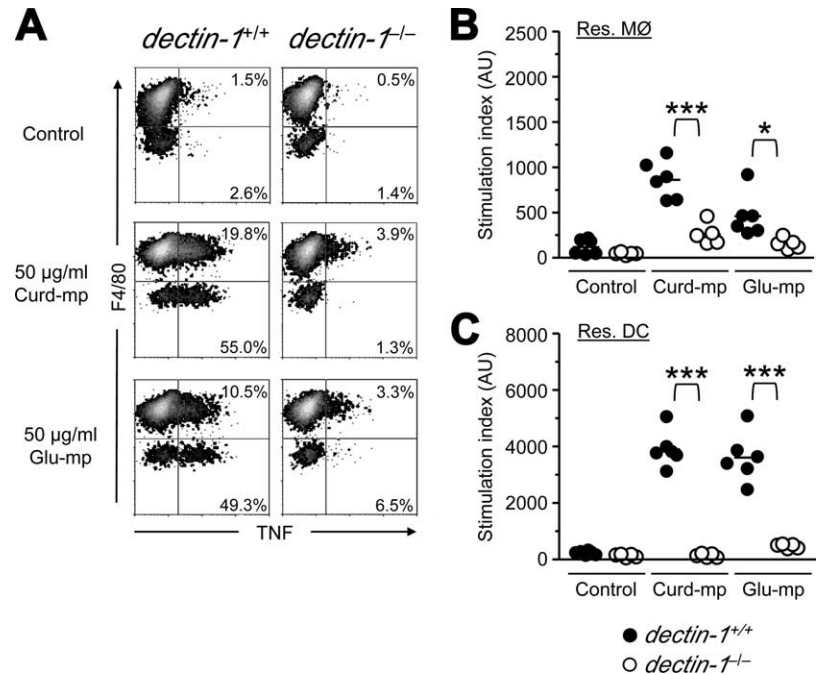
In wild-type mice, 10 µg of Glu-mp induced an inflammatory response that was characterized by a substantial neutrophil and a detectable monocyte influx, the disappearance of resident perito-

neal MØ from the cavity, and the production of IL-6 (Fig. 6, A and C). Much of this inflammatory response occurred in the *dectin-1*<sup>-/-</sup> mice (recruitment of comparable numbers of neutrophils and monocytes); however, both the IL-6 production and the MØ-disappearance remained significantly reduced, indicating that cellular activation was still impaired in the absence of dectin-1.

Taken together, these data indicated that while curdlan-induced inflammation was essentially dectin-1-dependent, Glu-mp at higher doses were able to induce inflammation in the absence of dectin-1. Higher doses of Glu-mp were able to bypass the need for dectin-1 for neutrophil and monocyte recruitment, but myeloid cell activation was still impaired (as indicated by the failure of MØ disappearance and reduced IL-6 production in the absence of dectin-1). This indicated that alternate inflammatory processes were evident in the *dectin-1*<sup>-/-</sup> mice treated with higher doses of microparticles. These observations most likely reflect the combination of the consequence of an in vivo balance between effector functions, target:host cell ratios, and an impairment in the phagocytosis of Glu-mp in the absence of dectin-1.

Thus, both  $\beta$ -glucan particles can induce dectin-1-dependent inflammatory responses, which are characterized by inflammatory

**FIGURE 7.** Context-dependent response of tissue M $\phi$  and DC to  $\beta$ -glucan particles. *A*, Peritoneal cells from naive mice were recovered and stimulated with  $\beta$ -glucan particles. The representative FACS plots gated on resident peritoneal M $\phi$  (F4/80<sup>high</sup>) and DC (F4/80<sup>int</sup>; where “int” is “intermediate”) indicated that both the M $\phi$  and DC were capable of a glucan-induced cytokine responses. Percentages indicate the percentage of F4/80<sup>high</sup> or F4/80<sup>int</sup> cells that are producing TNF. *B* and *C*, The data from individual wild type (●) and *dectin-1*<sup>-/-</sup> (○) mice pooled from two independent experiments demonstrated the importance of dectin-1 on resident peritoneal M $\phi$  (Res. M $\phi$ ) (*B*) for cytokine responses to  $\beta$ -glucan particles and an absolute requirement for the dectin-1 on the resident DC (Res. DC) (*C*) response to  $\beta$ -glucan particles. Data represent individual mice analyzed by Student's *t* test and are expressed in the form of a stimulation index (percentage of cells responding  $\times$  mean fluorescence of responding cells) in arbitrary units (AU). \*, *p* < 0.05; \*\*, *p* < 0.01; \*\*\*, *p* < 0.001.



cell recruitment, cytokine production, and resident M $\phi$  activation. Most notably, the physical differences between the two particles allow curdlan to be used in a high dose, driving a potent inflammatory response in a dectin-1-restricted manner. In contrast, Glu-mp, which is also dectin-1-specific at lower doses, have a greater capacity to bypass dectin-1 for some aspects of the inflammatory response when present at higher numbers.

#### *$\beta$ -Glucan-induced cellular activation and the initiation of inflammation*

The ability to convert M $\phi$  into cells that can produce cytokines in response to particulate  $\beta$ -glucans reinforces the idea that M $\phi$  are very heterogeneous cells and that the *in vivo* context of M $\phi$  subsets and their prior programming may dictate M $\phi$  responsiveness to such stimuli. Having observed significant dectin-1-mediated activation of resident peritoneal M $\phi$  *in vivo* after challenge with particulate  $\beta$ -glucans, we wanted to examine the capacity of these cells to respond directly to  $\beta$ -glucan stimulation. We conducted immediate *ex vivo* analysis of resident peritoneal M $\phi$  and DC, measuring the TNF response to microparticulate  $\beta$ -glucan preparations to assess cellular activation. We used microparticulates because of the high particle:cell ratios and, hence, greater cellular involvement achievable in these rapid *ex vivo* assays. In these mixed cell assays M $\phi$  outnumber DC by ~10–20-fold as they do *in vivo*. Both M $\phi$  and DC from wild-type mice responded to  $\beta$ -glucan microparticles with TNF production (Fig. 7). The *dectin-1*<sup>-/-</sup> resident peritoneal M $\phi$  showed a substantial reduction in response to microparticulate  $\beta$ -glucans compared with the wild-type cells (Fig. 7, *A* and *B*). The ability of these M $\phi$  to respond immediately to particulate  $\beta$ -glucans in a dectin-1-dependent manner confirms the speculation that subsets of M $\phi$  *in vivo* may already be competent to mount a cytokine response to dectin-1 ligation and that a context-dependent consideration of M $\phi$  activity is essential. The DC were almost totally dependent on dectin-1 for responses to microparticulate  $\beta$ -glucans (Fig. 7*C*). Thus, both resident peritoneal DC and M $\phi$  are competent to respond immediately with a dectin-1-mediated cytokine response to particulate  $\beta$ -glucan stimulation.

#### Discussion

We and others have recently demonstrated the importance of dectin-1 in host defense against fungal infection (23, 27). The *dectin-1*<sup>-/-</sup> mice exhibited impaired production of inflammatory mediators and inflammatory cell recruitment in response to an initial infection with *C. albicans* and to challenge with the yeast model zymosan particle also (27). However, the role of dectin-1 in the orchestration of the host-protective inflammatory response remains unclear. Notably, we showed that the specific ligation of dectin-1 with particulate  $\beta$ -glucans was insufficient for the induction of cytokine production by M $\phi$  but was able to act in synergy with the TLR-mediated cytokine responses (25). However, others have reported that DC stimulated with particulate  $\beta$ -glucans are induced to produce substantial levels of cytokines in a MyD88-independent manner (19, 24). In this study, we sought to clarify the mechanism underlying dectin-1-mediated cellular activation that results in an inflammatory response. Understanding the mode of action of dectin-1 would serve as a model for other non-Toll-like pattern recognition receptors and would also have implications for studies directed toward the therapeutic manipulation of the dectin-1 system, such as clinical administration of  $\beta$ -glucans as immunostimulants.

We have shown that particulate  $\beta$ -1,3-glucans are dectin-1 specific at the cellular level and that myeloid cells (M $\phi$  and DC) are “preprogrammed” as to whether they are capable of responding to particulate  $\beta$ -glucans with cytokine production. This is readily achieved *in vitro* with GM-CSF with bona fide thioglycollate-elicited M $\phi$  and M $\phi$  derived from bone marrow progenitors as well as GM-CSF-derived BMDC. It is likely that the programming of the cells with GM-CSF alters the availability of downstream signaling components of the Syk/CARD9 and NF- $\kappa$ B pathways, rendering the cells permissive to respond in a manner similar to that reported for LPS (44). It is unlikely that this cellular programming effect would be restricted to GM-CSF, and other candidates, such as IFN- $\gamma$ , could also be expected to promote this kind of signaling response. The complex *in vivo* inflammatory setting would likely in many cases ensure activation of M $\phi$  for a robust  $\beta$ -glucan-induced cytokine response. It has recently been shown that



microglia are unable to mount a cytokine response to particulate  $\beta$ -glucans (45), and this is reminiscent of the results we have previously observed with most M $\phi$  populations (22, 25, 26). Reprogramming of specialized subsets of the mononuclear phagocyte system such as these with growth factors and inflammatory cytokines may also convert their potential to respond to  $\beta$ -glucans and other microbial components with important consequences during inflammatory responses.

On an individual cell basis, curdlan particles (aggregates of microparticulate  $\beta$ -glucan) were substantially more potent than  $\beta$ -glucan microparticles at stimulating a cytokine response from M $\phi$  or DC. This was found to be directly caused by the inability of the phagocyte to ingest such large material. It was already known that dectin-1-mediated (TLR2-dependent) cytokine production in response to zymosan can occur at the cell surface without the need for phagocytosis (11). We have demonstrated in this study that frustrated phagocytosis leads to significantly enhanced dectin-1-mediated cell activation. These observations could be of fundamental importance to the way myeloid cells recognize invasive fungal infections *in vivo*, where fungal hyphae have established a network of non-phagocytosable material within the tissues and protracted dectin-1 mediated cellular activation further drives inflammation. It is likely that the cellular activation events that occur during frustrated fungal phagocytosis will not only depend on the nature of the pathogen, its ligand exposure, and susceptibility to phagocytosis, but also on the availability of alternate activation receptors such as dectin-2 (46, 47), which can preferentially recognize hyphal *C. albicans* ligands (48, 49). All of these factors will control the level of the inflammatory response, pathogen containment, and the consequential host tissue damage.

One of our primary objectives was to determine how dectin-1 induced inflammatory responses *in vivo*, having observed defects in an *in vivo* fungal challenge model (27). We used the two pure glucan preparations to establish the mechanism of a dectin-1 inflammatory response in the absence of the additional confounding factors, such as TLR activation, that would be evident with live yeast or complex yeast particle experiments. Although both curdlan and Glu-mp were specific for dectin-1 *in vitro*, this was not the case *in vivo* where only curdlan induced dectin-1-specific inflammation even at high doses.

Given the *in vitro* specificity of Glu-mp for dectin-1, we could speculate that a non-cell-associated inflammatory mechanism such as complement may be involved in the dectin-1-independent response to Glu-mp, as has been recently shown in the case of zymosan (50). Both glucan particles were able to fix complement (data not shown), indicating that this was a valid possibility. The very low surface area:volume ratio of curdlan could theoretically limit complement activation compared with the high available surface area of Glu-mp. In the absence of its primary non-opsonic receptor, the Glu-mp are likely to persist in the fluid phase of the peritoneal cavity longer after injection (as they do *in vitro*), and this could lead to more sustained complement activation in *dectin-1*<sup>-/-</sup> mice than would be seen in wild-type animals. Thus, the inflammatory response in the *dectin-1*<sup>-/-</sup> animals challenged with higher doses of Glu-mp, at least in part, most likely reflects an artifactual inflammatory process brought about by the failed clearance of the particles. This is evidenced by the data (Fig. 5B); at low doses of Glu-mp dectin-1 is required for an inflammatory response when the dose is not sufficiently high to invoke alternative processes. These principles are most likely broadly applicable to the interpretation of *in vivo* pathogen recognition in general and very important for the interpretation of *in vivo* models of disease. Thus, to directly and specifically agonize dectin-1 function *in vivo*, a large non-phagocytosable ligand with relatively low surface area

would be the most potent agonist on a single cell basis. Microparticulate agonists have the advantage that they are broadly applicable and much more readily distributed to higher numbers of phagocytes; but there is also the drawback that excess particulates, particularly in the experimental absence of the primary clearance mechanism, may still drive inflammation.

The accessibility of the peritoneal cavity makes it a useful model of inflammatory responses and has in our studies highlighted the importance of dectin-1 on the resident cells in driving the inflammatory response. The primary sources of dectin-1 in the peritoneal cavity are the resident M $\phi$  and the rarer resident DC populations, as is the case in many peripheral tissues. Critically, we have shown that both populations of cells are capable of rapid response to particulate  $\beta$ -glucan stimulation. Both cell types were heavily reliant on dectin-1 for the response to particulate  $\beta$ -glucans. The predominance of M $\phi$  may suggest that they play a more substantial role in the inflammatory response as defined by the recruitment of inflammatory leukocytes and the production of inflammatory mediators, but this remains to be addressed. These studies also emphasize the importance of a context-dependent understanding of myeloid cell heterogeneity for a physiologically relevant dissection of inflammatory response mechanisms.

In summary, we aimed to define the mechanism by which dectin-1 engagement leads to an inflammatory response and to explain the initial dectin-1-mediated events that govern the antifungal inflammatory response. We have demonstrated that the isolated ligation of dectin-1 on M $\phi$  populations and DC is sufficient to drive a potent proinflammatory cytokine response. After initial "infection," the ligation of dectin-1 results in rapid activation of the tissue-resident M $\phi$  and DC, leading to recruitment of the neutrophils and monocytes that are important for pathogen containment. This myeloid cell activation is deficient in the absence of dectin-1 (this study and Ref. 27), and this is associated with susceptibility to live fungal infection (27). Furthermore, we found that this response to particulate  $\beta$ -glucans is controlled at least at two levels: by the cellular programming of distinct myeloid cell subsets and by the progression through the phagocytic process. Select M $\phi$ , and theoretically specific DC subsets, are programmed to respond to dectin-1 engagement with proinflammatory cytokines, and this most likely proceeds through Syk/CARD9 pathways. Other M $\phi$  populations are not so programmed, and this indicates a mechanism of controlling the M $\phi$  response to fungi *in vivo* in different microenvironments. Those M $\phi$ , which are incapable of mounting a notable cytokine response to particulate  $\beta$ -glucans, can augment TLR-mediated responses (25) but can also be programmed to respond *in vitro* with GM-CSF. These proinflammatory responses are further enhanced by "frustration" of phagocytosis. Thus, by establishing an *in vivo* inflammatory model that was dectin-1 specific (and the parameters that define this), we have elucidated the initial mechanistic role of M $\phi$  dectin-1 in the orchestration of the inflammatory response after infection. These processes are able to act concurrent to and in synergy with TLR-mediated processes and explain how the initiation of an inflammatory response to fungal infection is impaired in the absence of dectin-1.

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## Disclosures

The authors have no financial conflict of interest.

## References

1. Romani, L. 2004. Immunity to fungal infections. *Nat. Rev. Immunol.* 4: 1–23.

2. Netea, M. G., G. D. Brown, B. J. Kullberg, and N. A. Gow. 2008. An integrated model of the recognition of *Candida albicans* by the innate immune system. *Nat. Rev. Microbiol.* 6: 67–78.
3. Di Carlo, F. J., and J. V. Fiore. 1958. On the composition of zymosan. *Science* 127: 756–757.
4. Gantner, B. N., R. M. Simmons, and D. M. Underhill. 2005. Dectin-1 mediates macrophage recognition of *Candida albicans* yeast but not filaments. *EMBO J.* 24: 1277–1286.
5. Chen, J., and R. Seviour. 2007. Medicinal importance of fungal  $\beta$ -(1 $\rightarrow$ 3), (1 $\rightarrow$ 6)-glucans. *Mycol. Res.* 111: 635–652.
6. Ross, G. D., V. Vetvicka, J. Yan, Y. Xia, and J. Vetvickova. 1999. Therapeutic intervention with complement and  $\beta$ -glucan in cancer. *Immunopharmacology* 42: 61–74.
7. Tzianabos, A. O. 2000. Polysaccharide immunomodulators as therapeutic agents: structural aspects and biological function. *Clin. Microbiol. Rev.* 13: 523–533.
8. Ariizumi, K., G. L. Shen, S. Shikano, S. Xu, R. Ritter III, T. Kumamoto, D. Edelbaum, A. Morita, P. R. Bergstresser, and A. Takashima. 2000. Identification of a novel, dendritic cell-associated molecule, dectin-1, by subtractive cDNA cloning. *J. Biol. Chem.* 275: 20157–20167.
9. Brown, G. D., and S. Gordon. 2001. Immune recognition: a new receptor for  $\beta$ -glucans. *Nature* 413: 36–37.
10. Brown, G. D., P. R. Taylor, D. M. Reid, J. A. Willment, D. L. Williams, L. Martinez-Pomares, S. Y. Wong, and S. Gordon. 2002. Dectin-1 is a major  $\beta$ -glucan receptor on macrophages. *J. Exp. Med.* 196: 407–412.
11. Brown, G. D., J. Herre, D. L. Williams, J. A. Willment, A. S. Marshall, and S. Gordon. 2003. Dectin-1 mediates the biological effects of  $\beta$ -glucans. *J. Exp. Med.* 197: 1119–1124.
12. Gersuk, G. M., D. M. Underhill, L. Zhu, and K. A. Marr. 2006. Dectin-1 and TLRs permit macrophages to distinguish between different *Aspergillus fumigatus* cellular states. *J. Immunol.* 176: 3717–3724.
13. Hohl, T. M., H. L. Van Epps, A. Rivera, L. A. Morgan, P. L. Chen, M. Feldmesser, and E. G. Pamer. 2005. *Aspergillus fumigatus* triggers inflammatory responses by stage-specific  $\beta$ -glucan display. *PLoS Pathog.* 1: e30.
14. Steele, C., L. Marrero, S. Swain, A. G. Harmsen, M. Zheng, G. D. Brown, S. Gordon, J. E. Shellito, and J. K. Kolls. 2003. Alveolar macrophage-mediated killing of *Pneumocystis carinii* f. sp. muris involves molecular recognition by the Dectin-1  $\beta$ -glucan receptor. *J. Exp. Med.* 198: 1677–1688.
15. Steele, C., R. R. Rapaka, A. Metz, S. M. Pop, D. L. Williams, S. Gordon, J. K. Kolls, and G. D. Brown. 2005. The  $\beta$ -glucan receptor dectin-1 recognizes specific morphologies of *Aspergillus fumigatus*. *PLoS Pathog.* 1: e42.
16. Viriyakosol, S., J. Fierer, G. D. Brown, and T. N. Kirkland. 2005. Innate immunity to the pathogenic fungus *Coccidioides posadasii* is dependent on Toll-like receptor 2 and Dectin-1. *Infect. Immun.* 73: 1553–1560.
17. Gantner, B. N., R. M. Simmons, S. J. Canavera, S. Akira, and D. M. Underhill. 2003. Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. *J. Exp. Med.* 197: 1107–1117.
18. Rogers, N. C., E. C. Slack, A. D. Edwards, M. A. Nolte, O. Schulz, E. Schweighoffer, D. L. Williams, S. Gordon, V. L. Tybulewicz, G. D. Brown, and C. Reis e Sousa. 2005. Syk-dependent cytokine induction by Dectin-1 reveals a novel pattern recognition pathway for C type lectins. *Immunity* 22: 507–517.
19. LeibundGut-Landmann, S., O. Gross, M. J. Robinson, F. Osorio, E. C. Slack, S. V. Tsoni, E. Schweighoffer, V. Tybulewicz, G. D. Brown, J. Ruland, and C. Reis e Sousa. 2007. Syk- and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17. *Nat. Immunol.* 8: 630–638.
20. Underhill, D. M., E. Rosnagle, C. A. Lowell, and R. M. Simmons. 2005. Dectin-1 activates Syk tyrosine kinase in a dynamic subset of macrophages for reactive oxygen production. *Blood* 106: 2543–2550.
21. Herre, J., A. S. Marshall, E. Caron, A. D. Edwards, D. L. Williams, E. Schweighoffer, V. Tybulewicz, C. Reis e Sousa, S. Gordon, and G. D. Brown. 2004. Dectin-1 uses novel mechanisms for yeast phagocytosis in macrophages. *Blood* 104: 4038–4045.
22. Suram, S., G. D. Brown, M. Ghosh, S. Gordon, R. Loper, P. R. Taylor, S. Akira, S. Uematsu, D. L. Williams, and C. C. Leslie. 2006. Regulation of cytosolic phospholipase A<sub>2</sub> activation and cyclooxygenase 2 expression in macrophages by the  $\beta$ -glucan receptor. *J. Biol. Chem.* 281: 5506–5514.
23. Saijo, S., N. Fujikado, T. Furuta, S. H. Chung, H. Kotaki, K. Seki, K. Sudo, S. Akira, Y. Adachi, N. Ohno, et al. 2007. Dectin-1 is required for host defense against *Pneumocystis carinii* but not against *Candida albicans*. *Nat. Immunol.* 8: 39–46.
24. Yoshitomi, H., N. Sakaguchi, K. Kobayashi, G. D. Brown, T. Tagami, T. Sakihama, K. Hirota, S. Tanaka, T. Nomura, I. Miki, et al. 2005. A role for fungal  $\beta$ -glucans and their receptor Dectin-1 in the induction of autoimmune arthritis in genetically susceptible mice. *J. Exp. Med.* 201: 949–960.
25. Dennehy, K. M., G. Ferwerda, I. Faro-Trindale, E. Pyz, J. A. Willment, P. R. Taylor, A. Kerrigan, S. V. Tsoni, S. Gordon, F. Meyer-Wentrup, et al. 2008. Syk kinase is required for collaborative cytokine production induced through dectin-1 and toll-like receptors. *Eur. J. Immunol.* 38: 500–506.
26. Kikkert, R., I. Bulder, E. R. de Groot, L. A. Aarden, and M. A. Finkelman. 2007. Potentiation of Toll-like receptor-induced cytokine production by (1 $\rightarrow$ 3)- $\beta$ -D-glucans: implications for the monocyte activation test. *J. Endotoxin Res.* 13: 140–149.
27. Taylor, P. R., S. V. Tsoni, J. A. Willment, K. M. Dennehy, M. Rosas, H. Findon, K. Haynes, C. Steele, M. Botto, S. Gordon, and G. D. Brown. 2007. Dectin-1 is required for  $\beta$ -glucan recognition and control of fungal infection. *Nat. Immunol.* 8: 31–38.
28. Ensley, H. E., B. Tobias, H. A. Pretus, R. B. McNamee, E. L. Jones, I. W. Browder, and D. L. Williams. 1994. NMR spectral analysis of a water-insoluble (1 $\rightarrow$ 3)- $\beta$ -D-glucan isolated from *Saccharomyces cerevisiae*. *Carbohydr. Res.* 258: 307–311.
29. Hunter, K. W., Jr., R. A. Gault, and M. D. Berner. 2002. Preparation of micro-particulate  $\beta$ -glucan from *Saccharomyces cerevisiae* for use in immune potentiation. *Lett. Appl. Microbiol.* 35: 267–271.
30. Taylor, P. R., G. D. Brown, D. M. Reid, J. A. Willment, L. Martinez-Pomares, S. Gordon, and S. Y. Wong. 2002. The  $\beta$ -glucan receptor, dectin-1, is predominantly expressed on the surface of cells of the monocyte/macrophage and neutrophil lineages. *J. Immunol.* 169: 3876–3882.
31. Hirsch, S., J. M. Austyn, and S. Gordon. 1981. Expression of the macrophage-specific antigen F4/80 during differentiation of mouse bone marrow cells in culture. *J. Exp. Med.* 154: 713–725.
32. Inaba, K., M. Inaba, N. Romani, H. Aya, M. Deguchi, S. Ikehara, S. Muramatsu, and R. M. Steinman. 1992. Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. *J. Exp. Med.* 176: 1693–1702.
33. Kitamura, T. 1998. New experimental approaches in retrovirus-mediated expression screening. *Int. J. Hematol.* 67: 351–359.
34. Wang, G. G., K. R. Calvo, M. P. Pasillas, D. B. Sykes, H. Hacker, and M. P. Kamps. 2006. Quantitative production of macrophages or neutrophils ex vivo using conditional Hoxb8. *Nat. Methods* 3: 287–293.
35. Tora, L., A. Mullick, D. Metzger, M. Ponglikitmongkol, I. Park, and P. Chambon. 1989. The cloned human oestrogen receptor contains a mutation which alters its hormone binding properties. *EMBO J.* 8: 1981–1986.
36. Taylor, P. R., G. D. Brown, J. Herre, D. L. Williams, J. A. Willment, and S. Gordon. 2004. The role of SIGNR1 and the  $\beta$ -glucan receptor (dectin-1) in the nonopsonic recognition of yeast by specific macrophages. *J. Immunol.* 172: 1157–1162.
37. Heinsbroek, S. E., P. R. Taylor, M. Rosas, J. A. Willment, D. L. Williams, S. Gordon, and G. D. Brown. 2006. Expression of functionally different dectin-1 isoforms by murine macrophages. *J. Immunol.* 176: 5513–5518.
38. Rosas, M., S. Gordon, and P. R. Taylor. 2007. Characterisation of the expression and function of the GM-CSF receptor  $\alpha$ -chain in mice. *Eur. J. Immunol.* 37: 2518–2528.
39. Taylor, P. R., G. D. Brown, A. B. Geldhof, L. Martinez-Pomares, and S. Gordon. 2003. Pattern recognition receptors and differentiation antigens define murine myeloid cell heterogeneity ex vivo. *Eur. J. Immunol.* 33: 2090–2097.
40. Willment, J. A., H. H. Lin, D. M. Reid, P. R. Taylor, D. L. Williams, S. Y. Wong, S. Gordon, and G. D. Brown. 2003. Dectin-1 expression and function are enhanced on alternatively activated and GM-CSF-treated macrophages and are negatively regulated by IL-10, dexamethasone, and lipopolysaccharide. *J. Immunol.* 171: 4569–4573.
41. Henson, P. M. 1971. Interaction of cells with immune complexes: adherence, release of constituents, and tissue injury. *J. Exp. Med.* 134: 114s–135s.
42. Barth, M. W., J. A. Hendrzak, M. J. Melnickoff, and P. S. Morahan. 1995. Review of the macrophage disappearance reaction. *J. Leukocyte Biol.* 57: 361–367.
43. Nelson, D. S., and S. V. Boyden. 1963. The loss of macrophages from peritoneal exudates following the injection of antigens into guinea-pigs with delayed-type hypersensitivity. *Immunology* 6: 264–275.
44. Fleetwood, A. J., T. Lawrence, J. A. Hamilton, and A. D. Cook. 2007. Granulocyte-macrophage colony-stimulating factor (CSF) and macrophage CSF-dependent-macrophage phenotypes display differences in cytokine profiles and transcription factor activities: implications for CSF blockade in inflammation. *J. Immunol.* 178: 5245–5252.
45. Shah, V. B., Y. Huang, R. Keshwara, T. Ozment-Skelton, D. L. Williams, and L. Keshwara. 2008.  $\beta$ -glucan activates microglia without inducing cytokine production in Dectin-1-dependent manner. *J. Immunol.* 180: 2777–2785.
46. Ariizumi, K., G. L. Shen, S. Shikano, R. Ritter III, P. Zukas, D. Edelbaum, A. Morita, and A. Takashima. 2000. Cloning of a second dendritic cell-associated C-type lectin (dectin-2) and its alternatively spliced isoforms. *J. Biol. Chem.* 275: 11957–11963.
47. Taylor, P. R., D. M. Reid, S. E. Heinsbroek, G. D. Brown, S. Gordon, and S. Y. Wong. 2005. Dectin-2 is predominantly myeloid restricted and exhibits unique activation-dependent expression on maturing inflammatory monocytes elicited in vivo. *Eur. J. Immunol.* 35: 2163–2174.
48. McGreal, E. P., M. Rosas, G. D. Brown, S. Zamze, S. Y. Wong, S. Gordon, L. Martinez-Pomares, and P. R. Taylor. 2006. The carbohydrate-recognition domain of Dectin-2 is a C-type lectin with specificity for high mannose. *Glycobiology* 16: 422–430.
49. Sato, K., X. L. Yang, T. Yudate, J. S. Chung, J. Wu, K. Luby-Phelps, R. P. Kimberly, D. Underhill, P. D. Cruz, Jr., and K. Ariizumi. 2006. Dectin-2 is a pattern recognition receptor for fungi that couples with the Fc receptor  $\gamma$  chain to induce innate immune responses. *J. Biol. Chem.* 281: 38854–38866.
50. Mullaly, S. C., and P. Kubes. 2007. Mast cell-expressed complement receptor, not TLR2, is the main detector of zymosan in peritonitis. *Eur. J. Immunol.* 37: 224–234.