

Fungal zymosan induces leukotriene production by human mast cells through a dectin-1-dependent mechanism

Timothy J. Olynych, PhD,^a David L. Jakeman, PhD,^c and Jean S. Marshall, PhD^{a,b}

Halifax, Nova Scotia, Canada

Background: Fungi are capable of causing or exacerbating inflammatory disease in the airways. We have previously demonstrated that zymosan and peptidoglycan induce the production of cysteinyl leukotrienes from human mast cells. However, the mechanisms of pathogen-induced leukotriene production by immune effector cells are very poorly understood. The coreceptor dectin-1, through a Syk tyrosine kinase-dependent pathway, can mediate some responses to fungal challenge, but its expression by mast cells and its involvement in lipid mediator responses have not been assessed. **Objective:** In the current study, the potential role of dectin-1 in zymosan-induced leukotriene production from human mast cells was examined.

Methods: Human mast cells were examined for dectin-1 mRNA and protein expression. Human mast cells were incubated with either zymosan or peptidoglycan in the presence or absence of specific inhibitors for dectin-1 or Syk tyrosine kinase and mediator production examined.

Results: Human mast cells were found to express a functional isoform of dectin-1. Both peptidoglycan and zymosan induced significant amounts of leukotriene (LT)-B₄ and LTC₄. The dectin-1 inhibitors laminarin and glucan phosphate reduced the LTC₄ response to zymosan by more than 60% but did not alter peptidoglycan responses. Inhibitors of Syk tyrosine kinase activity significantly decreased LTC₄ production in response to both peptidoglycan and zymosan.

Conclusion: These data demonstrate mast cell expression of the coreceptor dectin-1 and a role for this molecule in the generation of cysteinyl leukotrienes.

Clinical implications: These findings suggest new approaches to the selective inhibition of lipid mediator production in response to fungal infection or exposure. (J Allergy Clin Immunol

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Key words: Fungal infection, mast cells, lipid mediators, GM-CSF, IL-1 β , allergy, bronchoconstriction

The mechanisms whereby fungal organisms such as *Aspergillus fumigatus* alter lung function and lead to features such as reversible bronchoconstriction are complex.¹ Pathogen-induced leukotriene (LT) production in the airways could contribute to asthma initiation or exacerbations associated with these or other infections. Fungal products from certain occupational environments may also induce local leukotriene generation.² The cysteinyl leukotrienes have potent bronchoconstrictive effects, cause edema through vasodilation and increased vascular permeability, and enhance the recruitment of effector cells.³⁻⁵ LTB₄ has been shown to be important for neutrophil recruitment to the site of infection and also assists in CD8⁺ T-cell recruitment.⁶⁻⁸

Mast cells are now known to be critical effectors of both allergic disease and host defense. Activation of mast cells results in selective and specific release of preformed granule-associated mediators, lipid-derived mediators such as LTC₄ and prostaglandin (PG) D₂, and many potent cytokines and chemokines. Mast cell-deficient mice have decreased survival after bacterial infection.⁹ Mast cell-derived TNF and leukotrienes are critical for effector cell recruitment and host survival.⁹⁻¹¹ Several pathogens have been shown to exacerbate the symptoms of allergic asthma or are associated with chronic allergic disease.^{12,13} Recently, we described the ability of human mast cells to produce the potent bronchoconstrictor, LTC₄, in response to several pathogen products that are known to activate Toll-like receptors (TLRs).¹⁴

The mechanisms by which pathogen products induce leukotriene production from immune effector cells are unclear. The importance of TLR for mast cell responses to several pathogen products including LPS, peptidoglycan, zymosan, and Pam₃CysSerLys₄ has been clearly demonstrated.¹⁵⁻¹⁷ Both peptidoglycan and zymosan have been shown to signal through a TLR2/6 heterodimer¹⁸ leading to the activation of the mitogen-activated protein kinase pathway and the translocation of nuclear factor- κ B (NF- κ B) to the nucleus. However, lipid mediator production normally requires activation of calcium-dependent pathways¹⁹ that are not known to be associated with TLR signaling. TLR signaling usually occurs through large multimeric receptor complexes that may involve several coreceptors and adaptor molecules.

From ^athe Departments of Pathology and ^bMicrobiology and Immunology, Dalhousie Inflammation Group; and ^cthe College of Pharmacy, Dalhousie University.

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Reprint requests: Jean S. Marshall, PhD, Department of Microbiology and Immunology, Sir Charles Tupper Medical Building, College St, Dalhousie University, Halifax, Nova Scotia, Canada, B3H1X5. E-mail: jean.marshall@dal.ca.

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Abbreviations used

CBMC:	Cord blood–derived mast cell
LT:	Leukotriene
ITAM:	Immunoreceptor tyrosine activation motif
NF- κ B:	Nuclear factor- κ B
PG:	Prostaglandin
SCF:	Stem cell factor
Syk:	Spleen tyrosine kinase
TLR:	Toll-like receptor

Dectin-1 is a lectinlike receptor, expression of which was initially thought to be restricted to dendritic cells, but it has since been shown to be expressed on monocytes, macrophages, and neutrophils.^{20,21} It is found in several isoforms, only 2 of which (A and B) have been demonstrated to have functional activity.²² Work by Brown and Gordon²⁰ and Brown et al^{23,24} demonstrated that dectin-1 is the receptor on macrophages that allows them to bind the yeast cell wall component zymosan. Further work by Gantner et al²⁵ and Brown et al²³ demonstrated that the binding of zymosan to dectin-1 modulated cytokine production²⁶ and phagocytosis and is responsible for zymosan-induced reactive oxygen species. More recently, it has been described to have a role in lipid mediator production in macrophages.²⁷ Dectin-1 has also been demonstrated to contribute to murine cytokine responses to conidia of *A. fumigatus*.²⁸

Dectin-1 possesses an intracellular immunoreceptor tyrosine activation motif (ITAM)–like motif which suggests signaling capabilities including the elevation of intracellular calcium. Spleen tyrosine kinase (Syk) is known to associate with ITAM motifs in other receptor systems. Its association with phospholipase C γ can lead to the release of intracellular calcium stores.²⁹ Rogers et al²⁶ have demonstrated that dectin-1 can lead to the activation of Syk and that Syk mediates certain cytokine responses to zymosan in murine dendritic cells. Although dectin-1 can signal independently, it is known to function as a coreceptor for TLR2, with both dectin-1 and TLR-mediated signals contributing to the effective cytokine response to pathogen challenge.^{23,25,26}

In this study, human mast cell expression of dectin-1 and leukotriene production in response to zymosan is examined as a model of fungal-induced leukotriene generation. A role for both dectin-1 and Syk in zymosan-induced LTC₄ production is demonstrated. In contrast, dectin-1 does not significantly mediate zymosan-induced production of the cytokines GM-CSF and IL-1 β from human mast cells or LTC₄ generation in response to bacterial peptidoglycan.

METHODS

Reagents

Yeast zymosan from *Saccharomyces cerevisiae*, *Staphylococcus aureus*–derived peptidoglycan, and laminarin were purchased from Sigma (St Louis, Mo) and contained <0.0025 ng/ μ L endotoxin as assessed by using the Limulus amoebocyte lysate test. Glucan

phosphate was prepared as previously described.³⁰ Briefly, barley derived β -glucan (Sigma) was treated with dimethyl sulfoxide, urea, and phosphoric acid, boiled for 6 hours, filtered, dialyzed, shell-frozen, and lyophilized. The β -glucan phosphate purity was comparable with that described previously,²⁵ as observed in the proton-decoupled ¹³C and ³¹P solution-state nuclear magnetic resonance spectra recorded on a Bruker DMX 500 MHz spectrometer (Bruker, Billerica, Mass), in deuterated dimethyl sulfoxide. Syk inhibitor (catalog #574711) and Piceatannol were purchased from Calbiochem (San Diego, Calif). Piceatannol, as described by the manufacturer, inhibits rat liver protein kinase A catalytic subunit, Protein Kinase C (PKC), and myosin light-chain kinase at similar concentrations to those used in the current study. Syk inhibitor is a oxindole derivative that has demonstrated potent inhibition of Syk tyrosine kinase.³¹ It has not been described to inhibit other kinases at the concentrations we used. Antihuman CD48 blocking antibody was purchased from Neomarkers (Freemont, Calif).

Mast cells

Cord blood–derived mast cells (CBMCs) were prepared by long-term culture of human cord blood progenitor cells as previously described,³² using a modification of the method described by Saito et al.³³ Cord blood samples were obtained from the umbilical cords obtained after elective caesarian sections after protocol approval by the ethics review board of the Izaak Walton Killam-Grace Health Center. Mononuclear cells isolated from cord blood were cultured in RPMI 1640 supplemented with supernatant from CCL-204 cells American Type Culture Collection [ATCC] as a source of IL-6, PGE₂ (10^{−6} mol/L), 15% fetal calf serum (FCS), stem cell factor (SCF, 100 ng/mL; Peprotech, Ottawa, Ontario, Canada), and penicillin/streptomycin (Life Technologies, Burlington, Ontario, Canada). Medium was changed once weekly. After 5 to 8 weeks of culture, the purity of mast cells was determined morphologically and by the presence of metachromatic granules (toluidine blue staining). Only >95% pure mast cell preparations were used. Mast cells were placed in mast cell growth medium devoid of PGE₂ and containing 10 ng/mL SCF for >16 hours before use.

RT-PCR

Primers used for PCR amplification of RT RNA samples for human dectin-1 were as follows: (1) sense, 5'-ATACTGGTGATAGCTGTGGTCCTG-3'; (2) antisense 5'-ATCAGGTTGGGAAGACACTTG-3'. These primers amplify the A, B, and H isoforms of dectin-1, which could be differentiated by product size. cDNA was subjected to RT-PCR with advantage enzyme (Clontech, Mississauga, Ontario, Canada) for 35 cycles. The identity of the PCR products was confirmed by direct sequencing.

Mast cell activation

Mast cells were washed and resuspended at a final concentration of 1 $\times$ 10⁶ cells/mL in RPMI containing 10% FCS and, for 24-hour activations, 1 ng/mL SCF and 100 μ g/mL soybean trypsin inhibitor. The cells were treated with media alone, 0.5 μ mol/L calcium ionophore (A23187), 50 μ g/mL peptidoglycan, or 100 μ g/mL zymosan for 20 minutes (leukotriene production) or 24 hours (cytokine production) at 37°C. Cell free supernatants were obtained by centrifugation and assayed for mediator content.

Cytokine analysis

IL-1 β and GM-CSF production was examined from cell free supernatants harvested after 24-hour incubation of the CBMC with TLR2 activators or controls at 37°C. When using an inhibitor or blocking antibody, mast cells were pretreated for 30 minutes at 4°C. The cytokine content of supernatants was measured via ELISA as

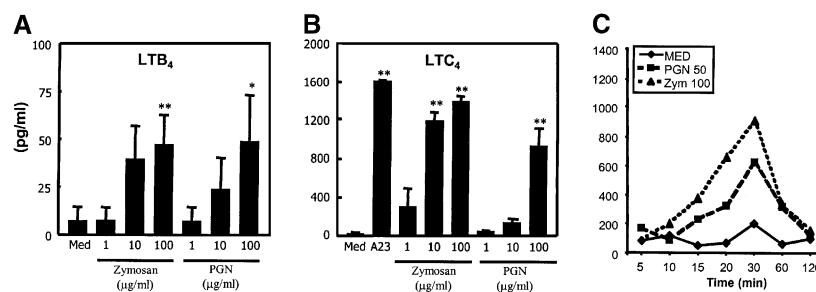


FIG 1. Leukotriene response to zymosan and peptidoglycan. Treatment of CBMCs with zymosan (Zym; $\mu\text{g}/\text{mL}$), peptidoglycan (PGN; $\mu\text{g}/\text{mL}$), or A23187 (A23; 1×10^{-7} mol/L) as indicated. Induction of **A**, LTB₄ ($n = 6$). **B**, LTC₄ ($n = 5$). * $P < .05$; ** $P < .01$. **C**, LTC₄ production from CBMCs treated with media (MED), zymosan 100 $\mu\text{g}/\text{mL}$, or peptidoglycan 50 $\mu\text{g}/\text{mL}$ for indicated times (representative of 2 independent experiments).

previously described.³² Sensitivities of the IL-1 β and GM-CSF were <20 pg/mL and <10 pg/mL, respectively. None of the activating agents used significantly interfered with the ELISA assays.

Leukotriene assessment

Cord blood–derived mast cells ($1 \times 10^6/\text{mL}$) were incubated for a predetermined optimal time of 20 minutes at 37°C with activating agents or controls. Supernatants were harvested by centrifugation, snap-frozen, and later analyzed for LTC₄ using an ELISA, as previously described by Volland et al,³⁴ using the 6E7 clone antibody (Neomarkers, Fremont, Calif). LTC₄ is quickly broken down by γ -glutamyl transpeptidase to LTD₄ and by dipeptidases to LTE₄.³⁵ Leukotrienes are also notoriously unstable in oxygen and in exposure to direct light. Therefore, our samples were frozen immediately in colored Eppendorf tubes to prevent degradation. The sensitivity of the LTC₄ assay was <30 pg/mL. LTB₄ was measured from the same supernatants using a commercial kit from Amersham Biosciences (Piscataway, NJ) or Cayman Chemicals (Ann Arbor, Mich). The sensitivities of the 2 LTB₄ assays were <30 pg/mL and <10 pg/mL, respectively.

Western blot analysis

Resting CBMC and differentiated KU-812 protein lysates were analyzed for the presence of dectin-1. Briefly, KU-812 cells were differentiated by culture with sodium butyrate (0.3 mmol/L) for 3 days or IL-4 (20 ng/mL) for 24 hours. These treatments have been shown to induce the differentiation of KU-812 cells to express more mature mast cell–like characteristics such as enhanced mast cell tryptase expression.³⁶ Resting CBMC, KU-812, and differentiated KU-812 were homogenized in lysis buffer (150 mol/L NaCl, 20 mmol/L Tris(hydroxymethyl)aminomethane (TRIS) pH 7.4, 1 mmol/L ethylenediaminetetra acetic acid [EDTA], 1 mmol/L EGTA, 1% Triton X-100, 100 mmol/L sodium orthovanadate, 5 mg/mL leupeptin, 5 mg/mL pepstatin A, 25 mg/mL aprotinin, and 1 mmol/L phenylmethylsulfonyl fluoride [PMSF]). The protein supernatants were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were then transferred to nitrocellulose and probed with antihuman dectin-1 (R&D Systems, Minneapolis, Minn) or anti-total p38 (Cell Signaling Technologies, Beverly, Mass) as loading control. Antibody binding was detected by using the Visualizer development kit (Upstate Cell Signaling Solutions, Lake Placid, NY).

RESULTS

Mast cell LTC₄ and LTB₄ production in response to zymosan

Cord blood–derived mast cells were treated with either zymosan or peptidoglycan for 20 minutes, and the release

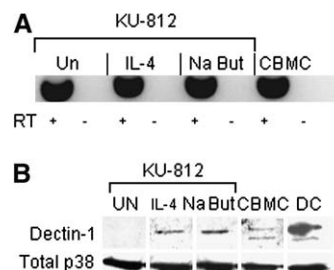


FIG 2. Dectin-1 is expressed on KU-812 and CBMC. **A**, RNA derived from KU-812 (untreated [un], IL-4 or sodium butyrate [NaBut] treated), CBMC, and dendritic cells (DC) was subjected to PCR, with and without (+/-) RT, with primers designed to recognize the A and B isoforms. Sequencing confirmed dectin-1 RNA expression. **B**, Dectin-1 protein expression by Western blot using human monocyte–derived dendritic cells as control.

of LTB₄ was assessed via ELISA. Both zymosan and peptidoglycan were capable of inducing significant levels of LTB₄ and LTC₄; however, the levels of LTB₄ were substantially less than the levels of LTC₄ produced under the same conditions (Fig 1, A and B). The time course of LTC₄ production was found to be similar to that described for IgE-mediated mast cell activation,³⁷ with the majority of the LTC₄ response occurring within 20 minutes (Fig 1, C).

Human mast cells express dectin-1

The expression of functional isoforms of dectin-1 by human mast cells was initially assessed via RT-PCR. Primers were designed specifically to amplify the 2 functional β -glucan receptor isoforms, A and B, as well as isoform H. On the basis of predicted product size, we observed RNA for the functional B isoform in CBMC and KU-812 (Fig 2, A). This was confirmed by using direct sequencing of the PCR product obtained, which demonstrated complete identity to the published B-isoform sequence (GenBank accession number, AF400596). Using commercially available polyclonal antibodies (R&D Systems), the expression of dectin-1 by human mast cells and differentiated KU-812, but not untreated KU-812 cells, was confirmed at the protein level using Western blot analysis (Fig 2, B). A lower band of 28-kD molecular weight was observed in CBMC and dendritic cell lysates,

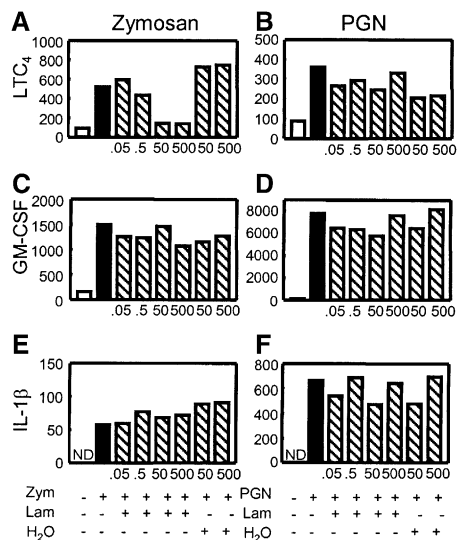


FIG 3. Effect of laminarin on CBMC mediator responses. CBMCs were pretreated with laminarin (*Lam*; $\mu\text{g/mL}$ as indicated) or diluent control for 30 minutes before the addition of zymosan (*Zym*; $\mu\text{g/mL}$; **A**, **C**, and **E**) or peptidoglycan (*PGN*; $\mu\text{g/mL}$; **B**, **D**, and **F**) for 20 minutes (LTC_4) or 24 hours (cytokines). Representative data from 1 of 5 independent experiments.

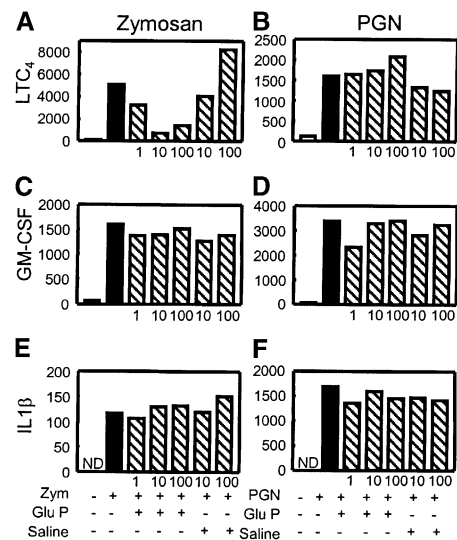


FIG 4. Effect of glucan phosphate on CBMC mediator responses. CBMCs were pretreated with glucan phosphate ($\mu\text{g/mL}$ as indicated) or diluent control for 30 minutes before the addition of either zymosan (*Zym*; $\mu\text{g/mL}$; **A**, **C**, and **E**) or peptidoglycan (*PGN*; $\mu\text{g/mL}$; **B**, **D**, and **F**) for 20 minutes (LTC_4) or 24 hours (cytokines). Representative data from 1 of 5 independent experiments.

TABLE I. Selective inhibition of LTC_4 responses to zymosan via dectin-1 blockade

	LTC_4		GM-CSF		IL-1 β		Number of experiments
	PGN	Zym	PGN	Zym	PGN	Zym	
Glu P 10	-22(50) [†]	65(17)*	4(10)	-10(10)	-14(16)	18(10)	4-5
Lam 50	3(12)	68(12)**	-30(26)	16(13)	6(8)	-38(69)	4-7
Lam 500	15(11)	64(14)*	-24(22)	46(8)	-15(12)	-6(25)	4-7

Glu P, Glucan phosphate; Lam, laminarin.

* $P < .05$; ** $P < .01$.

[†]Data are presented as % inhibition compared with zymosan (Zym)-treated or peptidoglycan (PGN)-treated cells (SEM). Negative values indicate that inhibitor treatment resulted in higher levels of production on average.

which likely represents the B isoform of dectin-1, whereas the larger band is consistent with the larger stalk region containing A isoform. Notably, mast cells demonstrated a much lower level of dectin-1 expression than human monocyte-derived dendritic cells.

LTC_4 production in response to zymosan is inhibited by dectin-1 blockade

In view of the significant amounts of LTC_4 produced after zymosan treatment of human mast cells, the mechanism of this response was investigated by using the well defined dectin-1 inhibitors laminarin and glucan phosphate.^{20,23,24} The role of dectin-1 in the longer-term production of cytokines IL-1 β and GM-CSF was also assessed. Pretreatment of CBMC with laminarin for 30 minutes, before the addition of zymosan, significantly decreased the LTC_4 response of zymosan-treated mast cells (Fig 3, *A* and *B*). Interestingly, laminarin treatment had no significant effect on the production of either GM-CSF or IL-1 β in response to zymosan treatment (Fig 3, *C* and *E*) or on either lipid mediator or cytokine responses to

peptidoglycan treatment (Fig 3, *D* and *F*). Glucan phosphate, an alternate inhibitor of dectin-1, also significantly and selectively inhibited zymosan induced LTC_4 responses (Fig 4, *A-F*). Table I shows the mean percentage inhibition of LTC_4 and cytokine production for both laminarin and glucan phosphate-pretreated mast cells in a series of experiments using cells obtained from 4 to 7 independent donors. Notably, there was a very consistent and selective inhibition of LTC_4 production by both glucan phosphate and laminarin in zymosan-treated mast cells from all subjects. The extent to which cytokine responses were altered by dectin-1 blockade was highly variable between subjects and did not reach statistical significance. Mean mediator production in the absence of glucan phosphate or laminarin treatment (mean $\pm$ SEM in pg/mL) were as follows: LTC_4 with peptidoglycan activation, 1165 ± 611 ; zymosan activation, 5224 ± 1332 ; GM-CSF with peptidoglycan activation, 3606 ± 879 ; zymosan activation, 1783 ± 492 ; IL-1 β with peptidoglycan activation, 789 ± 143 ; and zymosan activation, 163 ± 41 .

In similar experiments, the ability of laminarin to inhibit the relatively low levels of LTB₄ production by zymosan activated mast cells was examined. Laminarin blockade at concentrations of as much as 500 μ g/mL did not consistently inhibit LTB₄ production (data not shown).

Syk tyrosine kinase mediates LTC₄ production to zymosan and peptidoglycan

Syk tyrosine kinase is known to associate with ITAMs in other receptor systems and can mediate downstream effects such as intracellular calcium release. Therefore, the importance of Syk in zymosan and peptidoglycan-induced LTC₄ production was assessed. CBMCs were pretreated with Syk inhibitor or Piceatannol for 30 minutes at 4°C before the addition of either zymosan or peptidoglycan. Either Syk inhibitor or Piceatannol dose-dependently decreased the amount of LTC₄ produced in response to zymosan treatment for 20 minutes in human mast cells (Fig 5, *A and C*, respectively). Inhibition of Syk with either Syk inhibitor or Piceatannol also inhibited LTC₄ production from human mast cells in response to peptidoglycan (Fig 5, *B and D*). Levels of LTC₄ produced in the absence of Syk inhibition were (pg/mL $\pm$ SEM) (*A*) 1073 $\pm$ 356, (*B*) 250 $\pm$ 111, (*C*) 1050 $\pm$ 93, and (*D*) 692 $\pm$ 256, respectively. These data suggest that Syk is critical for the dectin-1-mediated production of LTC₄ from human mast cells in response to zymosan and may also play a role in LTC₄ responses to peptidoglycan. In other systems, Syk has been demonstrated to be critical for the activation of phospholipase C γ and generation of an intracellular calcium signal.²⁹

DISCUSSION

Human mast cells have previously been demonstrated to respond to the TLR2 activators peptidoglycan and zymosan with the release of LTC₄.¹⁴ Other TLR activators share this effect.³⁸ In the current study, the production of significant levels of LTB₄ in response to these TLR2 activators is also observed. Dectin-1 was found to be expressed on human mast cells, and a dectin-1 like coreceptor was found to be critical for the production of LTC₄ in response to zymosan. Interestingly, dectin-1 inhibition did not significantly inhibit IL-1 β or GM-CSF production.

Dectin-1 expression on mast cells is in keeping with the critical role of these resident tissue cells as sentinels in host defense, particularly at mucosal surfaces. The much lower expression of dectin-1 on mast cells than on dendritic cells may have contributed to the expression of this molecule on mast cells not being previously recognized. In this study, we demonstrate leukotriene production in response to the yeast cell wall component zymosan; however, fungal products from more pathogenic fungi may be able to elicit bronchoactive effects in the lung through a similar mechanism. Indeed, *A fumigatus* infection has been associated with allergic bronchopulmonary aspergillosis and is found in as many as 57% of cystic fibrosis cases.³⁹ Rodent

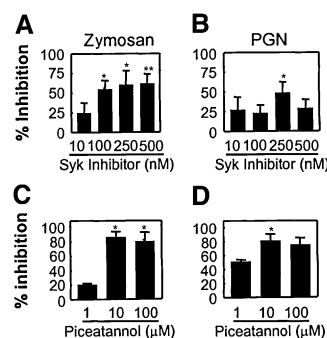


FIG 5. Effect of Syk inhibition on LTC₄ production. CBMCs were pretreated with Syk inhibitor (nmol/L; *A* and *B*) or Piceatannol (mmol/L; *C* and *D*) for 30 minutes, then with zymosan (*A* and *C*) or peptidoglycan (PGN; *B* and *D*). Percent inhibition data vs diluent control ($\pm$ SEM). ($n = 5$). * $P < .05$; ** $P < .01$.

studies suggest that dectin-1 plays an important role in responses to this organism.²⁸ Dectin-1 has also been shown to bind and mediate phagocytosis of the opportunistic fungal pathogen *Candida albicans*.²⁰ More studies are needed to address the clinical significance of dectin-1 mediated responses to investigate the potential of dectin-1 blockade in the treatment of fungi-associated airways inflammation.

It is notable that both LTB₄ and LTC₄ production occurred under activation conditions that do not lead to significant mast cell degranulation.¹⁴ The ability of human mast cells to produce large amounts of leukotrienes in response to zymosan suggests potential for an antibody-independent mechanism of LTB₄ and LTC₄ generation, which could play a role in the development of acute airways symptoms after environmental or occupational exposure to certain fungi.

Both zymosan and peptidoglycan are known to use TLRs, usually a TLR2/TLR6 heterodimer for signaling.¹⁸ However, current models of TLR2/TLR6 signaling do not provide a clear mechanism whereby this receptor system would generate the elevation of intracellular calcium levels usually considered necessary for 5-lipoxygenase (5-LO) translocation to the nuclear envelope and subsequent leukotriene generation.^{40,41} Because dectin-1 has an intracellular ITAM motif and potential to induce a calcium signal, it was an excellent candidate coreceptor for mediating cysteinyl leukotriene generation in this system. Syk kinase is known to associate with the ITAMs of T-cell and B-cell receptors. The role of this kinase in the LTC₄ response to zymosan and peptidoglycan was investigated. Inhibition of Syk, using 2 independent inhibitors, significantly and dose-dependently decreased the amount of LTC₄ production in response to zymosan and peptidoglycan. In other cell systems, Syk has been repeatedly demonstrated to be critical for the activation of phospholipase C γ leading to the generation of an intracellular calcium signal.²⁹

Laminarin and glucan phosphate are structurally closely related molecules that demonstrate a remarkable degree of specificity for dectin-1. These molecules do not

block the activity of the mannose receptor or the complement receptor 3 (CR3) receptor²⁴ and have been reliably used to confirm the role of dectin-1 in other systems.^{23,25} Blocking antibodies are not available in human beings for dectin-1. Using laminarin and glucan phosphate, mast cell production of LTC₄ in response to zymosan but not peptidoglycan was demonstrated to be dependent on dectin-1-like function. However, the role of dectin-1 in the production of LTB₄ remains uncertain. The levels of LTB₄ produced in a series of 3 experiments were relatively low, making assessment of inhibition by laminarin difficult. These data also suggest that, in general, dectin-1 function is not critical for the production of GM-CSF or IL-1 β from mast cells. This is consistent with studies in other cell types that suggest TLR can activate NF- κ B and induce the production of these cytokines independent of dectin-1 activity.²⁵ In some individual subjects, inhibition of GM-CSF and IL-1 responses was observed. This may reflect cooperativity between dectin-1 and TLR signaling pathways in the activation of NF- κ B.²⁵

Overall, these studies demonstrate a novel role for a dectin-1-like coreceptor in the generation of LTC₄ by human mast cells via a Syk tyrosine kinase-dependent mechanism. It is likely that similar mechanisms for lipid mediator production may exist in other dectin-1-expressing cells such as monocytes and dendritic cells, although this remains to be determined experimentally. Understanding the role of lectinlike receptors such as dectin-1 in lipid mediator production may provide important new opportunities to modulate innate immune and inflammatory responses to pathogens and modify inappropriate responses to environmental or occupational exposure to a variety of microorganisms.

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