Dectin-2 mediates Th2 immunity through the generation of cysteiny1 leukotrienes

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The innate signaling pathways for Th2 immunity activated by inhaled antigens are not well defined. We previously identified Dectin-2 as a receptor for glycans in allergen extracts from the house dust mite Dermatophagoides farinae (Df) that mediates cysteiny1 leukotriene (cys-LT) generation from pulmonary CD11c+ cells and from GM-CSF–cultured bone marrow cells (BMCGM-CSF). Using lentiviral knockdown of Dectin-2 in BMCGM-CSF and adoptive transfer of Df–pulsed BMCGM-CSF to sensitize naive mice, we now report that Dectin-2 is critical for the development of Df–elicited eosinophilic and neutrophilic pulmonary inflammation and Th2 cytokine generation in the lungs and restimulated lymph nodes. Sensitization with Df–pulsed BMCGM-CSF from Ltc4 synthase (Ltc4;S)–deficient mice or type 1 cys-LT receptor (CysLT1;R)–deficient mice demonstrated that both proteins were required for Df–elicited eosinophilic pulmonary inflammation and Th2 cytokine generation in the lungs and restimulated lymph nodes. Direct sensitization and challenge of Ltc4s−/− and Cysltr1−/− mice confirmed that cys-LTs mediate these parameters of Df–elicited Th2 pulmonary inflammation. Thus, the Dectin-2–cys-LT pathway is critical for the induction of Th2 immunity to a major allergen, in part through CysLT1;R. These findings identify a previously unrecognized link between a myeloid C-type lectin receptor and Th2 immunity.

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Dectin-2 is a member of the myeloid CLR family that couples to the FeRγ chain to initiate ITAM (immunoreceptor tyrosine-based activation motif)-dependent signaling and the recruitment and activation of spleen tyrosine kinase (Syk; Ariizumi et al., 2000; Sato et al., 2006). The Dectin-2–Syk pathway activates the CARD9 adaptor protein in DCs to generate NF-κB–dependent IL-2, IL-10, TNF, and IL-23p19 and to promote Th17 immune responses to C. albicans (Robinson et al., 2009). We have previously demonstrated that the Dectin-2–Syk pathway also triggers the generation of cysteinyl leukotrienes (cys-LTs) from pulmonary CD11c+ cells and from GM-CSF–cultured BM cells (BMCsGM-CSF) in response to glycans in allergen extracts from the house dust mite (HDM) species Dermatophagoides farinae (Df) and Dermatophagoides pteronyssinus (Dp; Barrett et al., 2009), the most common aeroallergens to elicit sensitization in humans and potent triggers of Th2- and Th17-dependent bronchial inflammation in mice (Cates et al., 2004; Krishnamoorthy et al., 2008; Hanmad et al., 2009; Phipps et al., 2009). Thus, we hypothesized that Dectin-2 is a PRR coupled to the generation of Th2 immune responses via cys-LT signaling.
CD11c+ DCs in the bronchial mucosa sample inhaled antigens and then migrate to the lung-draining lymph nodes while simultaneously activating a maturation program that results in antigen-driven T cell division. This sensitization can be mimicked by the intratracheal or intranasal adoptive transfer of antigen-pulsed myeloid DCs to naive recipient mice and subsequent antigen challenge (Lambrecht et al., 2000a,b,c). We took advantage of this adoptive transfer system and the ability to knock down Dectin-2 in BMCsGM-CSF to examine the role of the Dectin-2–cys-LT pathway in Df-induced Th2 and Th17 pulmonary inflammation. In this paper, we demonstrate that Dectin-2 mediates Df-elicted generation of cys-LTs and cytokines, including TNF, IL-6, IL-10, and IL-23, using lentiviral knockdown of Dectin-2 in BMCsGM-CSF (kdBMCsGM-CSF). Moreover, WT mice sensitized with Df-pulsed kdBMCsGM-CSF and challenged intranasally with Df had significantly reduced pulmonary inflammation and Th2 cytokine generation from lung and restimulated parabronchial lymph nodes as compared with mice sensitized with Df-pulsed BMCsGM-CSF infected with a vector control (cBMCsGM-CSF), thereby identifying Dectin-2 as a critical PRR for Th2 immunity elicited by HDM. Furthermore, we show that the genetic absence of either LTC3 synthase (LTCS3), the critical enzyme in cys-LT generation, or the type 1 cys-LT receptor (CysLT1R) on Df-pulsed BMCsGM-CSF similarly impairs Df-induced Th2 pulmonary inflammation in lung and lymph nodes, thereby demonstrating that cys-LT production and CysLT1R-mediated function promote Th2 sensitization.

**RESULTS**

Dectin-2 mediates Df-elicted production of IL-6, IL-10, IL-23, and TNF by BMCsGM-CSF

Before assessing the role of Dectin-2 in Df-initiated cytokine responses in vitro and in vivo using GM-CSF–expanded BM cultures, we further characterized the BMCsGM-CSF from WT BALB/c mice. At day 7, BMCsGM-CSF were 74.9 ± 0.7% CD11c+ by flow cytometry. The CD11c+ cell population expressed CD11b (97.7 ± 0.4%) and MHCII (88.5 ± 0.2%; Fig. S1 A and not depicted). Although 89 ± 3.6% of CD11c+ cells expressed Dectin-2, the range of expression was broad with CD11c+MHCII+ cells expressing the highest levels (Fig. S1 A). 92.4 ± 0.2% of Dectin-2+ cells were CD11c+ (Fig. S1 B). 7.6 ± 0.2% of Dectin-2+ cells were CD11c− and expressed CD11b and the monocyte marker Ly6C (Fig. S1 B). Analysis of stained cytospins revealed that 12.4 ± 0.5% of Dectin-2+ cells were CD11c+ MHCIIlow cells expressing high levels of Dectin-2 (Fig. S1 C).

As we assessed the role of Dectin-2 in Df-induced cytokine production, we infected day-1 BMCsGM-CSF with lentivirus expressing a short hairpin (sh) RNA for Dectin-2 (kdBMCsGM-CSF), a nontargeting sequence (nontargeting control BMCsGM-CSF [ntcBMCsGM-CSF]), or an empty vector alone (cBMCsGM-CSF). After puromycin selection of infected cells, kdBMCsGM-CSF demonstrated a significant 43.9% reduction in Dectin-2 expression by flow cytometry, as compared with cBMCsGM-CSF (Fig. 1 A, left). A CD11c+MHCII+ subgroup, which was 5 ± 0.8% of total cells, expressed high levels of Dectin-2 in control vector BMCGM-CSF cultures and demonstrated a significant 73.6% reduction in Dectin-2 expression in knockdown BMCGM-CSF cultures (Fig. 1 A, left). Dectin-2 expression on all BMCGM-CSF- and on CD11cMHCII+ cells from ntcBMCsGM-CSF was comparable to cBMCsGM-CSF and distinct from the reduction observed in kdBMCsGM-CSF (Fig. 1 A, right). Day-7 cBMCsGM-CSF, ntcBMCsGM-CSF, and kdBMCsGM-CSF showed similar expression of CD11c and MHCII (Fig. 1 B). The knockdown was confirmed by bioassay, in which kdBMCsGM-CSF demonstrated a 79.6% reduction in cys-LT generation after stimulation for 1 h with 100 µg/ml Df (Fig. 1 C). In parallel, we stimulated cells at 0, 10, or 50 µg/ml Df for 4 or 30 h. At 4 h, kdBMCsGM-CSF stimulated with Df had significant reductions in the generation of IL-6, IL-10, IL-23, and TNF, as compared with cBMCsGM-CSF (Fig. 1 C). There was no reduction in IL-2. At 30 h, kdBMCsGM-CSF stimulated with Df had significant reductions in the generation of IL-6, IL-10, IL-23, and TNF, as compared with cBMCsGM-CSF and a lower level of IL-23 that did not reach statistical significance (Fig. S2 A). kdBMCsGM-CSF showed no reduction in TNF generation in response to LPS or to curdian (Fig. S2 B) and no reduction in cys-LT generation in response to curdian (Fig. S2 B), indicating the functional integrity of TLR4 and Dectin-1.

**Figure 2.** BMCsGM-CSF Dectin-2 is required for Df-elicted Th2 pulmonary inflammation. WT mice were sensitized by intranasal administration of 10^6 saline-pulsed or Df-pulsed kdBMCsGM-CSF or cBMCsGM-CSF at day 8 of culture, challenged with 2 µg Df on day 22 and day 23, and analyzed on day 25. (A) Cells from the BAL fluid were counted, cytospin preparations were stained, and 400 cells/slide were counted for specific cell types. Results are means ± SEM (n = 13–14 mice per group) from three independent experiments. **, P = 0.001; *, P = 0.001. Significance was determined with an unpaired Student’s t test. (B) ELISA was performed on BAL fluid supernatants. Results are means ± SEM (n = 14 mice per group) from three independent experiments. †, P = 0.05.
To determine whether the reduction in BAL fluid inflammatory cells and cytokines reflected an impairment in the generation of Th2 and/or Th17 immune responses in the lymph nodes, we assessed cytokine generation from the lung-draining lymph nodes of the three groups of mice. Upon ex vivo restimulation with 0, 1, or 5 µg/ml Df for 72 h, recipients sensitized with Df-pulsed cBMCsGM-CSF generated robust production of T cell cytokines, as compared with recipients of saline-pulsed cBMCsGM-CSF. Lymph node cells from recipients sensitized with Df-pulsed kdBMCsGM-CSF had significant reductions at the 5-µg/ml dose in the total generation of IL-4, IL-5, and IL-13 per mouse of 51.7, 59.6, and 42.2%, respectively, compared with recipients of Df-pulsed cBMCsGM-CSF (Fig. 3). IL-17A and IFN-γ were not different between the Df-pulsed groups. The reduction in IL-4, IL-5, and IL-13 generated by recipients of Df-pulsed kdBMCsGM-CSF was the result of both a reduction in concentration of each cytokine per million cells plated and an 18.9% reduction in the total lymph node cell count (Fig. 3). The reductions in concentration were significant for IL-5 and IL-13, whereas the trend for IL-4 did not reach significance. Thus, the cytokine findings of a reduced Th2 response in the restimulated nodal cells reflected the reduced eosinophil count and the reduced IL-13 level in the BAL fluid. There was no reduction in IL-17A generation to account for the notable neutrophil reduction in the BAL fluid of mice sensitized with kdBMCsGM-CSF.

BMCsGM-CSF Dectin-2 is required for Df-elicited eosinophilic pulmonary inflammation. WT mice were sensitized by intranasal administration of 10⁴ Df-pulsed WT or Ltc4s⁻/⁻ BMCsGM-CSF, challenged with 2 µg Df on day 22 and day 23, and analyzed on day 25. Cells from the BAL fluid were counted, cytospin preparations were stained, and 400 cells/slide were counted for specific cell types. Results are means ± SEM (n = 13–14 mice per group) from four independent experiments. ***, P = 0.0001. Significance was determined with an unpaired Student’s t test.

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BMCsGM-CSF Dectin-2 mediates Df-elicited Th2 pulmonary inflammation

We next assessed whether Dectin-2 influenced the ability of BMCsGM-CSF to initiate a Df-induced immune response in an adoptive transfer model. We stimulated day-7 kdBMCsGM-CSF and cBMCsGM-CSF with 100 µg/ml Df or saline for 24 h, adoptively transferred 10⁴ cells into WT recipients by intranasal injection on day 8, challenged recipients with 2 µg Df intranasally on day 22 and day 23, and euthanized the recipients for assay on day 25. Df-pulsed cBMCsGM-CSF were able to sensitize recipients for Df challenge, inducing a robust inflammatory response with recruitment of mononuclear cells, neutrophils, and eosinophils in bronchoalveolar lavage (BAL) fluid. After Df challenge, recipients sensitized with kdBMCsGM-CSF had significant reductions in total cell counts of 43.6%, in mononuclear cells of 28.9%, in neutrophils of 65.9%, and in eosinophils of 70.3%, as compared with recipients of cBMCsGM-CSF (Fig. 2 A). To determine whether the reduction in BAL fluid inflammation was associated with a reduction in T cell cytokines, ELISA was performed on the BAL fluid supernatants for IL-13, IL-17A, and IFN-γ (Fig. 2 B). Recipients sensitized with kdBMCsGM-CSF had a significant 59.7% reduction in BAL fluid IL-13, as compared with mice sensitized with cBMCsGM-CSF. The levels of IL-17A and IFN-γ were below the limits of detection.

BMCsGM-CSF cys-LT generation mediates Df-elicited Th2 pulmonary inflammation

As Dectin-2 can elicit generation of both cys-LTs and pro-inflammatory cytokines, we next determined the contribution of these lipid mediators to Df-induced pulmonary inflammation in the adoptive transfer model. We generated BMCsGM-CSF pulsed with Df.
produced IL-4, IL-5, IL-17A, and IFN-γ (Fig. 5 B and Fig. S3). There was no significant induction of these cytokines from CD8+ T cells. Recipients of Df-pulsed Ltc4s−/− BMCsGM-CSF had a significant 68.6% reduction in the number of IL-4+ CD4+ T cells and a significant 73.0% reduction in the number of IL-5+ CD4+ T cells, as compared with mice sensitized with Df-pulsed WT BMCsGM-CSF, whereas the numbers of IL-17A+ and IFN-γ+ CD4+ T cells were unchanged. Thus, the selective decline in BAL fluid eosinophils was paralleled by a selective reduction in Th2 cells recruited to lung.

We next assessed the lung-draining lymph nodes to determine whether the profile of cytokine production was similarly impaired. Cells from the lung-draining lymph nodes of sensitized and challenged recipients were restimulated ex vivo with Df at 0, 1, or 5 µg/ml, and cytokines in the supernatant were measured at 72 h. Recipients sensitized with Df-pulsed WT BMCsGM-CSF generated robust production of T cell cytokines, as compared with recipients of saline-pulsed WT BMCsGM-CSF. Mice sensitized with Df-pulsed Ltc4s−/− BMCsGM-CSF had a selective 78.4% reduction in the recruitment of eosinophils to the BAL fluid with Df challenge (Fig. 4).

To determine whether the attenuation of eosinophil recruitment in mice sensitized with Ltc4s−/− BMCsGM-CSF reflected a decrement in Th2 cells in the lung, we characterized the CD4+ and CD8+ T cell populations recruited to the lung in another cohort sensitized and challenged in the same manner. Pulmonary mononuclear cells were isolated, stimulated with PMA and ionomycin in the presence of monensin, permeabilized, and stained for cell surface expression of CD4 and CD8 (A) and for intracellular expression of IL-4, IL-5, IL-17A, and IFN-γ (B), and analyzed by flow cytometry. Gating on cell size, on CD4 and CD8 expression, the total number of cells recruited to the lung is shown. Results are means ± SEM (n = 11 mice per group) from two experiments. *, P = 0.02; **, P = 0.005; ***, P = 0.002; ****, P = 0.0008. Significance was determined with an unpaired Student’s t test.

Figure 5. BMCsGM-CSF Ltc4s is required for Df-elicited CD4+ Th2 cell recruitment to the lung. WT mice were sensitized with saline-pulsed or Df-pulsed WT or Ltc4s−/− BMCsGM-CSF and challenged with Df as described in the legend to Fig. 4. Pulmonary mononuclear cells were isolated, stimulated with 50 ng/ml PMA and 1 µM ionomycin for 10 h in the presence of 2.5 µM monensin, stained for cell surface expression of CD4 and CD8 (A) and for intracellular expression of IL-4, IL-5, IL-17A, and IFN-γ (B), and analyzed by flow cytometry. Gating on cell size, on CD4 and CD8 expression, the total number of cells recruited to the lung is shown. Results are means ± SEM (n = 11 mice per group) from two experiments. *, P = 0.02; **, P = 0.005; ***, P = 0.002; ****, P = 0.0008. Significance was determined with an unpaired Student’s t test.

Figure 6. BMCsGM-CSF Ltc4s is required for lymph node Th2 cytokine production elicited by Df. WT mice were sensitized with saline-pulsed or Df-pulsed WT or Ltc4s−/− BMCsGM-CSF and challenged with Df as described in the legend to Fig. 4. Parabronchial lymph node cells were isolated, counted, and restimulated for 72 h with 0, 1, or 5 µg/ml Df, and cytokines in the supernatant were measured by ELISA. Total cytokine production per mouse is shown. Results are means ± SEM (n = 12–15 mice per group) from three experiments. Triangles under x axes indicate 0, 1, and 5 µg/ml Df, from left to right. *, P = 0.04; **, P = 0.001; ***, P = 0.0001. Significance was determined with an unpaired Student’s t test.

from BALB/c WT and Ltc4s-deficient (Ltc4s−/−) mice, pulsed them with Df, sensitized WT recipients via intranasal transfer of Df-pulsed BMCsGM-CSF, and challenged recipients intranasally with Df to elicite memory responses. Whereas WT BMCsGM-CSF sensitized recipients for robust pulmonary inflammation with recruitment of mononuclear cells, neutrophils, and eosinophils to the airway, mice sensitized with Df-pulsed Ltc4s−/− BMCsGM-CSF had a selective 78.4% reduction in the recruitment of eosinophils to the BAL fluid with Df challenge (Fig. 4).

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We next assessed the lung-draining lymph nodes to determine whether the profile of cytokine production was similarly impaired. Cells from the lung-draining lymph nodes of sensitized and challenged recipients were restimulated ex vivo with Df at 0, 1, or 5 µg/ml, and cytokines in the supernatant were measured at 72 h. Recipients sensitized with Df-pulsed WT BMCsGM-CSF generated robust production of T cell cytokines, as compared with recipients of saline-pulsed WT BMCsGM-CSF. Mice sensitized with Df-pulsed Ltc4s−/− BMCsGM-CSF had significant reductions in the total generation of IL-4, IL-5, and IL-13 per mouse of 61.5, 76.4, and 78.2%, respectively, as compared with mice sensitized with
Df-pulsed WT BMCsGM-CSF (Fig. 6). The reduction in IL-4, IL-5, and IL-13 generation by recipients of Df-pulsed Ltc4s−/− BMCsGM-CSF was the result of both a reduction in concentration of each cytokine per million cells plated and a 20.5% reduction in the total lymph node cell count (Fig. 6). The reductions in concentration were significant for IL-5 and IL-13, whereas the trend for IL-4 did not reach significance. There was no difference in the total generation of IL-17A or IFN-γ per mouse. Thus, the attenuation in pulmonary eosinophil and Th2 cell recruitment to the lung in mice sensitized with Df-pulsed Ltc4s−/− BMCsGM-CSF appeared to reflect a selective defect in Th2 immunity to Df.

To confirm that MHCIIdependent antigen presentation by BMCsGM-CSF was required to sensitize recipient mice for the generation of eosinophilic pulmonary inflammation and Th2 cytokine induction after Df challenge, we sensitized WT C57BL/6 mice with Df-pulsed WT or MHCIIdeficient (MHCIId−/−) BMCsGM-CSF. After Df challenge, recipients of Df-pulsed MHCIId−/− BMCsGM-CSF had significant reductions in BAL fluid total cells of 88.3%, in monocytes of 66.9%, in neutrophils of 97%, and in eosinophils of 99%, as compared with recipients of Df-pulsed WT BMCsGM-CSF (Fig. S4 A). Lymph node cells were isolated, stimulated with PMA and ionomycin in the presence of monensin, permeabilized, and stained for CD4, CD8, IL-4, IL-5, IL-17A, and IFN-γ. As shown for a representative plot (Fig. S4 B), IL-4, IL-5, and IL-17A were induced in CD4+ lymphocytes from recipients of WT, but not MHCIId−/− BMCsGM-CSF. We did not detect IL-4, IL-5, or IL-17A in any other cell population. Thus, MHCIId-mediated antigen presentation from transferred BMCsGM-CSF was necessary to sensitize WT recipients for the appearance of a cellular infiltrate in the BAL fluid and CD4+ Th2 cells in the draining lymph nodes.

Th2 differentiation may be altered by the maturation of DCs, their expression of costimulatory molecules, their production of proinflammatory cytokines, or their ability to migrate to the lung-draining lymph nodes. Flow cytometry revealed comparable cell surface expression of CD80, CD86, CD40, OX40L, CCR7, PD-L1, and ICOSL on CD11c+MHCIId+ WT and Ltc4s−/− BMCsGM-CSF after Df loading (Fig. S5). We assessed cytokine production by WT and Ltc4s−/− BMCsGM-CSF stimulated with Df and found no significant difference in the ability to generate TNF, IL-10, IL-6, IL-2, or IL-23 (Fig. S6). IL-12p70 was not detected from WT or Ltc4s−/− BMCsGM-CSF (unpublished data). BMCsGM-CSF can chemotax to LTD4 in a CysLT1R-dependent fashion, and cys-LTs have been reported to enhance DC migration to the DC chemotactic ligand for CCR7, CCL19 (Robbiani et al., 2000). We conjugated Df to Alexa Fluor 647 and assessed the migration of intranasally transferred Alexa Fluor 647–Df-pulsed BMCsGM-CSF to the regional lymph nodes 24 h later. We found that Alexa Fluor 647–Df-pulsed WT and Ltc4s−/− BMCsGM-CSF trafficked to the regional lymph nodes to a similar extent and comprised 7.9 ± 0.75 and 13.5 ± 4.7% of CD11c+MHCIId+ cells, respectively, at 24 h over two experiments (Fig. S7).

CysLT1R function conditions BMCsGM-CSF for Th2 immune responses
We next sought to determine whether the Th2 priming function of cys-LTs was mediated through CysLT1R. WT recipients were sensitized by intranasal transfer of Df-pulsed WT or CysLT1R-deficient (Cysltr1−−) BMCsGM-CSF and directly challenged with Df. Although Df-pulsed WT BMCsGM-CSF initiated a characteristic robust pulmonary inflammation with the recruitment of mononuclear cells, neutrophils, and eosinophils to the airway, mice sensitized with Df-pulsed Cysltr1−− BMCsGM-CSF had a selective 45.1% reduction in eosinophil recruitment to the BAL fluid (Fig. 7). Restimulation of lung-draining lymph node cells demonstrated that recipients sensitized with Df-pulsed WT BMCsGM-CSF generated robust production of Th2 cytokines, as compared with recipients of saline-pulsed WT BMCsGM-CSF. At 5 µg/ml Df, mice sensitized with Df-pulsed Cysltr1−− BMCsGM-CSF had significant reductions in the total generation of IL-5 and IL-13 per mouse of 53.7 and 47.4%, respectively, as compared with recipients of Df-pulsed WT BMCsGM-CSF, and a 69.3% fall in IL-4 that did not reach statistical significance (Fig. 8). The reductions in IL-5 and IL-13 were the result of a significant 36.7% reduction in the total lymph node cell count (Fig. 8). There was no significant difference in the generation of IL-17A or IFN-γ between mice sensitized with WT BMCsGM-CSF or Cysltr1−− BMCsGM-CSF. Thus, Cysltr1−− BMCsGM-CSF had a selective impairment in the generation of Th2 immunity that was consistent with the findings with Ltc4s−/− BMCsGM-CSF.

Cys-LTs mediate Df-elicited active sensitization and Th2 pulmonary inflammation
To demonstrate that cys-LT generation and CysLT1R function are each required for Th2 pulmonary inflammation to Df in a direct immunization model, we sensitized and challenged...
sensitized and challenged BALB/c mice with intranasal Df and treated them with a monoclonal antibody to Dectin-2 or the rat IgG2a isotype control on days 0, 2, 15, and 17. Mice treated with the Dectin-2 antibody had significant reductions in BAL fluid total cells (64%), mononuclear cells (61%), neutrophils (78%), and eosinophils (80%), as compared with mice treated with the isotype control (Fig. 10 A). Cytokines from restimulated lung-draining lymph node cells demonstrated that mice treated with the Dectin-2 antibody had significant reductions in the total generation of IL-4, IL-5, IL-13, and IL-17A per mouse of 47, 55, 44, and 90%, respectively, as compared with mice treated with the isotype control (Fig. 10 A). Dectin-2 mediates Df-elicited active sensitization and Th2 pulmonary inflammation

Finally, to assess the role of Dectin-2 in Df-elicited Th2 pulmonary inflammation in the direct immunization model, we sensitized and challenged BALB/c mice with intranasal Df and treated them with a monoclonal antibody to Dectin-2 or the rat IgG2a isotype control on days 0, 2, 15, and 17. Mice treated with the Dectin-2 antibody had significant reductions in BAL fluid total cells (64%), mononuclear cells (61%), neutrophils (78%), and eosinophils (80%), as compared with mice treated with the isotype control (Fig. 10 A). Cytokines from restimulated lung-draining lymph node cells demonstrated that mice treated with the Dectin-2 antibody had significant reductions in the total generation of IL-4, IL-5, IL-13, and IL-17A per mouse of 47, 55, 44, and 90%, respectively, as compared with mice treated with the isotype control (Fig. 10 A). Cytokines from restimulated lung-draining lymph node cells showed significant reductions in IL-4 and IL-5 in Ltc4s−/− and Cysltr1−/− mice, respectively, as compared with WT (Fig. 9 B). Cysltr1−/− mice had a significant 63% reduction in IL-13, whereas in Ltc4s−/− mice the reduction did not reach statistical significance. There were additional reductions in IL-17A and in IFN-γ from Cysltr1−/− mice that did not reach statistical significance.

Figure 8. Df-elicited Th2 cytokine generation is dependent on BMCSM-CyBA and CysLT1R. WT mice were sensitized with saline-pulsed or Df-pulsed WT or Cysltr1−/− BMCSM-CyBA and challenged with Df as described in the legend to Fig. 7. Parabronchial lymph node cells were isolated, counted, and restimulated for 72 h with 0, 1, or 5 µg/ml Df, and cytokines in the supernatant were measured by ELISA. Total cytokine production per mouse is shown. Results are means ± SEM (n = 7 mice per group) from two experiments. Triangles under x axes indicate 0, 1, and 5 µg/ml Df, from left to right. *, P = 0.03; **, P = 0.02. Significance was determined with an unpaired Student’s t test.

Figure 9. Df-elicited eosinophilic pulmonary inflammation and Th2 cytokine generation is dependent on LTC4S and CysLT1R. WT (black), Ltc4s−/− (white), or Cysltr1−/− (gray) mice were sensitized with 10 µg Df or saline on days 0 and 1, challenged with 10 µg Df on days 15 and 16, and analyzed on day 18. (A) Cells from the BAL fluid were counted, cytospin preparations were stained, and 400 cells/slide were counted for specific cell types. Results are means ± SEM (n = 7–12 mice per group) from three experiments. *, P = 0.05; **, P = 0.01. (B) Parabronchial lymph node cells were isolated, counted, and restimulated for 72 h with 0 or 5 µg/ml Df, and cytokines in the supernatant were measured by ELISA. Total cytokine production per mouse is shown. Results are means ± SEM (n = 7–12 mice per group) from three experiments. *, P = 0.05; **, P = 0.01; ***, P = 0.001. Significance was determined with a one-way ANOVA.
compared with mice treated with the isotype control (Fig. 10 B).
The reduction in IL-4, IL-5, and IL-13 generation by mice
treated with the Dectin-2 antibody was the result of a 72%
reduction in the total lymph node cell count, whereas the re-
duction in IL-17A was the result of both a reduction in the
concentration of each cytokine per million cells plated and
the reduction in the total lymph node cell count (Fig. 10 B).

**DISCUSSION**

PRR signaling is central to the initiation of Th1 and Th17
adaptive immune responses. We have previously identified
Dectin-2 on pulmonary CD11c+ cells and on BMCsGM-CSF as
a PRR for clinically relevant allergens including Df, Dp, and
Aspergillus fumigatus extract (Barrett et al., 2009). In this study,
we demonstrate that Dectin-2 activation elicited by Df in
both adoptive transfer and direct sensitization and challenge
models triggers Th2 pulmonary inflammation and that this
coupling is mediated through the specific actions of cys-LTs.
Notably, TLR4 on lung stromal cells is the only PRR previ-
ously identified that links HDM recognition to Th2 pulmo-
nary inflammation (Hammad et al., 2009). Arachidonic acid
release and cys-LT generation can be elicited by signaling
from other myeloid CLRs, such as Dectin-1 and DC-SIGN
(Olynych et al., 2006; Suram et al., 2006; Valera et al., 2007),
and although these receptors recognize glycans that can serve
as adjuvants for a Th2 response (Shreffler et al., 2006; Inoue
et al., 2009), no mechanism for this linkage has been suggested.
Herein, we observe a link to Th2 immunity not only via
Dectin-2–mediated generation of cys-LTs from BMCsGM-CSF
but also via CysLT1R function on these cells.

Both human DCs and mouse BMCsGM-CSF express the
CysLT1R, and two prior pharmacologic studies with CysLT1R
antagonists show that cys-LTs can augment Th2 sensitization
in BALB/c mice (Machida et al., 2004; Okunishi et al., 2004).
In WT mice sensitized by the adoptive transfer of Df-pulsed
BMCsGM-CSF and challenged with Df, the recruitment of
eosinophils to lung and the levels of IL-5 in BAL fluid were
increased by costimulation with LTD4 and suppressed by the
presence of a CysLT1R antagonist during the in vitro Df pulsa-
ting (Machida et al., 2004). Systemic administration of a
CysLT1R antagonist during OVA sensitization reduced the
levels of BAL fluid IL-4, IL-5, and IFN-γ elicited by OVA
challenge (Okunishi et al., 2004). However, in the current
study, the reductions in Th2 cytokines in restimulated lymph
node cells were not accompanied by a reduction in IFN-γ
production in WT recipients sensitized with BMCsGM-CSF
lacking LTC4S or CysLT1R.

Our findings for a CysLT1R function on BMCsGM-CSF
in Th2 sensitization prompt a consideration of possible path-
ways. We did not find evidence for impaired generation of
cytokines such as TNF from Ltc4s−/− BMCsGM-CSF in response
to Df (Fig. S6). Nor did we detect any difference in
maturation or expression of costimulatory markers between
Df-pulsed WT and Ltc4s−/− BMCsGM-CSF (Fig. S5). Cys-LTs
can specifically modulate human and mouse DC migration
(Robbiani et al., 2000; Thivierge et al., 2006), but we did not
see any impairment in the migration of Alexa Fluor 647–
pulsed BMCsGM-CSF in re-

**Figure 10.** Df-elicited pulmonary inflammation and Th2 cytokine
generation is dependent on Dectin-2. WT mice were sensitized and
challenged as described in the Fig. 9 legend. Mice were injected intraperi-
toneally with 200 µg Dectin-2 antibody or a rat IgG2a isotype control on
days 0, 2, 15, and 17, and analyzed on day 18. (A) Cells from the BAL fluid
were counted, cytospin preparations were stained, and 400 cells/slide were
counted for specific cell types. (B) Parabronchial lymph node cell counts.
Results are means ± SEM (n = 4 mice per group) and are representative of
two independent experiments. *, P = 0.04; **, P = 0.02; ***, P = 0.01.
(B) Parabronchial lymph node cells were isolated, counted, and restimu-
lated for 72 h with 0 or 5 µg/ml Df, and cytokines in the supernatant were
measured by ELISA. Total cytokine production per mouse is shown. Results
are means ± SEM (n = 4 mice per group) and are representative of two
independent experiments. *, P = 0.04; **, P = 0.03; ***, P = 0.01; †, P =
0.0001. Significance was determined with an unpaired Student’s t test.
GM-CSF-expanded BM cultures at day 7 provide a mixed population of cells, including Dectin-2+ granulocytes (12.4 ± 0.5%; Fig. S1 C) and Dectin-2–CD11c− cells (7.0 ± 0.5%) that express CD11b and Ly6C, presumably identifying Dectin-2+ monocytes in this culture. The dominant CD11c+ cell population in these cultures expresses CD11b (97.7 ± 0.4%) and MHCII (88.5 ± 2%). Such cells, termed inflammatory DCs, were recently identified as the critical antigen-presenting cells to drive Th2 responses to HDM (Hammad et al., 2010), and our findings suggest that Dectin-2 expression on these cells may be central to this function. As Dectin-2 is highly expressed on immature CD11c<sub>hi</sub>MHCII<sub>low</sub> cells (Fig. 1 A and Fig. S1 A), further studies are needed to determine whether Dectin-2 expression identifies a particular subset of inflammatory DCs in vivo that prime for Th2 immunity.

We found a significant role for Dectin-2–mediated cys-LT production and CysLT<sub>R</sub> function in the generation of Th2 immunity to Df in both the adoptive transfer studies with Df-pulsed BMCSGM-CSF and the direct sensitization and challenge of LTC4S− and CysLT1R-deficient strains. How-
to the protocol for BM-derived DC generation by Lutz et al. (1999). In brief, LM cells were harvested and resuspended at 4 x 10⁵ cells/ml in RPMI 1640 medium supplemented with 10% heat-inactivated FCS, 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM L-glutamine, 50 µM 2-mercaptoethanol, and 40 ng/ml of recombinant mouse GM-CSF (Peprotech). 10 ml of this suspension was plated in each Petri dish and cultured at 37°C in a 5% CO₂ incubator. On day 3 of culture, 10 ml of complete media with GM-CSF was added to each plate. On day 6 of culture, 10 ml of media with GM-CSF was exchanged from each plate. The floating cell population was harvested at day 7, washed, and counted. For cell stimulation, ΔF₅₉ was used at the concentrations noted with a final cell concentration of 10⁶ cells/ml.

Cytospin and immunocytochemistry. To assess the cell composition of day-7 cultures, 1.5 x 10⁵ cells in 200 µl were cytospun, fixed, and stained with Hema 3 Stain Set (Thermo Fisher Scientific). Cells were identified by morphological criteria. 400 cells per slide were counted. To assess Dectin-2 expression by immunocytochemistry, cells were fixed in 4% paraformaldehyde, washed, cytospun, and stained using the rat ABC staining system (Santa Cruz Biotechnology, Inc.), according to the manufacturer’s instructions. In brief, the slides were blocked with 1.5% goat serum, incubated with rat anti-Dectin-2 (Abd Serotec), or a rat IgG₂a isotype control Ab (BD) at 1:20 at 25°C for 1 h, washed, and incubated with biotin-conjugated goat anti-rat antibody for 30 min. Detection was accomplished using avidin-biotinylated horseradish peroxidase and diaminobenzidine substrate. Hematoxylin was used for counterstaining. The pictures were taken by a digital camera (DMX 1200; Nikon) with ACT-1 (version 2.70; Nikon) image acquisition software. 500 cells per slide were assessed.

Flow cytometry. To assess Dectin-2 expression on day 7, BMCGM-CSF were harvested, washed, blocked in PBS containing 1 mM EDTA and 10% donkey serum (Jackson ImmunoResearch Laboratories, Inc.), and serially stained with rat anti-Dectin-2 (Abcam) or rat IgG₂a isotype control Ab (BD) at 1 µg/0.2 µl/10⁵ cells, donkey anti-rat IgG-alkaline phosphocaymin (Jackson ImmunoResearch Laboratories, Inc.) at 1:100 dilution, and either rat anti-mouse CD11c-PE, CD80-PE, CD86-PE, CD40-PE, OX40L-PE, CCR7-PE, PD-L1-PE, and ICOSL-PE (BD) or rat anti-mouse CD11c-Pacific Blue, MHCIIEFITC, CD11b-PECy7, and Ly6c-APC/Cy7 (eBioscience). MHCIIE-PE staining was performed at nonsaturating antibody concentrations. Analyses were performed on a FACScanto II flow cytometer (BD), and data were analyzed with FlowJo (7.5; Tree Star, Inc.).

Lentiviral knockdown of Dectin-2 in BMCGM-CSF. Infectious viral particles were prepared and titered as previously described (Barrett et al., 2009) using the shRNA knockdown TRCN0000066785 for mouse Dectin-2 in the pLKO1 lentiviral vector (Open Biosystems), an empty vector control (Open Biosystems), and a nontargeting sequence (Sigma-Aldrich). BMCGM-CSF cultures were begun on day 0, infected on day 1 at a multiplicity of infection of 4, and selected with 2 µg/ml puromycin from days 4–7. BMCGM-CSF cultures were begun on day 0, infected on day 1 at a multiplicity of infection of 40 ng/ml of recombinant mouse GM-CSF (Peprotech). 10 ml of this suspension was plated in each Petri dish and cultured at 37°C. 2.5 µM monensin was added and the cells were incubated for an additional 6 h. Cells were harvested, treated with DNase I at a concentration of 60 µg/ml for 15 min at 37°C, washed, fix with fixation buffer (eBioscience) for 15 min, washed again, and blocked in 300 µl permeabilization buffer, and 1 mM sodium pyruvate; plated at 2 x 10⁵ cells/ml in 24-well plate; and stimulated with 30 µg/ml PMA and 1 µM ionomycin for 2 h at 37°C. 2.5 µM monensin was added and the cells were incubated for an additional 8 h. Cells were harvested, treated with DNase I at a concentration of 60 µg/ml for 15 min at 37°C, washed, fix with fixation buffer (eBioscience) for 15 min, washed again, and blocked in 300 µl permeabilization buffer. Cells were either 1 µg/ml IgG (Sigma-Aldrich) or 1 µg/ml IgG (eBioscience) with lower limits of detection at 78.8, 78.5, and 31.2 pg/ml, respectively.

BAL. Mice were killed, the trachea was exposed and cannulated with a 22-gauge angiocatheter, and the bronchoalveolar space was lavaged three times with 0.75 ml PBS with 1 mM EDTA. The BAL fluid was centrifuged, and the pelleted cells were resuspended in PBS with 1% FCS and counted. Then, 1.5 x 10⁶ cells in 200 µl were cytospun, fixed, and stained with Hema 3 Stain Set (Thermo Fisher Scientific). Cells were identified by morphological criteria. 400 cells per slide were counted. IL-13, IL-17A, and IFN-γ levels in the BAL fluid supernatants were measured by ELISA (eBioscience) with lower limits of detection at 7.8, 7.8, and 31.2 pg/ml, respectively.

Intracellular cytokine staining of pulmonary mononuclear cells and lymph node cells. For pulmonary mononuclear cell assessment, mice were killed, and the lungs were perfused with 10 ml of sterile PBS, homogenized with sterile scalpels, and digested in a shaker for 30 min with 500 U/ml of type IV collagenase and 0.02 mg/ml DNase I at 37°C. The cells were washed, filtered through a 70-µm cell strainer, and separated on a NycoPrep gradient (Axis-Shield) at 600 g for 30 min at 4°C. Harvested mononuclear cells were washed; resuspended in RPMI 1640 medium supplemented with 10% heat-inactivated FCS, 100 U/ml penicillin, 100 µg/ml streptomycin, 0.1 mM nonessential amino acids, 0.043 mM 2-ME, 2 mM l-glutamine, 0.025 M Heps buffer, and 1 mM sodium pyruvate; plated at 2 x 10⁶ cells/ml in 24-well plate; and stimulated with 30 µg/ml PMA and 1 µM ionomycin for 2 h at 37°C. 2.5 µM monensin was added and the cells were incubated for an additional 8 h. Cells were harvested, treated with DNase I at a concentration of 60 µg/ml for 15 min at 37°C, washed, fixed with fixation buffer (eBioscience) for 15 min, washed again, and blocked in 300 µl permeabilization buffer. Cells were either 1 µg/ml IgG (Sigma-Aldrich) or 1 µg/ml IgG (eBioscience) with lower limits of detection at 78.8, 78.5, and 31.2 pg/ml, respectively.

Cytokine assays from lung-draining lymph nodes. Single cell suspensions from the parabranchial lymph nodes were excised from each mouse. Single cell suspensions were generated, filtered through a 70-µm cell strainer, washed, resuspended in the RPMI medium described in this section, and counted. Cell stimulation, washing, fixation, permeabilization, and staining were as in this section with the exception that PMA, ionomycin, and then monensin treatment was done for a total of 6 h, and cells were stained with anti-mouse CD4-PE/Cy7 (clone RM4-5; BD), anti-mouse CD8α-alkaline phosphocaymin (clone 53–6.7; BD), anti-mouse IL-4–PE (clone 11B11; BD), anti-mouse IL-5–PE (clone TRFK5; BD), anti-mouse IL-17A–PE (clone TC11-18H10; BD), anti-mouse IFN-γ–PE (clone XMG1.2; eBioscience), or rat IgG1-PE isotype control Ab (BD) at a concentration of 0.2 µg/0.1 ml/2 x 10⁶ cells. Analyses were performed on a FACScanto II flow cytometer, and data were analyzed with FlowJo. For lymph node cell assessment, the parabronchial lymph nodes were excised from each mouse. Single cell suspensions were generated, filtered through a 70-µm cell strainer, washed, resuspended in the RPMI medium described in this section, and counted. Cell stimulation, washing, fixation, permeabilization, and staining were as in this section with the exception that PMA, ionomycin, and then monensin treatment was done for a total of 6 h, and cells were stained with anti-mouse CD4-PE/Cy7 (clone RM4-5; BD) and anti-mouse CD8α–PE-Cy7 (clone 53–6.7; BD).

Cytokine assays from lung-draining lymph nodes. Single cell suspensions from the parabranchial lymph nodes were generated. Lymph node cells were cultured at 4 x 10⁵ cells/ml with 0, 1, or 5 µg/ml ΔF₅₉ for 72 h at 37°C in RPMI medium with 10% FCS. The concentrations of IL-4, IL-5, IL-13, IL-17A, and IFN-γ in the supernatants were measured with ELISA kits (eBioscience) with lower limits of detection at 39 pg/ml, 117 pg/ml, 390 pg/ml, 39 pg/ml, and 156 pg/ml, respectively. The total concentration of cytokine generated per mouse is reported.

BMCGM-CSF migration. ΔF₅₉ was conjugated to Alexa Fluor 647 with a Protein Labeling kit (Invitrogen) according to the manufacturer’s protocol and
was dialedyzed with a 10K membrane (Thermo Fisher Scientific) against PBS for 6 h. Day-7 BMCsGM-CSF were stimulated with 100 µg/ml Alexa Fluor 647-D for 18 h, and 10^5, 10^6, or 10^7 cells were transferred intranasally into mice. 24 and 48 h later, the lymph nodes were harvested, digested with collagenase IV and DNase I for 30 min at 37°C, and filtered. Single cell suspensions were blocked in PBS supplemented with 0.5% heat-inactivated FCS, 0.05% NaN3, 1% mouse IgG, and 1% anti-mouse CD16/CD32. The cells were stained with anti-mouse CD11c-PE-Cy7 (clone HL3; BD) and anti-mouse MHCII-PE (clone M5/114.15.2; BD) at 0.2 µg/100 µl/10^6 cells.

Statistical analysis. All experiments were repeated at least three times, except where noted. Results are expressed as means ± SEM. The Student’s t test was used for statistical analysis, except where noted. A value of P < 0.05 was considered significant.

Online supplemental material. Fig. S1 depicts CD11c, MHCII, Dectin-2, CD11b, and Ly6C expression on BMCsGM-CSF. Fig. S2 shows Df-elicted cytokine production by BMCsGM-CSF at 30 h and the generation of cys-ELTs and TNF in response to curdian and LPS. Fig. S3 shows a representative plot of intracellular cytokine staining in CD4+ lymphocytes from the lung of WT recipients sensitized with Df-pulsed WT and Ln46–/– BMCsGM-CSF and challenged with Df. Fig. S4 shows the difference in BAL fluid cell infiltrate and in intracellular cytokine staining in CD4+ lung node cells from WT recipients sensitized with Df-pulsed WT or MHCII–/– BMCsGM-CSF. Fig. S5 shows comparable cell surface expression of costimulatory markers in WT and Ln46–/– BMCsGM-CSF. Fig. S6 shows comparable production of Df-elicted cytokines at 4 and 24 h by WT and Ln46–/– BMCsGM-CSF. Fig. S7 shows migration of Alexa Fluor 647–Df-pulsed WT and Ln46–/– BMCsGM-CSF into the lung-draining lymph nodes in WT mice. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20100793/DC1.

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REFERENCES

Altmann, F. 2007. The role of protein glycosylation in allergy. Int. Arch. Allergy Immunol. 142:99–115. doi:10.1159/000091144

Aragane, Y., A. Maeda, A. Schwarz, T. Tezuka, K. Ariizumi, and T. Schwarz. 2007. The role of protein glycosylation in allergy. Ann. Allergy Asthma Immunol. 99:585–593. doi:10.2506/annall.99.5.585-593

Instanes, C., H. Ormsbad, B. Rydjord, H.G. Wiker, and G. Hetland. 2004. Involvement of dectin-2 in ultraviolet radiation-induced tolerance. J. Biol. Chem. 279:22608–22613. doi:10.1074/jbc.M401357200

Meliton, A.Y., N.M. Muñoz, C.M. Osan, L.N. Meliton, and A.R. Leff. 2010. Cysteinyl leukotriene D4 activates β2-integrin adhesion in human polymorphonuclear leukocytes. Eur. Respir. J. 35:402–409. doi:10.1183/09031936.0009309

Meyer, S., B. Tefsen, A. Imberty, R. Geyer, and I. van Die. 2007. The C-type lectin L-SIGN differentially recognizes glycans antigens on egg...
glycosphingolipids and soluble egg glycoproteins from *Schistosoma mansoni*. *Glyobiology*. 17:1104–1119. doi:10.1093/glycob/cwm073

Okano, M., A.R. Satoskar, K. Nishizaki, M. Abe, and D.A. Harn Jr. 1999. Induction of Th2 responses and IgE is largely due to carbohydrates functioning as adjuvants on *Schistosoma mansoni* egg antigens. *J. Immunol.* 163:6712–6717.

Okano, M., A.R. Satoskar, K. Nishizaki, and D.A. Harn Jr. 2001. Lacto-N-fucopentaose III found on *Schistosoma mansoni* egg antigens functions as adjuvant for proteins by inducing Th2-type response. *J. Immunol.* 167:442–450.

Okunishi, K., M. Dohi, K. Nakagome, R. Tanaka, and K. Yamamoto. 2004. A novel role of cysteinyl leukotrienes to promote dendritic cell activation in the antigen-induced immune responses in the lung. *J. Immunol.* 173:6393–6402.

Olynch, T.J., D.L. Jakeman, and J.S. Marshall. 2006. Fungal zymosan induces leukotriene production by human mast cells through a dectin-1-dependent mechanism. *J. Allergy Clin. Immunol.* 118:837–843. doi:10.1016/j.jaci.2006.06.008

Peng, J., X.O. Yang, S.H. Chang, J. Yang, and C. Dong. 2010. IL-23 signaling enhances Th2 polarization and regulates allergic airway inflammation. *Cell Res.* 20:62–71. doi:10.1038/cr.2009.128

Phipps, S., C.E. Lam, G.E. Kaiko, S.Y. Foo, A. Collison, J. Mattes, J. Barry, S. Davidson, K. Oreó, L. Smith, et al. 2009. Toll/IL-1 signaling is critical for house dust mite-specific helper T cell type 2 and type 17 [corrected] responses. *Am. J. Respir. Crit. Care Med.* 179:883–893. doi:10.1164/rccm.200806-974OC

Rincón, M., J. Anguita, T. Nakamura, E. Fikrig, and R.A. Flavell. 1997. Interleukin (IL)-6 directs the differentiation of IL-4-producing CD4+ T cells. *J. Exp. Med.* 185:461–469. doi:10.1084/jem.185.3.461

Robbiani, D.F., R.A. Finch, D. Jäger, W.A. Muller, A.C. Sartorelli, and G.J. Randolph. 2000. The leukotriene C(4) transporter MRP1 regulates CCL19 (MIP-3β, ELC)-dependent mobilization of dendritic cells to lymph nodes. *Cell*. 103:757–768. doi:10.1016/S0092-8674(00)01797-3

Robinson, M.J., F. Osorio, M. Rosas, R.P. Freitas, E. Schweighoffer, O. Gross, J.S. Verbeek, J. Ruuland, V. Tybulewicz, G.D. Brown, et al. 2009. Dectin-2 is a Syk-coupled pattern recognition receptor crucial for Th17 responses to fungal infection. *J. Exp. Med.* 206:2037–2051. doi:10.1084/jem.20082818

Saito, 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. Arizumi. 2010. Dectin-2 recognition of alpha-mannans and induction of Th17 cell differentiation is essential for host defense against *Candida albicans*. *Immunity*. 32:681–691. doi:10.1016/j.immuni.2010.05.001

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. Arizumi. 2010. Dectin-2 is a pattern recognition receptor for fungi that couples with the Fc receptor γ chain to induce innate immune responses. *J. Biol. Chem.* 281:38854–38866. doi:10.1074/jbc.M605422200

Shreffler, W.G., R.R. Castro, Z.Y. Kucuk, Z. Charlap-Powers, G. Grishina, S. Yoo, A.W. Burks, and H.A. Sampson. 2006. The major glycoprotein allergen from *Arachis hypogaea*, Ara h 1, is a ligand of dendritic cell-specific ICAM-grabbing nonintegrin and acts as a Th2 adjuvant in vitro. *J. Immunol.* 177:3677–3685.

Soram, S., G.D. Brown, M. Ghosh, S. Gordon, R. Loper, P.R. Taylor, S. Akira, S. Uematsu, D.L. Willams, and C.C. Leslie. 2006. Regulation of cytosolic phospholipase A2 activation and cyclooxygenase 2 expression in macrophages by the β-glucan receptor. *J. Biol. Chem.* 281:5506–5514. doi:10.1074/jbc.M509824200

Thivierge, M., J. Stankova, and M. Rola-Pleszczynski. 2006. Toll-like receptor agonists differentially regulate cysteinyl-leukotriene receptor 1 expression and function in human dendritic cells. *J. Allergy Clin. Immunol.* 117:1155–1162. doi:10.1016/j.jaci.2005.12.1342

Valera, I., A.G. Vigo, S. Alonso, L. Barbolla, M.S. Crespo, and N. Fernández. 2007. Peptidoglycan and mannose-based molecular patterns trigger the arachidonic acid cascade in human polymorphonuclear leukocytes. *J. Leukoc. Biol.* 81:925–933. doi:10.1189/jlb.0706451

Williams, M., A. Nathan, K. Page, and C.L. Karp. 2010. New insights into innate immune mechanisms underlying allergy. *Mucosal Immunol.* 3:104–110. doi:10.1038/mi.2009.138

Yamasaki, S., E. Ishikawa, M. Sakuma, H. Haru, K. Ogata, and T. Saito. 2008. Mincle is an ITAM-coupled activating receptor that senses damaged cells. *Nat. Immunol.* 9:1179–1188. doi:10.1038/ni.1651