

✎ Harnessing the Antiinflammatory Power of MyD88 to Reduce Allergic Fungal Inflammation?

Diverse groups of pattern recognition receptors (PRRs) are expressed on dendritic cells (DCs) including Toll-like receptors (TLRs), C-type lectins including DC-SIGN and Dectins, scavenger receptors, and nucleotide oligomerization domain (NOD)-like receptors that sense and recognize a variety of pathogens leading to appropriate or even disproportional activation of immune responses (1). *Aspergillus fumigatus* is a ubiquitous fungal pathogen associated with the induction of several disease entities ranging from serious infections in immunocompromised hosts to intense inflammatory responses or hypersensitivity reactions such as allergic bronchopulmonary aspergillosis and allergic asthma (2). *A. fumigatus* is primarily recognized by TLR2, Dectin-1 and -2 via pathogen-associated molecular patterns from airborne spores (conidia), filamentous branches (hyphae), and β -glucans on its cell wall (3). Studies have shown that a combined action of TLRs and Dectin-1/2 in recognizing the β -glucans from *A. fumigatus* is required for efficient activation of NF- κ B-dependent production of inflammatory cytokines (4) and reactive oxygen species through a respiratory burst (5). Recognition of *A. fumigatus* with TLR2 involves the adaptor protein MyD88 (6), whereas Dectin-1 and -2 use mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) adaptor molecules for signal transduction (7). The TLR/MyD88 signaling pathway is also recognized for its complex role in airway inflammatory diseases whereby exposure to microbial motif-enriched environments (e.g., agriculture related) can be protective against the development of allergic asthma (8) or alternatively exert negative consequences such as chronic bronchitis and exacerbation of existing disease (9–11).

In this issue of the *Journal*, Percier and colleagues (pp. 39–49) report on investigation of bone marrow-derived DCs (BMDCs) pulsed with *A. fumigatus* from mice that were deficient in either MyD88 or MALT1 to understand how these signaling pathways were involved in the pathogenesis of *A. fumigatus*-associated allergic asthma (12). Even though MyD88 and MALT1 were both involved with induction of proinflammatory cytokines including IL-6 and IL-1 β of BMDCs, MyD88 (but not MALT1) was critical to the allergic hypersensitivity responses. Specifically, mice sensitized with BMDCs derived from MyD88 knockout mice pulsed with *A. fumigatus* demonstrated an exaggerated allergic response including elevated IL-4, IL-5, and IL-13 with increased eosinophil influx as compared with control mice sensitized with wild-type-derived BMDCs pulsed with *A. fumigatus*. The authors

also found this response to be associated with IL-13-producing CD4⁺ CD44⁺ lung T cells, and *A. fumigatus*-induced IL-13 production from mediastinal lymph node cells was potentiated in the setting of MyD88 deficiency.

Using knockout mice each for TLR2, TLR4, and IL-1R, the authors confirmed that TLR2 (not TLR4 or IL-1R) recognition of *A. fumigatus* mediated the reduced T-helper cell type 2 responses. The scientific rigor of these observations was enhanced by studies using antibody to block TLR2 and studies stimulating TLR2 signaling via the agonist Pam₃CSK₄. The response was specific to *A. fumigatus* and not to the nonpathogenic *Clostridium sphaerospermum*. However, additional studies are warranted to understand if this feature is restricted to only allergenic or pathogenic molds. Percier and colleagues explored an explanation for the MyD88-dependent antiinflammatory results, finding a role for IL-10 production of DCs. *A. fumigatus*-induced IL-10 levels were markedly reduced in BMDCs derived from MyD88- or TLR2-deficient mice, and supplementing MyD88-deficient BMDCs with exogenous IL-10 reversed the exaggerated airway inflammatory response.

This study investigated how the *in vitro* differentiated BMDCs could modulate the immune response to decipher the role of TLR2/MyD88-dependent signaling in *A. fumigatus* sensitization and allergic airway disease. Importantly, a critical role was established for TLR/MyD88-induced IL-10 pathway signaling in DCs for regulating allergic inflammation. Others have shown that CD11c⁺ DCs expressing MyD88 as opposed to IL-10-producing T-regulatory cells or Foxp3⁺ T cells had beneficial effects with allergen-specific immunotherapy in the setting of CpG-ODN (TLR9 agonist) administration (13). Moreover, the protective effect of microbial-motif-enriched Amish dust extract in experimental allergic asthma was also ascribed to MyD88 and TIR-domain-containing adapter-inducing interferon- β (TRIF) signaling in mice, and this protective response was not explained by regulatory T cells (8). Thus, promoting IL-10-producing lung DCs through engagement of the TLR/MyD88 signaling pathway represents a potential therapeutic approach in allergic asthma.

Although signaling mechanisms were not investigated in the present study, TLR2/MyD88-dependent activation of IL-10 production has been shown to be regulated by the transcription factor CREB in macrophages (14). In addition, scavenger receptor A receptor (CD204) signaling has been demonstrated to enhance IL-10 responsiveness following microbial-enriched dust extract exposures through PKC zeta activation to inhibit TNF- α

✎ This article is open access and distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives License 4.0 (<http://creativecommons.org/licenses/by-nc-nd/4.0/>). For commercial usage and reprints, please contact Diane Gern (dgern@thoracic.org).

Supported by the National Institute of Environmental Health Sciences grant R01ES019325 (J.A.P.) and the National Institute for Occupational Safety and Health grant U54OH010162 (J.A.P.).

Originally Published in Press as DOI: 10.1165/rcmb.2020-0442ED on October 26, 2020

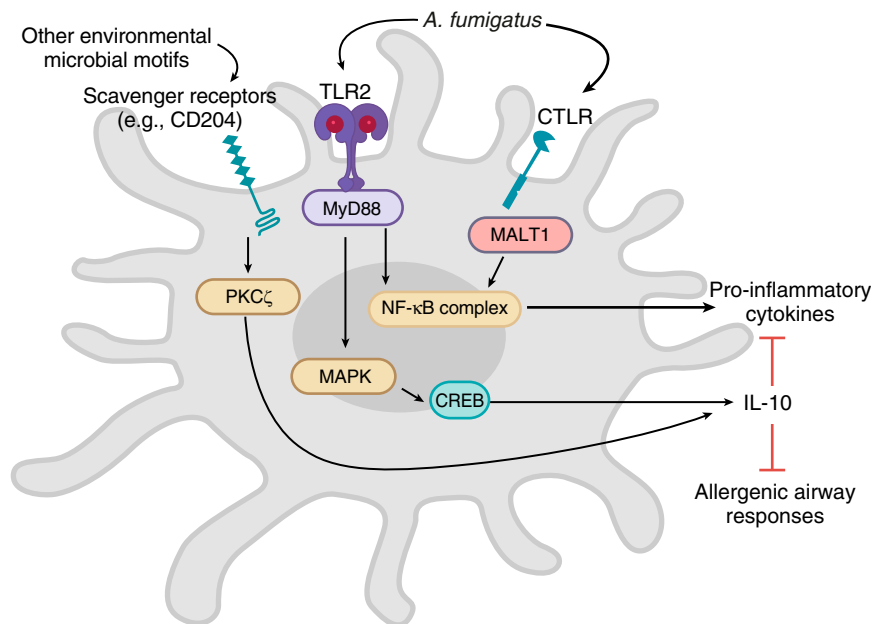


Figure 1. Conceptual overview of potential mechanistic pathway of *Aspergillus fumigatus* recognition through pattern recognition receptors in dendritic cells to upregulate IL-10 production and to downregulate allergic hypersensitivity reactions. *A. fumigatus* is recognized to signal through TLR2 and CTLRs (e.g., dectins). The current study demonstrates that MALT signaling was dispensable and TLR2/MyD88 was critical to lung eosinophilia and T-helper cell type 2 allergy responses. Scavenger receptor signaling has been demonstrated to mediate IL-10 responses to complex environmental pathogen-associated molecular patterns. Created in Biorender.com. *A. fumigatus* = *Aspergillus fumigatus*; CREB = cAMP response element-binding protein; CTLRs = C-type lectin receptors; MALT1 = mucosa-associated lymphoid tissue lymphoma translocation protein 1; MAPK = mitogen-activated protein kinase; PKC ζ = protein kinase C zeta; TLR2 = Toll-like receptor 2.

production (15). Thus, it is possible that other PRR-sensing pathways such as scavenger receptor signaling are also important in regulating allergic asthma and could be investigated in future studies. Other hallmarks of allergic inflammation including mucus production and/or mucociliary clearance were not investigated, and these pathways may also be important in regulating inflammatory responses. It has been recently shown that mucins (i.e., MUC5AC protein) are dependent upon MyD88 signaling following microbial motif-enriched dust extract exposure marked by increased expression in MyD88-deficient mice (16). Although expected to be of a plasmacytoid-like DC that favors tolerance (1), further characterization of the lung myeloid versus plasmacytoid DCs programming and maturation leading to reversal of an allergic phenotype is also warranted. A proposed conceptual schematic of how *A. fumigatus*-induced TLR2/MyD88-dependent pro- versus antiinflammatory signaling effects may be regulated is shown (Figure 1).

In summary, this study provides mechanistic insights into the role of TLR2 and MyD88 signaling in reducing allergenic responses, particularly with exposure to *A. fumigatus*. The novel findings in this paper will pave the way for a better understanding of the antiinflammatory driving mechanisms following TLR/MyD88 signaling in DCs to potentially lead to the development of targeted therapies to reduce fungal-driven allergic diseases such as allergic bronchopulmonary aspergillosis and allergic airway inflammation. ■

Rohit Gaurav, Ph.D.
Jill A. Poole, M.D.
Department of Internal Medicine
University of Nebraska Medical Center
Omaha, Nebraska

ORCID IDs: 0000-0003-4753-2680 (R.G.); 0000-0002-6236-2563 (J.A.P.).

References

- Gaurav R, Agrawal DK. Clinical view on the importance of dendritic cells in asthma. *Expert Rev Clin Immunol* 2013;9:899–919.
- Homma T, Kato A, Bhushan B, Norton JE, Suh LA, Carter RG, et al. Role of *Aspergillus fumigatus* in triggering protease-activated receptor-2 in airway epithelial cells and skewing the cells toward a T-helper 2 bias. *Am J Respir Cell Mol Biol* 2016;54:60–70.
- Neveu WA, Bernardo E, Allard JL, Nagaleekar V, Wargo MJ, Davis RJ, et al. Fungal allergen β -glucans trigger p38 mitogen-activated protein kinase-mediated IL-6 translation in lung epithelial cells. *Am J Respir Cell Mol Biol* 2011;45:1133–1141.
- Walachowski S, Tabouret G, Foucras G. Triggering dectin-1-pathway alone is not sufficient to induce cytokine production by murine macrophages. *PLoS One* 2016;11:e0148464.
- Gaurav R, Bewtra AK, Agrawal DK. Chloride channel 3 channels in the activation and migration of human blood eosinophils in allergic asthma. *Am J Respir Cell Mol Biol* 2015;53:235–245.
- Bretz C, Gersuk G, Knoblauch S, Chaudhary N, Randolph-Habecker J, Hackman RC, et al. MyD88 signaling contributes to early pulmonary responses to *Aspergillus fumigatus*. *Infect Immun* 2008;76:952–958.
- Loures FV, Röhm M, Lee CK, Santos E, Wang JP, Specht CA, et al. Recognition of *Aspergillus fumigatus* hyphae by human plasmacytoid

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

- dendritic cells is mediated by dectin-2 and results in formation of extracellular traps. *PLoS Pathog* 2015;11:e1004643.
8. Stein MM, Hrusch CL, Gozdz J, Igartua C, Pivniouk V, Murray SE, *et al*. Innate immunity and asthma risk in amish and hutterite farm children. *N Engl J Med* 2016;375:411–421.
 9. Warren KJ, Dickinson JD, Nelson AJ, Wyatt TA, Romberger DJ, Poole JA. Ovalbumin-sensitized mice have altered airway inflammation to agriculture organic dust. *Respir Res* 2019;20:51.
 10. Eduard W, Pearce N, Douwes J. Chronic bronchitis, COPD, and lung function in farmers: the role of biological agents. *Chest* 2009;136:716–725.
 11. Poole JA, Wyatt TA, Kielian T, Oldenburg P, Gleason AM, Bauer A, *et al*. Toll-like receptor 2 regulates organic dust-induced airway inflammation. *Am J Respir Cell Mol Biol* 2011;45:711–719.
 12. Percier P, De Prins S, Tima G, Beyaert R, Grooten J, Romano M, *et al*. *Aspergillus fumigatus* recognition by dendritic cells negatively regulates allergic lung inflammation through a TLR2/MyD88 pathway. *Am J Respir Cell Mol Biol* 2021;64:39–49.
 13. Alberca-Custodio RW, Faustino LD, Gomes E, Nunes FPB, de Siqueira MK, Labrada A, *et al*. Allergen-specific immunotherapy with liposome containing CpG-ODN in murine model of asthma relies on MyD88 signaling in dendritic cells. *Front Immunol* 2020;11:692.
 14. Sanin DE, Prendergast CT, Mountford AP. IL-10 production in macrophages is regulated by a TLR-driven CREB-mediated mechanism that is linked to genes involved in cell metabolism. *J Immunol* 2015;195:1218–1232.
 15. Wyatt TA, Nemecek M, Chandra D, DeVasure JM, Nelson AJ, Romberger DJ, *et al*. Organic dust-induced lung injury and repair: Bi-directional regulation by TNF α and IL-10. *J Immunotoxicol* 2020;17:153–162.
 16. Dickinson JD, Sweeter JM, Staab EB, Nelson AJ, Bailey KL, Warren KJ, *et al*. MyD88 controls airway epithelial Muc5ac expression during TLR activation conditions from agricultural organic dust exposure. *Am J Physiol Lung Cell Mol Physiol* 2019;316:L334–L347.