Toll like receptor-2 modulates both innate and adaptive immune responses during chronic fungal asthma in mice

K. F. Buckland¹, E. O’Connor¹, L. A. Murray², C. M. Hogaboam¹

¹ Immunology Program, Department of Pathology, University of Michigan Medical School, Rm 4057, BSRR, 109 Zina Pitcher Place, Ann Arbor, MI, 48109-2200, USA, e-mail: hogaboam@med.umich.edu
² Immunobiology, Centocor Inc.

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Abstract. Objective and Design: We investigated the effect of TLR2 gene deletion in a murine model of chronic fungal asthma. Methods: TLR2 wildtype (TLR2⁺/⁺) and TLR2 deficient (TLR2⁻/⁻) mice were sensitized to soluble A. fumigatus antigens and challenged with live A. fumigatus conidia, and the extent of allergic airways disease was analyzed in both groups of mice at 3, 7, 14, and 30 days after conidia. Results: At day 7 post-conidia, TLR2⁻/⁻ mice exhibited significantly lower airway hyperresponsiveness, airway inflammation, and whole lung Th2 cytokine levels compared with the TLR2⁺/⁺ group. TLR2 deletion also significantly reduced mucus cell metaplasia and peribronchial fibrosis at day 30 after conidia. However, fungal material persisted in the TLR2⁻/⁻ group, and at day 30 after conidia TLR2⁻/⁻ mice exhibited enhanced airway neutrophil recruitment and airway hyperresponsiveness. Conclusion: Thus, during chronic fungal asthma in mice, TLR2 is a major contributor to the maintenance of the adaptive Th2-cytokine driven and anti-fungal innate responses.

Key Words: innate immunity – Fungal asthma – TLR2

Introduction

Airborne A. fumigatus conidia exacerbate allergic and asthmatic diseases [1–3]. The mechanism through which A. fumigatus exerts this effect is unknown, but one explanation lies in the immune response evoked by this pathogen. Experimental and clinical data show that the pulmonary immune response to A. fumigatus conidia is skewed toward Th2 immunity, rather than an anti-fungal Th1 immune response [4]. Directing the immune response toward Th1 immunity is a therapeutically attractive approach to containing the pathological consequences of fungal exacerbation of allergy airways disease and/or asthma.

The immune responses required for the recognition and elimination of fungus such as A. fumigatus remain to be fully elucidated. Accordingly, the toll like receptors (TLRs) have been shown to provide important immune signals for the anti-fungal responses by immune cells such as macrophages, neutrophils, and dendritic cells [5, 6]. Several receptors mediate the recognition of fungus in the host, it is the TLRs that evoke unique immune activating properties [7]. For example, TLR2, working through the central adaptor protein MyD88 [8], has been shown to collaborate with recognition receptors such as dectin-1 to recognize fungal components associated with A. fumigatus conidia [9, 10], thereby contributing to the phagocytosis and containment of this pathogen. The relative importance of TLR2 or MyD88 in the anti-Aspergillus immune process remains controversial since its absence does not necessarily lead to impaired anti-fungal immune responses in vivo [6] or in vitro [11, 12]. However, neutrophil depleted TLR2 gene deficient (TLR2⁻/⁻) mice exhibit increased susceptibility to invasive aspergillosis compared with wildtype [13]. Together, these data suggest that the role of TLR2 is complex, and requires further investigation.

In the present study, we addressed the role of TLR2 in the innate and adaptive immune responses elicited by A. fumigatus in a model of chronic fungal asthma. We have previously observed dynamic changes in TLR2 transcript expression during chronic fungal asthma [14, 15]. Herein, we show that TLR2 deficiency modulates the initiation and maintenance of features of Th2 immunopathology during fungal asthma, including the elaboration of Th2 cytokines, mucus cell metaplasia and peribronchial fibrosis. However, TLR2 expression was necessary for elimination of fungal components from the lungs of TLR2⁻/⁻ mice, and the persistence of fungus in these mice promoted chronic changes in airway physiology. Thus, TLR2 contributes to both innate and adaptive pulmonary immune responses to A. fumigatus.
Materials and methods

Mice

Male and female, homozygous TLR2 gene deficient (TLR2−/−) fully backcrossed ten generations onto a C57BL/6 background and wildtype C57BL/6 (TLR2+/+) mice at 6-8 weeks of age were purchased from Jackson Laboratory (Bar Harbor, ME) and were maintained in a specifically pathogen free facility. Prior approval for mouse usage was obtained from the University Laboratory of Animal Medicine facility.

Chronic fungal asthma model

We have previously described a model of chronic allergic airway disease induced by *A. fumigatus* conidia [16]. TLR2+/+ and TLR2−/− mice were similarly sensitized to a commercially available preparation of soluble *A. fumigatus* antigens (Greer Laboratories, Lenoir, NC) as previously described [16].

Assessment of airway physiology

Immediately prior to and at days 3, 7, 14, and 30 after an intratracheal *A. fumigatus* conidia challenge, bronchial hyperresponsiveness was assessed in a BuxcoTM plethysmograph (Buxco, Troy, NY) (16). After the assessment of airway hyperresponsiveness, approximately 500 µl of blood was removed from each mouse and centrifuged at 900 g for 10 min to yield serum. A bronchoalveolar lavage (BAL) was then performed using 1 ml of filter-sterilized normal saline. Finally, whole lungs were dissected from each mouse and snap frozen in liquid N2 or fixed in 10% formalin for histological analysis (see below).

Whole lung RNA isolation and TAQMAN analysis

Total RNA was isolated from homogenized mouse lungs using Trizol reagent (Invitrogen/ Life Technologies, Carlsbad, CA) as previously described in detail [17]. Purified RNA was treated with DNase and reverse transcribed into cDNA using TAQMAN Reverse Transcription Reagents (Foster City, CA). Pre-developed TAQMAN Gene Expression Assays were used to quantify TLR2, TLR4, TGF-β, and procollagen III transcripts as per manufacturer’s (Applied Biosystems) instructions. The fold changes in transcript expression were calculated via the comparison of gene expression in naive whole lung samples, which were assigned a value of 1 to that in whole lung samples from naive mice.

Serum immunoglobulin analysis

Serum levels of IgE and IgG2a at days 7, 14, and 30 after conidia in the TLR2+/+ and TLR2−/− groups were analyzed using complementary capture and detection antibody pairs for IgE and IgG2a (PharMingen, San Diego, CA). Immunoglobulin ELISAs were performed according to the manufacturer’s directions.

Whole lung proteomic analysis

Murine TNF-α, IL-12, MIP-2, IL-4, IFN-γ, IL-5, IL-10, CCL17, and KC were determined in 50-µl samples from whole lung homogenates using a standardized sandwich ELISA technique previously described in detail [18]. The cytokine and chemokine levels in each sample were normalized to total protein levels measured using the Bradford assay.

![Fig. 1](image-url) A) Fold increase in whole lung TLR2 transcript levels at days 3, 7, 14, and 30 after *A. fumigatus* conidia intratracheal injection into *A. fumigatus*-sensitized TLR2 wildtype (+/+ mice. RNA was isolated from whole lung samples as described in the Materials and Methods section, and quantitative TAQMAN was used to quantify TLR2 transcript levels. TLR2 transcript levels were measured in whole lung samples from non-sensitized TLR2−/− mice, gene expression in these mice was normalized to an arbitrary value of 1 (dashed line), and fold changes in transcript expression in the fungal asthma groups were calculated by dividing transcript levels in this group by 1. Data are mean ± SEM of n = 3-5/group. B) Airway hyperresponsiveness in *A. fumigatus*-sensitized TLR2 wildtype (+/) and TLR2-deficient (−/) mice at various times after an *A. fumigatus* conidia challenge. Airway resistance (units = cm H2O/ml/sec) was calculated at each time point prior to (dashed line) and after methacholine (420 µg/kg; i.v.). Values are expressed as mean ± SE; n = 5–10/group/time point. *, P <0.05, demonstrates a significant difference in airway hyperresponsiveness between TLR2+/+ and TLR2−/− mice. C) Serum total IgE and IgG2A levels in *A. fumigatus*-sensitized TLR2 wildtype (+/) and TLR2-deficient (−/) mice at various times after *A. fumigatus* conidia challenge. Total IgE and IgG2a were measured using a specific ELISA as described in the Materials and Methods section. Data are expressed as mean ± SEM; n = 5/group/time point. * P ≤0.05, ** P ≤0.01, *** P ≤0.001 compared with values measured in the TLR2+/+ groups at the same time after the conidia challenge.