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Forum Minireview

Progress in Allergy Signal Research on Mast Cells: Regulation of Allergic Airway Inflammation Through Toll-Like Receptor 4–Mediated Modification of Mast Cell Function

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Abstract. In a mouse experimental asthma model, the administration of bacterial lipopolysaccharide (LPS), particularly at low doses, enhances the levels of ovalbumin (OVA)-induced eosinophilic airway inflammation. In an effort to clarify the cellular and molecular basis for the LPS effect, we demonstrate that the OVA-induced eosinophilic inflammation in the lung is dramatically increased by administration of LPS at the priming phase in wild-type mice, whereas such an increase was not observed in mast cell deficient mice. Adoptive transfer of bone marrow-derived mast cells (BMMC) from wild type but not from Toll-like receptor 4 (TLR4)-deficient mice restored the increased eosinophilic inflammation in mast cell–deficient mice. Moreover, in vitro analysis revealed that treatment of BMMC with LPS resulted in sustained up-regulation of GATA1 expression and increased production of Th2 cytokines (IL-4, IL-5, and IL-13) upon restimulation. Thus, mast cells appear to control allergic airway inflammation after their activation and modulation through TLR4-mediated induction of GATA1 proteins and subsequent increase in Th2 cytokine production.

Keywords: Toll-like receptor 4 (TLR4), mast cell, Th2 cytokine, NF-κB, GATA1, allergy

Introduction

It is well established that mast cells play a central role in anaphylactic reactions. Mast cells are activated by multivalent binding of antigens to receptor-bound IgE and release inflammatory mediators such as histamine, prostaglandins, and leukotrienes (1, 2). It is also known that mast cells regulate the levels of allergic inflammatory responses in the airways by producing cytokines, such as interleukin (IL)-4, IL-5, IL-6, IL-10, IL-13, and tumor necrosis factor (TNF)-α, which are important in the pathogenesis of various allergic reactions (3).

It is well recognized that respiratory infection modulates allergic airway inflammation (4). However, as for the role for exposure of bacterial components such as lipopolysaccharide (LPS) in allergic inflammation, there is apparent controversy evidenced by studies suggesting protective roles for LPS through Th1 cell induction and studies showing its exacerbating effects on asthma (5, 6). Recently, Th1/Th2 inflammatory responses were reported to be influenced by the levels of LPS exposure (7). The exposure to a high level of LPS with antigens resulted in increased antigen-specific Th1 responses, while a low dose of LPS resulted in Th2 sensitization. Collectively, these results suggest a unique biphasic effect for LPS in allergic inflammatory responses.

Recent progress has revealed that innate immune responses are initiated by various pattern recognition receptors, TLRs (8). TLRs comprise a family of proteins that enhance certain cytokine gene transcriptions in response to various pathogenic ligands and control acquired immune responses such as Th1 responses (9, 10). TLR4 was shown to be a receptor for LPS (11, 12).

Dendritic cells (DCs) are well recognized to play a central role in inflammatory reactions elicited by LPS (9). When DCs are activated by LPS through TLR4, they become mature and acquire an increased ability to prime T cells (13). In particular, mature DCs produce increased

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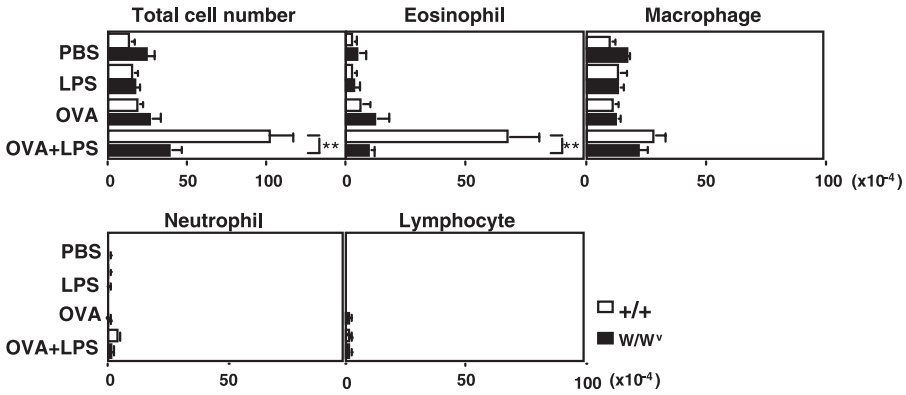

Fig. 1. LPS-mediated enhancement of eoshinophilic inflammation was not observed in mast cell-deficient mice. Wild type (+/+) and mast cell-deficient (W/W^v) mice were immunized with OVA (10 μg) in conjunction with 1 μg LPS intranasally, and 2 weeks later, OVA (25 μg) was again administrated intranasally. One day after the final OVA administration, BAL fluid was harvested and examined for infiltrating cells. Mean values, with standard deviation, of the numbers of total cells and each cell type are shown. $^{**}P<0.01$.

levels of IL-1, IL-12, and TNF-α that lead to the promotion of Th1-skewed responses (14). However, it is also well recognized in humans that LPS is a risk factor for asthma (14, 15). In some mouse models, LPS was reported to elicit airway inflammation (7, 16, 17). Recent studies on mouse mast cells (18, 19, 20) and human mast cells (20) suggested that LPS-induced activation was mediated through TLR4 expressed on mast cells.

In this review, we introduced our recent work describing the role of TLR4 on mast cells in allergic airway inflammation and in the production of Th2 cytokines, which may help explain the enhancement of allergic eosinophilic airway inflammation by LPS (21). From our studies presented here, we propose a new mechanism whereby mast cells play a crucial role for regulating eosinophilic airway inflammation. TLR4-mediated mast cell activation and modulation with increased production of Th2 cytokines such as IL-5 and IL-13 appear to control the severity of eosinophilic airway inflammation.

LPS-mediated enhancement of eosinophilic inflammation is not observed in W/W^v mice

In order to investigate the molecular basis underlying the LPS-induced mast cell activation and regulation of allergic eosinophilic airway inflammation, we used an experimental model with a low-dose LPS administration, in which Th2-dependent eosinophilic airway inflammation is selectively induced (7). To examine the effect of LPS on OVA-induced allergic inflammation, wild type (+/+) and mast cell–deficient W/W^v mice were treated intranasally with soluble OVA in conjunction with a low dose LPS (1 μg), and 2 weeks later, the mice were challenged intranasally with OVA. Under these conditions, Th2-skewed eosinophilic inflammation was reproducibly induced in the lung. Two days after the final OVA challenge, BAL fluid was harvested and examined for infiltrating leukocytes. A modest (approx. twofold) increase in the number of eosinophils was detected in mice treated with OVA alone as compared with those in PBS- or LPS-treated mice (Fig. 1). However, the numbers of total cells and eosinophils were significantly increased when wild type +/+ mice were treated with both OVA and LPS (Fig. 1). Intriguingly, when W/W^v mice were treated with OVA + LPS, the numbers of total infiltrating cells and eosinophils did not increase significantly as compared with those in +/+ mice (Fig. 1). These results point to an important role for mast cells in low-dose LPS–mediated enhancement of eosinophilic infiltration in BAL fluid.

Adoptive transfer of wild type (+/+) bone marrow-derived mast cells (BMMCs) reconstitutes LPS-mediated enhancement of eosinophilic airway inflammation in W/W^v mice

To further investigate the requirement of mast cells in enhanced eosinophilic inflammation induced by OVA + LPS, wild type mast cells were adoptively transferred into W/W^v mice. Bone marrow-derived mast cells (BMMCs) were generated by culturing bone marrow cells with IL-3 for four weeks. The administration of +/+ BMMCs resulted in the dramatic eosinophilic infiltration in the BAL fluid of W/W^v mice immunized OVA and LPS when compared to mice not given wild type (+/+) BMMCs (21). No apparent affect was observed in the numbers of macrophages, neutrophils, and lymphocytes with the +/+ BMMC transfer. The results thus far indicated that mast cells are critical for LPS-mediated enhancement of allergic airway eosinophilic inflammation.

Since TLR4 is a known receptor for LPS (22), we next examined the involvement of TLR4 molecules on mast cells in the LPS-mediated enhancement of airway inflammation. BMMCs prepared from wild type (+/+) or TLR4 KO mice were transferred into W/W^v mice. As anticipated, the levels of eosinophilic infiltration were enhanced by the administration of +/+ BMMCs but not

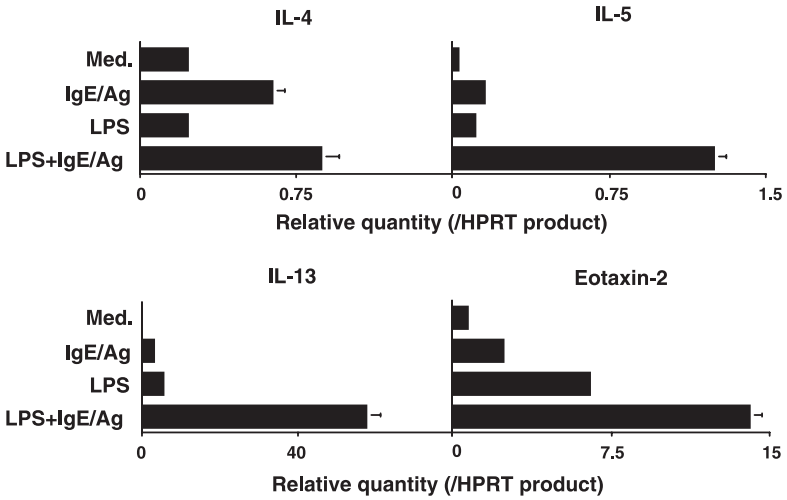

Fig. 2. Cytokine expression profiles in BMMCs treated with LPS. BMMCs were stimulated with combinations of LPS and IgE/Antigen. Transcriptional levels of IL-4, -5, and -13 and Eotaxin-2 were determined by quantitative RT-PCR analysis.

of TLR4 KO BMMCs (21). Collectively, these results clearly indicate that TLR4 on mast cells play a critical role in low dose LPS-mediated enhancement of airway eosinophilic inflammation.

Treatment with LPS modulates cytokine production profiles of mast cells

In order to analyze the molecular changes induced in mast cells by LPS treatment, BMMCs were stimulated with IgE/Antigen in the presence or absence of LPS, and mRNA expression of IL-4, IL-5, IL-13, and Eotaxin-2 was assessed. The obvious synergistic effects in the expression of IL-5, IL-13, and Eotaxin-2 were detected in LPS-treated BMMCs after co-stimulation with LPS and IgE/Ag (Fig. 2).

To assess the effect of LPS more preciously, BMMCs were cultured with or without LPS treatment (10 μg/ml) for one week in vitro, and then BMMCs were restimulated with PMA plus ionomycin, and the ability to produce various cytokines was assessed. The levels of IL-5, IL-13, and Eotaxin-2 were substantially increased after LPS treatment, whereas the production of IL-6 and TNF-α was only slightly increased (21).

LPS treatment induces the expression of GATA1 and GATA2 in BMMCs

Although GATA3 is critical for chromatin remodeling of the Th2 cytokine gene loci (23, 24) and transcription of the IL-5 and IL-13 genes in Th2 cells (25 – 27), GATA3 is not expressed in BMMCs. In contrast, GATA1 and GATA2 are expressed and play a key role in mast cell differentiation (28, 29). Therefore, the changes in the expression levels of protein expression of GATA1 and GATA2 were examined in BMMCs treated with LPS. The levels of GATA1 and GATA2 but not GATA3 were substantially increased in the BMMCs after LPS treatment (21). The LPS-induced increase in the levels of GATA1 and GATA2 protein was not detected in TLR4 KO BMMCs. Thus, TLR4-mediated signaling is critical for GATA1 and GATA2 up-regulation in BMMCs upon LPS treatment (21).

Finally, the role of GATA1 and GATA2 in transcription of Th2 cytokines in mast cells was examined by a reporter gene assay using a MC9 mast cell line. The introduction of GATA1 but not GATA2 or GATA3 into MC9 cells resulted in substantial induction of the reporter activities of IL-4 and IL-5 promoters (21). This result suggests that GATA1 could control the expression

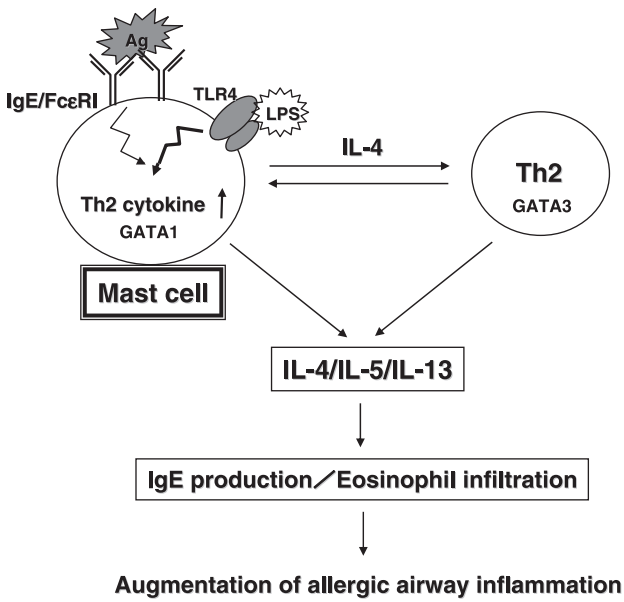

Fig. 3. Augmentation of allergic airway inflammation through the TLR4-mediated modification of mast cell functions (hypothesis).

level of Th2 cytokines in mast cells.

Conclusion

In conclusion, in a mouse allergic asthma model, the present findings show that mast cells play a crucial for LPS-mediated enhancement of the eosinophilic airway inflammation (Fig. 3). Moreover, TLR4 molecules on mast cells were critical for the LPS-induced mast cell activation and functional modulation (Fig. 3). Thus, a search for specific inhibitors acting on the TLR4-mediated signal transduction pathway could lead to a new approach for the treatment of inflammation in patients with bronchial asthma, particularly during respiratory infection.

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