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EXPERIMENTAL ALLERGY AND IMMUNOLOGY

The adjuvant-like activity of staphylococcal enterotoxin B in a murine asthma model is independent of IL-1R signaling

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Abstract

Background: Staphylococcal enterotoxin B (SEB) is a superantigen known to be a modulator of chronic airway inflammation in mice and humans, yet little is known about the mechanisms that regulate its interaction with the innate immune system. We investigated this mechanism in a murine model of allergic airway inflammation induced by OVA (ovalbumin) in the presence of SEB.

Methods: Superantigen-induced allergic inflammation was studied in IL-1R knock-out (KO) mice exposed to OVA+SEB. Multicolor flow cytometry was used to analyze the inflammatory cell profile in airways and lymph nodes. Production of IL-4, IL-5, IL-10, and IL-13 in lymph nodes was assessed by Luminex technology.

Results: In wild-type mice, endonasal instillation of OVA+SEB induced a pulmonary inflammation, characterized by an increase in the number of eosinophils, T cells, and dendritic cells and in the production of Th2 cytokines and OVA-specific IgE. In IL-1R KO mice exposed to OVA+SEB, attraction of CD4⁺ cells and production of Th2 cytokines were reduced. However, knocking out IL-1R did not affect any of the features of allergic airway inflammation, such as bronchial eosinophilia, OVA-specific IgE production and goblet cell metaplasia.

Conclusion: We provide new insights into the mechanisms of airways allergy development in the presence of bacterial superantigen. The asthma features induced by OVA+SEB, such as bronchial eosinophilia, goblet cell proliferation, production of OVA-specific IgE and increase in inflammatory dendritic cells, are IL-1R independent. Yet, IL-1R signaling is crucial for CD4 cell accumulation and Th2 cytokine production.

Severe chronic airway inflammation of the upper airways, also known as chronic rhinosinusitis (CRS), is often accompanied by increased *S. aureus* colonization, which is seen in about 64% of CRS patients with nasal polyps (CRSwNP), 67% of patients with CRSwNP and asthma and 87% of patients with aspirin-intolerant disease (1–3). Toxins produced by *S. aureus* have an immunomodulatory effect on airway inflammation in mice and in humans (4). Patients with CRS and asthma mount a specific IgE response to staphylococcal superantigens (3, 5), and in mice staphylococcal enterotoxin B (SEB) breaks tolerance to inhaled and contact allergens (6–8) and aggravates local inflammatory

responses in the airways induced by cigarette smoke (9). Indeed, it is well known that SEB and other superantigens can activate T cells *via* cross-linking of $\nu\beta$ chains of the TCR receptor on T cells to MHCII on antigen-presenting cells or by delivering an activation signal directly *via* MHC class II molecules (10). SEB is capable of polyclonal T-cell stimulation and induces the production of pro-inflammatory and Th2-polarizing cytokines such as IL-4, IL-5, IL-13, and other cytokines (6, 11, 12). Increased levels of eotaxin, a potent chemoattractant and survival factor for eosinophils, were observed in allergic murine airways after SEB treatment (12). We recently developed a mouse model that mimics the

immunomodulatory role of superantigens (e.g. SEB) when the airway is sensitized with ovalbumin (OVA) (7). By using this model, we demonstrated that SEB, but not other superantigens such as staphylococcal enterotoxin A and toxic shock syndrome toxin, aggravates allergic airway inflammation and bronchial hyperreactivity by a CD4-dependent mechanism in mice (7). Epithelial cells respond to SEB by secreting factors that induce eosinophil survival (13). SEB also promotes the induction of Th-2 cells *via* polarization of dendritic cells (DCs) (14). It also reinforces the Th-2 allergic inflammation *via* activation of epithelial cells and release of IL-4, IL-33, and thymic stromal lymphopoietin (14–16). All these data strongly indicate that SEB plays a role in modulation of severe airway inflammation.

Stimulation of human monocytes and epithelial cells by SEB leads to the production of IL-1 β and other pro-inflammatory cytokines (11, 17). IL-1 α and IL-1 β function by binding to IL-1R type I (IL-1R) downstream of which is the adapter protein MyD88 (18). Evidence indicates that the IL-1R–Myd88 pathway participates in the regulation of the allergic airway response (19). The role of the IL-1R pathway in allergic airway inflammation has been demonstrated in several mouse models of allergic airway inflammation. When the IL-1R pathway is blocked (IL-1R KO, IL-1 α KO, or IL-1 β KO mice), the bronchial eosinophilia caused by OVA sensitization and challenge is reduced due to decreased recruitment of Th2 cells to the lungs (20, 21). All these data suggest a role for the IL-1R pathway in the induction of the allergic airway response, but it is not known whether this pathway is required in the allergic airway response modulated by SEB. In this study, we show that SEB-induced allergic response involves IL-1R-dependent attraction of CD4⁺ lymphocytes and Th2 cytokine production. However, in contrast to asthma induced by allergens in the absence of adjuvants, in SEB-induced airway inflammation, IL-1R has no effect on the development of typical asthma features such as bronchial eosinophilia, goblet cell proliferation, production of OVA-specific IgE and attraction of inflammatory DCs.

Materials and methods

Mice and experimental protocol

Wild type (WT) Balb/c, WT C57/BL6, IL-1R KO mice and DO11.10 mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA). Animal experiments were approved by the Institutional Animal Ethical Committee of Ghent University.

Protocol of allergic airway sensitization

Briefly, mice were anesthetized by gaseous anesthesia with isoflurane/air and placed in a supine position. In the nose was instilled 50 μ l of 0.9% sodium chloride (SAL), OVA (10 mg/ml), SEB (10 μ g/ml), or SEB combined with OVA. At least five mice were included in each group. The applications were repeated seven times on alternate days according to the protocol established in our laboratory (7). On day fourteen, mice were killed by a lethal i.p. injection of Nembutal (Ceva, Sante Animale, Belgium).

Bronchoalveolar lavage, lung digestion, and histological analysis

Bronchoalveolar lavage and lung digest were performed as described before (7), and cells were used for the preparation of cytopins and for flow cytometry analysis. A list of the antibodies used in the study is in Table S1. After performing BAL, lungs were repeatedly perfused with 0.9% sodium chloride. Part of the left lung was fixed overnight in 10% formalin and embedded in paraffin. Tissue sections of 4 μ m were stained with hematoxylin and eosin and with periodic acid Schiff stain.

Measurement of ovalbumin-specific immunoglobulin E (IgE)

Peripheral blood was taken from anesthetized mice by retro-orbital bleeding on day 14. OVA-specific IgE in serum was analyzed as described before (7).

In vitro lymph node re-stimulation assay

Peribronchial and submandibular lymph nodes were dissected, and a single cell suspension was prepared using a cell strainer (70 μ m, Falcon, Becton Dickinson, Erembodegem, Belgium). Cells were resuspended in RPMI 1640 medium containing penicillin, streptomycin, 10% FCS, L-glutamine, and 0.1% β -mercaptoethanol. Cells were stimulated for 5 days at 37°C with OVA, SEB, or bovine serum albumin (BSA) (10 μ g/ml) in RPMI in the presence of 1×10^6 splenocytes from naive mice. The supernatants were collected, and the levels of IL-4, IL-5, IL-13 and IL-10 were determined using a Luminex (R&D Systems, Oxon, UK).

In vivo T-cell proliferation assay

Peripheral lymph nodes and spleens from naive OVA TCR-transgenic DO11.10 mice were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE) (Invitrogen, Merelbeke, Belgium) according to the manufacturer's instructions. Of these, 5×10^6 cells were injected intravenously (*i.v.*) into naive WT and IL-1R KO mice. After 24 h, the mice received intratracheally (*i.t.*) 100 μ g of OVA in 50- μ l PBS with or without 500-ng SEB. Five days later, peribronchial and submandibular lymph nodes were obtained, and the proliferation of CFSE-labeled OVA-specific TCR-transgenic T cells (TCR⁺CD4⁺CFSE⁺) was analyzed by FACS Canto II (BD). Division index was calculated by dividing the number of cells by CFSE content, after correction for the multiplying effect of division.

Results

IL-1R signaling is not required for eosinophilic inflammation, IgE production, and goblet cell hyperplasia, in the murine OVA+SEB model

Allergic airway inflammation was induced in mice by endo-nasal application of OVA (10 mg/ml), SEB (10 μ g/ml), or a combination of both. WT mice that received OVA+SEB developed airway inflammation. The total number of cells in

OVA+SEB WT group was statistically greater than in mice sensitized only with OVA or SAL (Fig. 1A). Significantly more lymphocytes and eosinophils were observed in BAL of the OVA+SEB group than in the OVA and the SEB groups (Fig. 1B,C). Moreover, in the lung tissue of WT mice, there was a tendency to an increase in eosinophils in the OVA+SEB group compared with the OVA group (Fig. 1E). In the lung tissue of IL-1R KO mice, OVA+SEB sensitization induced significantly more eosinophilia compared with mice that were sensitized only with OVA.

First, we examined whether endonasal SEB application induces IL-1 α or IL-1 β in our model. As shown in Fig. 2A, a combination of 500 μ g OVA and 500 ng of SEB increased the IL-1 β levels in lung tissue homogenates of WT mice suggestive for the role of IL-1R pathway in the control of SEB-induced airway inflammation. The levels of IL-1 α in lung homogenates were below detection limit.

The analysis of inflammatory cells in the lungs of IL-1R KO mice showed significantly fewer eosinophils after sole OVA treatment compared with WT mice. No difference in

the numbers of eosinophils could be found between IL-1R KO and WT mice after OVA+SEB application.

In BAL of WT mice, neutrophil numbers were higher in the groups treated with SEB or OVA+SEB (Fig. 1D) than with SAL or OVA alone. In BAL of mice sensitized with SEB, fewer neutrophils were found in IL-1R KO mice than in WT mice (Fig. 1D). A tendency to less neutrophilic inflammation in BAL was also seen in IL-1R KO treated with OVA+SEB. Similarly, after sensitization with OVA, the lungs of IL-1R KO mice had fewer neutrophils than that of WT mice. The number of neutrophils was not significantly different in the lungs of WT and IL-1R KO mice after OVA+SEB (Fig. 1F).

As we previously demonstrated, a combination of OVA+SEB resulted in increased serum levels of OVA-specific IgE as compared to OVA and SEB alone groups (7). The titers of OVA-specific IgE in IL-1R KO mice were comparable to WT mice (Fig. 2B). A semi-quantitative analysis of goblet cell proliferation did not show a difference between IL-1R KO and WT mice (Fig. 2C).

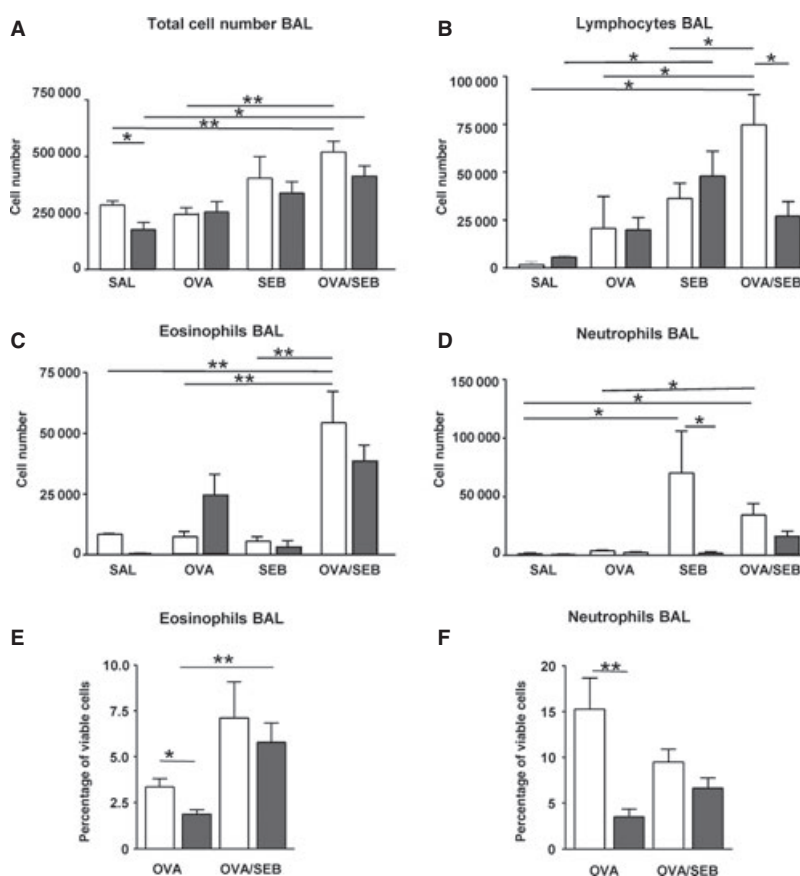


Figure 1 Analysis of airway inflammation. Cellular content of the bronchoalveolar lavage (BAL) was estimated from cytopins stained with May-Grunwald-Giemsa. Total cell numbers were counted in BAL (A), lymphocytes (B), eosinophils (C), and neutrophils (D). (* P < 0.05, ** P < 0.001). Wild-type mice (white bars) and IL-1R KO

mice (gray bars) were endonasally instilled with saline (SAL), 500 μ g ovalbumin (OVA), 500 ng of staphylococcal enterotoxin B (SEB) or OVA+SEB. (E) Lung eosinophils (GR^{low}CCR3⁺CD11b^{med}F4/80^{med}) and (F) lung neutrophils (GR^{high}CCR3⁺CD11b⁺F4/80⁺) were counted by flow cytometric analysis of enzymatically digested lung tissue.

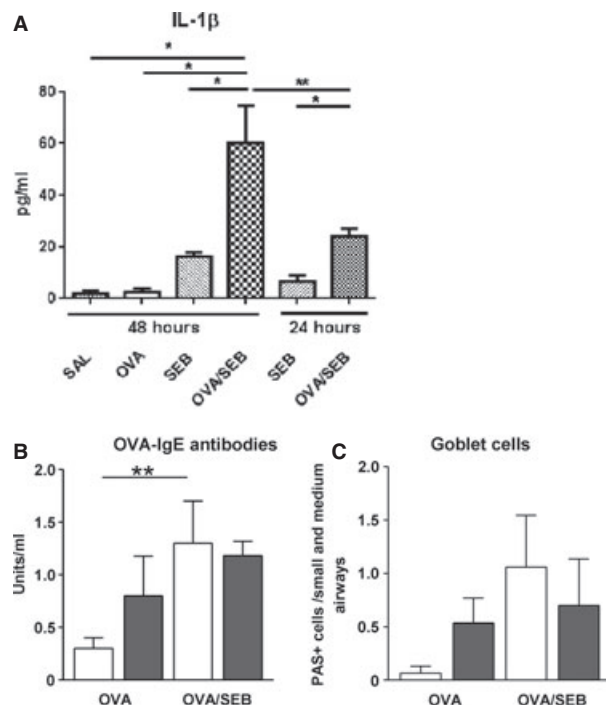


Figure 2 (A) Analysis of IL-1 β levels in lung homogenates of wild type mice that received single endonasal application of saline (SAL), 500 μ g ovalbumin (OVA), 500 ng of staphylococcal enterotoxin B (SEB) or OVA+SEB. (B) Analysis of OVA-specific IgE (Units/ml) production in serum. (C) Semi-quantitative analysis of goblet cells in small and medium airways in the lungs. Wild type mice (white bars) and IL-1R KO mice (gray bars) were endonasally instilled with OVA with or without SEB. $n = 5-6$ mice per group (* $P < 0.05$, ** $P < 0.001$).

Lymphocytes attraction to the airways upon allergic airway sensitization is diminished in IL-1R KO mice

In a previous study, we demonstrated that CD4 cells are essential mediators of SEB-induced allergic airway inflammation (7). So here, we analyzed T-lymphocyte populations in BAL and lungs of mice after induction of airway inflammation. Total lymphocyte percentage in cytopins of BAL of IL-1R KO mice was significantly lower than that of WT mice after OVA+SEB (Fig. 1B). Flow cytometric analysis of BAL showed that after treatment with OVA+SEB, IL-1R KO mice had fewer CD4⁺ T cells and activated CD4⁺ (CD4⁺CD25⁺) T-cells than WT mice (Fig. 3A,B). The numbers of Tregs were not decreased (data not shown). However, there was no difference in the numbers of CD3⁺CD8⁺ cells between the WT and IL-1R KO mice treated with OVA or OVA+SEB (Fig. 3C).

Likewise, the percentages of CD3⁺, CD3⁺CD4⁺, and CD4⁺CD25⁺ lymphocytes in single cell suspensions made from lungs of IL-1R KO mice treated with OVA+SEB were significantly less than in the corresponding WT mice (Fig. 3D-F).

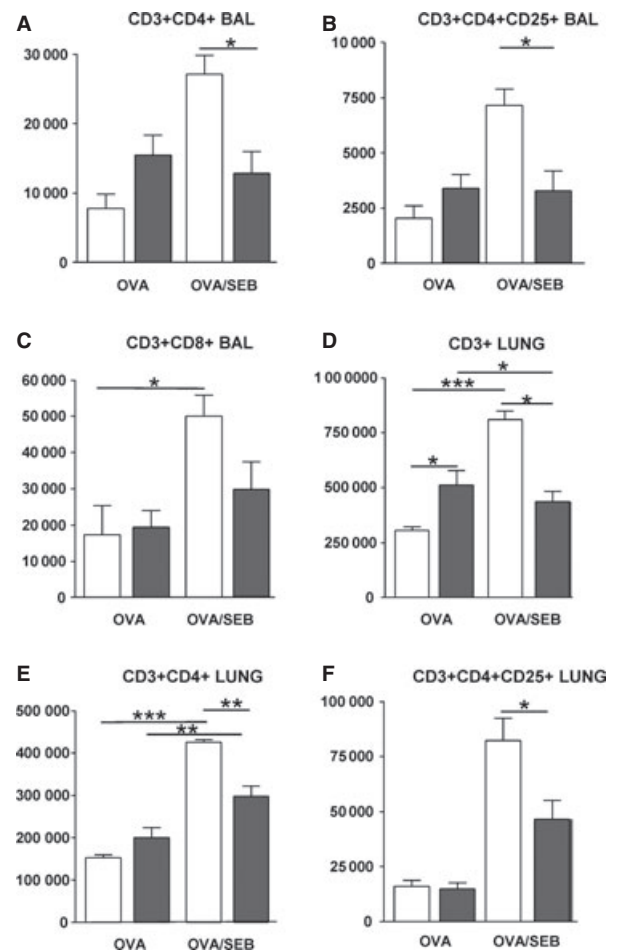


Figure 3 Flow cytometric analysis of lymphocyte subtypes. Bronchoalveolar lavage (A, B, C). Lungs (D, E, F). Wild-type mice (white bars) and IL-1R KO mice (gray bars) were endonasally instilled with 500- μ g ovalbumin (OVA) or OVA in combination with 500 ng of staphylococcal enterotoxin B (SEB). (* $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$).

Production of Th2 cytokines *in vitro* is reduced in IL-1R KO mice

As the T-cell activities causing inflammation were compromised in IL-1R KO mice sensitized with OVA+SEB, we presumed that T-cell priming is deficient in these mice. So, we compared the magnitude of the T-cell priming response to SEB and OVA in IL-1R KO and WT mice. The peribronchial lymph nodes of mice sensitized with OVA or OVA+SEB were re-stimulated *in vitro*, and the production of Th2 cytokines was analyzed. The re-stimulation of lymph nodes of mice sensitized with OVA or OVA+SEB resulted in a Th2 cytokine response after re-stimulation with OVA. Importantly, significantly less IL-10 and IL-5 were produced in IL-1R KO than in WT mice sensitized with OVA or OVA+SEB (Fig. 4 A,B). However, the levels of IL-4 and IL-13 were below the detection limit in all conditions re-stimulated with

OVA. Therefore, in addition to re-stimulation with OVA, we also used SEB (10 µg/ml), known for its polyclonal T-cells activation, to re-stimulate the lymph nodes from mice sensitized with OVA or with OVA+SEB. IL-1R KO mice produced significantly less of the key Th2 cytokines (IL-4, IL-5, IL-10, and IL-13) after re-stimulation with SEB in mice sensitized with OVA or OVA+SEB (Fig. 4 C–F).

T-cell proliferation is not affected by IL-1R deficiency

To analyze T-cell proliferation *in vivo*, we labeled OVA-specific TCR-transgenic OT-II T cells with CFSE and injected them in WT mice and IL-1R KO mice. We then sensitized the mice with OVA or OVA+SEB. CD4⁺ OT-II cells injected into WT mice tended to have higher proliferation rates after OVA+SEB treatment than after OVA treatment alone. However, the T-cell proliferation rates *in vivo* after OVA+SEB treatment were not different between WT and IL-1R KO (Fig. 4G).

OVA+SEB sensitization facilitates the migration of inflammatory DCs to the airways and local lymph nodes independently of IL-1R

DCs are among the first cells in the airways to sense allergens. They process and present the antigen and migrate from the tissues to the draining lymph node, where they come in contact with cells of adaptive immunity (22). We evaluated the attraction of different subtypes of dendritic cells to the airways after different sensitization protocols. Only few conventional DC and plasmacytoid DCs (pDCs) were present in the lungs of WT mice after SAL. The numbers of CD11c⁺MHCII^{hi} and CD11b⁺MHCII⁺CD103[−] DCs were significantly higher in the lungs of mice that received OVA+SEB as compared to OVA and SEB alone (Fig. 5A). Similarly, CD11c⁺MHCII^{hi} and CD11b⁺MHCII⁺CD103[−] DCs were increased in the BAL and lymph nodes of WT mice. Remarkably, more CD11c⁺MHCII^{hi} cells were also found in the lymph nodes of mice after OVA+SEB than after OVA or SEB alone (Fig. 5B). In addition, pDCs (PDCA1⁺CD11c⁺) were also induced in the lungs of WT mice after OVA+SEB than after SEB or OVA alone. No PDCA1⁺ pDCs are observed in the BAL of WT mice. We analyzed whether OVA+SEB induced the accumulation of DCs in IL-1R KO mice. However, no difference in the numbers of CD11c⁺MHCII^{hi} and CD11c⁺MHCII⁺CD11b⁺ DCs was found in the BAL and lungs of WT mice and IL-1R KO mice after OVA+SEB (Fig. 5 C–F).

Discussion

The respiratory tract is continuously challenged by inert protein antigens and environmental factors in addition to both pathogenic and nonpathogenic microorganisms. Exposure to allergens does not elicit clinical symptoms in nonallergic individuals but rather causes the induction of tolerance (23). We have demonstrated that bacterial factors such as SEB could break a state of tolerance and lead to the development of an

allergic immune response in the airways when inhaled together with an allergen (7).

In the current study, the mechanism of this altered immune tolerance was further studied with focus on the IL-1R

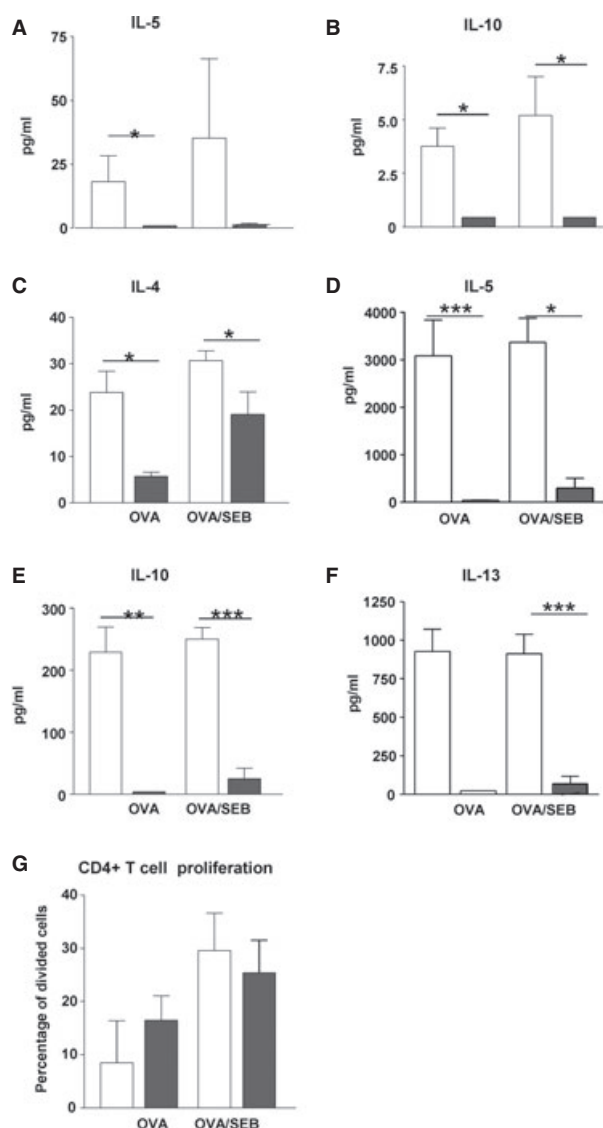


Figure 4 Lymph node re-stimulation assay. Following the different sensitization protocols, mediastinal lymph nodes were dissected and re-stimulated with 10 µg/ml ovalbumine (OVA) (A–B) or 10 µg/ml staphylococcal enterotoxin B (SEB) (C–F) for 5 days. IL-4, IL-5, IL-10, and IL-13 protein levels were determined in cell culture supernatants. Results are expressed as mean ± SEM. *n* = 5–6 mice per group (**P* < 0.05, ***P* < 0.001, ****P* < 0.0001). (G) Analysis of CD4⁺ T-cell proliferation *in vivo* using CFSE-labeled OVA-specific TCR-transgenic CD4⁺ OT-II T cells after 5 days *in vivo* in the presence of OVA or OVA+SEB. Percentage of viable CD4 cells expressing CFSE and Vα2 in each group represents cells that have undergone 0–8 cell divisions as calculated by the FlowJo software. The data are expressed as mean of divided CD4 + TCR+ cells as a percentage of viable CD4 cells ± SEM.

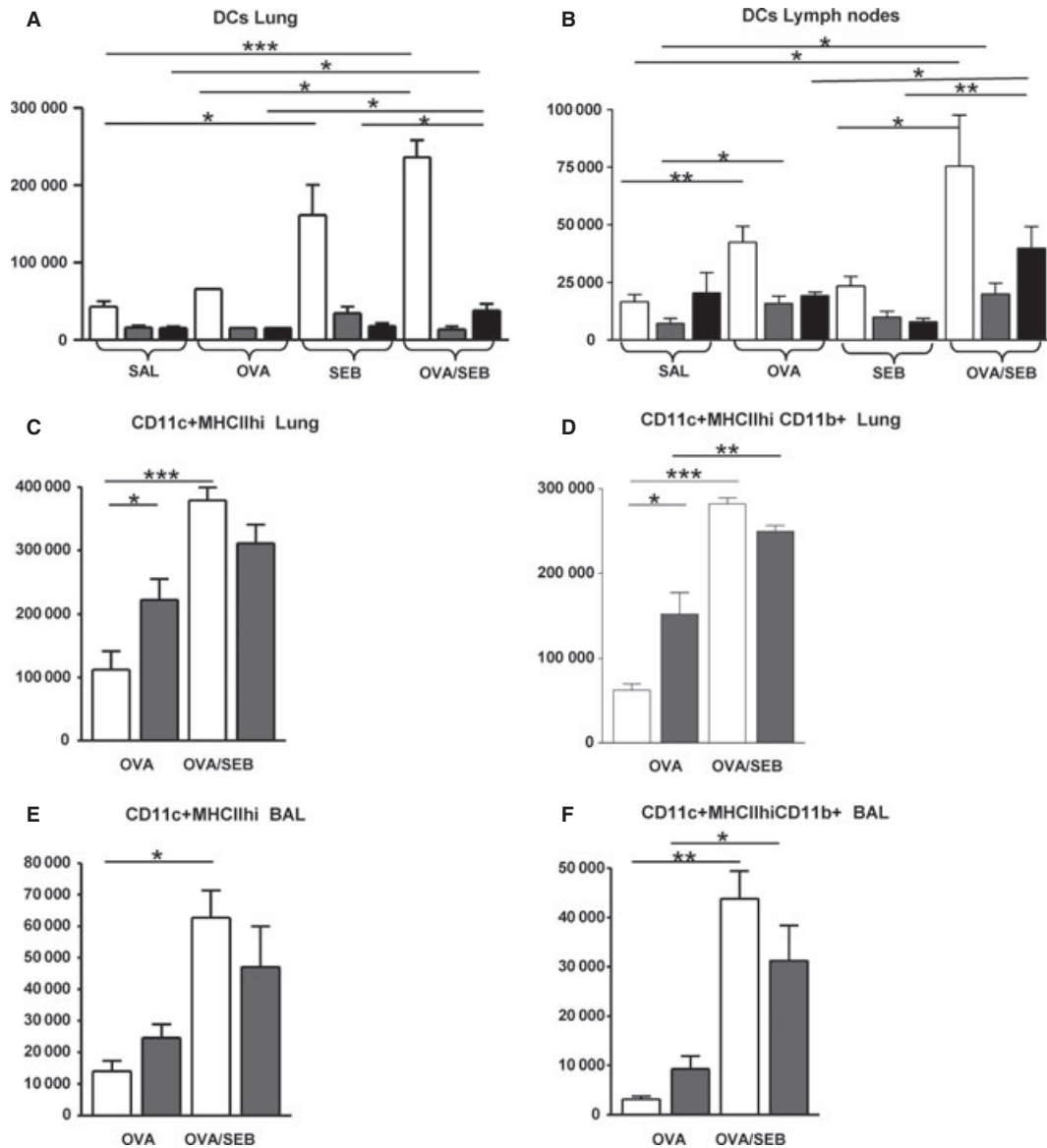


Figure 5 Flow cytometric analysis of dendritic cells. Analysis of dendritic cell subtypes in the lungs (A) and the lymph nodes (B) of wild-type mice that received endonasal application of saline (SAL), 500- μ g ovalbumin (OVA), 500 ng of staphylococcal enterotoxin B (SEB) or OVA+SEB. Total number of dendritic cells positive for CD11c⁺MHCII^{hi} (white bars) and CD103⁺CD11b⁺ (gray bars)

PDCA1⁺CD11c⁺ (black bars) gated on CD11⁺MHCII^{hi} cells. The dendritic cells in the lungs (C,D) and in BAL (E,F) of wild-type mice (white bars) and IL-1R KO mice (gray bars) that were endonasally instilled with OVA alone or OVA combined with SEB (* P < 0.05, ** P < 0.001, *** P < 0.0001).

pathway. We previously showed that SEB induces the production of IL-1 β in the human allergic mucosa, along with an increase in the production of other cytokines such as IL-2, IL-4, IL-5, and IL-13 (11). Here, we demonstrate that concomitant application of OVA and SEB in WT mice results in IL-1 β increase; however, no induction of IL-1 α was observed. Fewer T cells, and especially fewer CD4⁺ T cells and activated CD4⁺ T-cells, were attracted to the airways of IL-1R KO mice after OVA+SEB compared with controls. However, this significant but relatively small decrease in CD4⁺ T cells

does not cause corresponding deficits in goblet cells in the airways of IL-1R knockout mice. The re-stimulation of lymph nodes *in vitro* resulted in reduced levels of IL-4, IL-5, IL-10 in IL-1R KO mice, supporting the role of IL-1R in SEB-induced T-cell proliferation. It has been pointed out that the role of IL-1R signaling could differ in asthma models induced in the presence of adjuvant or in the absence thereof. A defective IL-1R pathway in a model of OVA-induced asthma in the absence of alum adjuvant was shown to cause an impairment of Th2 responses and to reduce the

production of IL-4, IL-5, and IL-13 (21,24). IL-1 α KO and IL-1 β KO mice were also shown to have reduced OVA-specific T-cell responses (25), which could be explained by the fact that IL-1 α and IL-1 β are both equally important for the attraction and proliferation of Th2 cells, because IL-1 β is required for IL-1 α secretion (26). IL-1 was shown to enhance the antigen-driven responses of CD4 and CD8 cells by increasing their survival (27). Moreover, in mice deficient in IL-1R antagonist (IL-1Ra), which is an endogenous IL-1R ligand blocking signaling of both IL-1 α and IL-1 β , the allergic response is aggravated, airway hyperreactivity is increased, and Th2 cells in the airways become more numerous (25).

However, in contrast to our findings, it has been shown that in the presence of an adjuvant, such as aluminum hydroxide, allergic asthma develops in IL-1R-independent manner. The asthma induced in IL-1R KO mice by treatment with OVA-alum is characterized by T-cell migration, pulmonary allergic Th2 responses, CD4 cells priming, eosinophilic inflammation, and the IgG, IgE, and IgA antibody responses, all of which are comparable to those seen in WT mice (24, 25, 28). It has been suggested that the OVA-alum protocol leads to cell death and the release of danger molecules such as uric acid and DNA (29, 30), which are sensed in an IL-1R-independent manner (31).

We show that WT mice receiving OVA+SEB develop eosinophil accumulation in the airways, in an IL-1R-independent fashion. Similarly, Myd88 KO and interferon responsive factor 3 (IRF3) KO mice that received OVA+SEB had eosinophil accumulation in airways comparable to controls (data not shown), supporting the observation that IL-1R is dispensable for eosinophil attraction after OVA+SEB treatment. Our data are in agreement with observations made by Schmitz et al. (24), who showed that in the presence of alum adjuvant, typical asthma features such as eosinophilia develop in an IL-1R-independent manner. This observation is probably due to the strong effect of SEB on eosinophil survival as shown by Huvenne et al. (13). In mice, staphylococcal enterotoxins were shown to have pro-inflammatory effects resulting in skin hypersensitivity reactions (32), asthma (6), and smoke-induced inflammation (9). There is also increasing evidence that *S. aureus* enterotoxins act as modulators in the pathogenesis of chronic rhinosinusitis with nasal polyps, because IgE specific for *S. aureus* enterotoxins was shown to be increased in CRS with nasal polyps and correlated with markers of eosinophil activation and recruitment (5). It has also been pointed out that the IL-1R pathway contributes to the control of eosinophil migration in several models of allergic airway inflammation (20, 21, 28).

DCs play a crucial role in the development of the allergic airway response. In allergic airways, uptake and presentation of the allergen by DCs to naive T cells is followed by a Th2 immune response characterized by production of IL-4, IL-5, and IL-13 (22). Migration of antigen-loaded DCs to lymph

nodes and their activation were shown to be strongly reduced in NLRP3-deficient mice, and these effects were accompanied by deficient production of IL-1 β , IL-33, TLSP, and IL-6 (21). Maturation and migration of DCs was shown to be dependent on presence of mature IL-1 β (21). Treatment of mice with the asthma protocol results in fewer myeloid DCs and plasmacytoid DCs in the lungs and local lymph nodes of Myd88 KO mice (19). These results suggest that IL-1R signaling could play a crucial role in the activation and migration of DCs in adjuvant-free models of allergic asthma. Therefore, we examined whether IL-1R depletion interferes with activation/migration of DCs in OVA+SEB model. In our previous study, we showed that the number of allergen-loaded DCs in the local lymph nodes was higher in mice that received OVA+SEB (7). Here, we analyzed different DC subtypes that are attracted after OVA+SEB treatment. In contrast to the study by Phipps et al. (19), we observed no difference in the attraction of CD11c⁺CD11b⁺MHCII⁺ and CD11c⁺ MHCII⁺ DCs to airways between IL-1 KO and Myd88 KO mice (unpublished data). IRF3 was reported to be essential for boosting of the canonical Th2 response in the OVA-alum model (33). However, in our model, IRF3 had no effect on DC migration, and the Th2 response was not altered (unpublished data).

In conclusion, our observations support the role of the IL-1R pathway in controlling the airway inflammation when an allergen is encountered in the absence of adjuvant. However, the IL-1R pathway is dispensable after exposure to both OVA and SEB. In clinical situations, bacterial infections are often present during asthma exacerbations, and thus allergic inflammation unlikely to be controlled by blocking of IL-1R.

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Conflict of interest

The authors declare no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. List of antibodies used for flow cytometry.

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antibody	clone	Company
CD3-PE	145-2C11	e-bioscience
CD4-FITC	RM 4-5	e-bioscience
CD8a PerCP	53.6-7	e-bioscience
CD11b-PerCP-Cy5.5	M1/70	e-bioscience
CD11b PE	M1/70	e-bioscience
CD11c PE-Cy7	HL3	BD Biosciences
CD16/32	93	e-bioscience
CD25-APC	PC61.5	e-bioscience
GR-1 FITC	RB6-8C5	e-bioscience
CCR3-PE	83103	BD Biosciences
MHCII FITC	M5/114.15. 2	e-bioscience
F4/80 APC	BM8	e-bioscience
CD103-biotin	2E-07	e-bioscience
PDCA-1-APC	JF05- 1C2.4.1	Miltenyi Biotec
CD40 PE	1C10	e-bioscience
CD80 biotin	B7-1	e-bioscience
CD86 APC	B7-2	e-bioscience
CD25 APC	PC 61.5	e-bioscience
Foxp3 PE	FJK-16s	e-bioscience

Suppl. Table 1. List of antibodies used for flow cytometry. Anti-mouse antibodies labeled with allophycocyanin (APC), fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein (PerCP) were used at concentrations recommended by the manufacturer to perform immunophenotyping of cells in airways. Granulocyte-differentiation antigen-1 (GR1), C-C chemokine receptor type 3 (CCR3), mouse plasmacytoid dendritic cell antigen-1 (mPDCA1), major histocompatibility complex (MHCII).