

Long-term exacerbation by interleukin 13 of IgE-mediated eosinophilia in rats

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Abstract

Recent work shows that at least two cycles of antigen challenge applied in a 7-day interval are required to yield tissue eosinophil accumulation in IgE-passively sensitized rats. Since interleukin (IL)-13 is widely regarded as a key mediator in eosinophilic responses associated with mast cells and IgE, we investigated whether this cytokine could replace the first cycle of sensitization and challenge in its proeosinophilic role. We found that IL-13 (25 and 50 ng/cavity) injected into the rat pleural space led to eotaxin generation and a dose-dependent accumulation of eosinophils following IgE-passive sensitization and challenge 7 days later. IL-13 failed to cause eosinophil chemotaxis in vitro but induced eosinophil accumulation into the pleural cavity of naïve rats, which peaked 1 day and faded 72 h post-challenge. No changes were found 1 week after intrapleural injection of IL-13, except a ~40–50% increase in the number of adhered and non-adhered pleural mast cells. As recovered from the pleural effluent 1 week after IL-13, mast cells expressed the same amount of IgE bound on their surface as compared to controls. However, they generated 3-fold more LTC₄ following IgE-sensitization and challenge in vitro, keeping intact the amount of histamine released. Finally, pretreatment with zileuton (50 µg/cavity) 1 h before allergen challenge prevented eosinophil accumulation in those animals injected with IL-13 1 week before. In conclusion, our findings show that IL-13 causes a long-term exacerbation of the IgE-mediated eosinophilic response in a mechanism associated with heightened cysteinyl-leukotriene (cys-LT) production by resident mast cells.

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1. Introduction

Allergic diseases such as asthma and rhinitis are increasing in prevalence in most countries around the world. One of the hallmarks of these diseases is the polarization of the Th2 responses and increased production of an array of type 2 cytokines including IL-4, IL-5, IL-9 and IL-13 [1–3]. This Th2 milieu favors the generation of IgE and activation of mast cells, lymphocytes and eosinophils leading to inflammation and disease [4–6]. It is now clear that allergen specific IgE is directly responsible for the synthesis and release of a variety of inflammatory mediators and cytokines that induce the early phase response [7,8]. However, despite the capacity of mast cell products to promote the traffic of leukocytes to challenged sites [9,10], the relative importance of the IgE–mast cell system to the installation and/or maintenance of leukocyte recruitment remains controversial.

For instance, it has been demonstrated that neutralization of serum IgE inhibited the recruitment of eosinophils into the lung and production of proallergic cytokines IL-4 and IL-5 [11]. Moreover, treatment of allergic asthmatic subjects with a non-anaphylactogenic anti-IgE antibody reduced circulating IgE and both early and late phase reactions [12]. However, studies using IgE knockout mice [13,14], B-cell deficient mice [15] and mast cell deficient mice [16] revealed that allergen-induced eosinophilic airway inflammation can occur in the absence of activation of the IgE–mast cell system. It has also been shown that a single cycle of IgE passive sensitization and challenge induced intense plasma leakage in mice [17] and rats [18,19] but failed to evoke eosinophil recruitment. Interestingly, we found a selective exacerbation of the eosinophilic response when, sites which had recovered from the first antigenic provocation, were re-stimulated within 7 days [19]. Since interleukin (IL)-13 is widely regarded as a key mediator in eosinophilic responses associated with mast cells and IgE [20–22], we investigated here whether IL-13 could replace the first cycle of sensitization and challenge in its proeosinophilic priming effect. We found that IL-13 is indeed able to promote a long-term exacerbation of the allergen-mediated eosinophil recruitment into the pleural space of IgE-passively sensitized rats, in a mechanism dependent on increased cysteinyl-leukotriene generation from resident mast cells.

2. Materials and methods

2.1. Animals

All experiments were performed in Wistar rats of both sexes, weighing between 180 and 220 g, obtained from the breeding colony of the Oswaldo Cruz Foundation, Rio de Janeiro, Brazil. The animals were housed in groups of five and maintained on a 12-h light/dark cycle, with water and food ad libitum until use. The Ethics Committee for Care and Use of Laboratory Animals of The Oswaldo Cruz Foundation approved the experimental protocols employed in this study (License no. 0085-02).

2.2. Antibodies and reagents

Mouse monoclonal IgE specific for dinitrophenol was kindly provided by Dr. Annie Prouvost-Danon (Unité d'Immuno-Allergie, Institut Pasteur, Paris France). Biotinylated and non-biotinylated anti-eotaxin as well as recombinant murine IL-13 were obtained from R and D Systems. Toluidine blue and trypan blue were from MERCK, eotaxin was from Peprotech, anti-murine biotinylated IgE monoclonal antibody was from Southern, neutravidin-peroxidase was from Pierce, K-blue was from Neogen and Zileuton was purchased from Abbott Laboratories (North Chicago, Ill, USA). Dinitrophenylated bovine serum albumin (DNP-BSA), bovine serum albumin (BSA), Hank's Balanced Salt Solution (HBSS), Percoll, histamine, EDTA, thimerosal, tween 20, sodium azide, streptavidin-FITC and biotinylated mouse IgG were purchased from Sigma (USA).

2.3. Pleurisy model and IL-13 administration

IgE-mediated pleurisy was carried out in rats passively sensitized with an intrapleural injection of a murine IgE mAb anti-DNP (1 µg/cavity) followed, 24 h later, by challenge with DNP-BSA (1 µg/cavity). In control groups, DNP-BSA injection was replaced by sterile isotonic 0.9% NaCl solution (saline). Recombinant murine IL-13 (25 and 50 ng/cavity), or its vehicle (saline), was injected intrapleurally into naïve rats 7 days before antigen challenge. Under this condition, in some experiments the animals were locally treated with the lipoxigenase inhibitor

zileuton (50 µg/ml), or saline, 5 min before antigen challenge (DNP-BSA). All intrapleural injections were performed under light ether anesthesia in a final volume of 0.1 ml using a 27.5-gauge needle adjusted to 3 mm in length. Four hours after antigen challenge, the animals were killed by asphyxiation over CO₂ atmosphere and the thoracic cavity was rinsed with 3 ml of saline containing heparin (20 IU/ml). The animals alternatively were killed 24 h, 72 h and 7 days after rIL-13 administration in order to evaluate inflammatory changes evoked by rIL-13 itself. In all cases, pleural washings were collected and their volume measured with a graduated syringe.

2.4. Leukocyte counts

Total leukocytes from the pleural cavity and intact mast cell counts were performed in a Neubauer chamber by means of an optical microscope after dilution with Türk solution (2% acetic acid) and toluidine blue dye, respectively. Differential cell analysis was made on cytopspin preparations stained with May-Grünwald Giemsa under an oil immersion objective.

2.5. Purification of rat eosinophils

Eosinophil purification was performed using Percoll density gradient according to the technique described by Martins et al. [23]. Briefly, eosinophils were obtained from the peritoneal cavity of anaesthetized normal rats and lavage cells were centrifuged at 1500 × g at 20 °C for 18 min. The supernatant was discarded and the pellet was re-suspended in RPMI-1640 medium (pH 7.2) containing 30 mM HEPES and 2mg/ml sodium bicarbonate. The cells were pooled, the suspension was layered onto discontinuous Percoll gradient and the tubes centrifuged at 2440 × g at 20 °C for 30 min. Eosinophils were collected and washed twice with RPMI-1640 medium. Cell viability was evaluated by trypan blue exclusion. Eosinophils of 80–95% purity and 96% viability were used in the following experiment.

2.6. Chemotaxis assay

Migration experiments were performed using 48-well microchemotaxis chamber (Neuro Probe, Inc.

USA) and a Toyo cellulose nitrate filters (3 µm pore) ([24]). PAF (5–5000 ng/ml), IL-13 (5–100 ng/ml) or RPMI-1640 medium containing bovine serum albumine were placed in the lower compartment and the eosinophil suspension (2×10^5 cells) was placed in the upper compartment of the chamber. The chamber was incubated at 37 °C in 5% CO₂:95% O₂ atmosphere for 2 h and the filter fixed and stained as described [24]. Eosinophils migrated at 40 µm from the upper surface of the filter were counted in 15 consecutive high-power fields (HPF) under an immersion objective. All experiments were done in duplicates.

2.7. Mast cell enumeration in the pleural mesothelium following IL-13 priming

Seven days after rIL-13 intrapleural challenge (50 ng/cavity), rats were killed by asphyxiation over CO₂ atmosphere, the thoracic cavity was opened and the pleural mesothelial membrane was removed and placed on glass slides to dry at room temperature. Then, glass slides were embedded in 5 ml of paraformaldehyde (4%) diluted with sucrose solution (4%). The mast cell population was identified in pleural membrane after staining with Giemsa dye [7,25]. The number of mast cells was counted under oil immersion by means of light microscope (Olympus BX50). The number of mast cells was expressed per square millimeter and at least 16 mm² were examined per pleural sample.

2.8. Analysis of mast cell reactivity in vitro following IL-13 priming in vivo

Seven days after rIL-13 intrapleural challenge (50 ng/cavity), rats were killed by asphyxiation over CO₂ atmosphere. Then, the thoracic cavity was opened and rinsed with 10 ml of HBSS (Ca⁺⁺- and Mg⁺⁺-free), after which the pleural washing was collected and centrifuged at 150 × g for 10 min. The pelleted cells were re-suspended in Hank's Balanced Salt Solution (HBSS) and then submitted to continuous Percoll gradient (72%) for mast cell isolation. Purity (95%) and viability (98%) were evaluated by means of toluidine blue and trypan blue exclusion staining, respectively. The cells were added to a 48-well plate (10⁵ cells/well) and incubated with IgE at 1

µg/ml overnight at 5% CO₂ atmosphere, 37 °C. Then, sensitized mast cells were challenged with DNP-BSA at 1 µg/ml for 30 min and the supernatants were recovered and stored (–20 °C) until quantification of histamine and LTC₄.

2.9. Measurement of histamine and LTC₄

Histamine in supernatant was quantified by fluorimetric assay as previously described [26], whereas LTC₄ was measured with an ELISA kit according to manufacture's instruction (Cayman Chemical).

2.10. Measurement of eotaxin

Pleural exudates from IgE sensitized and challenged animals, pretreated or not with rIL-13, were collected with 0.5 ml of 1% PBS containing EDTA (10 mM) 4 h after DNP-BSA challenge. Samples were centrifuged at 770 × *g* for 10 min and the supernatants stored at –20 °C until chemokine quantification by specific ELISA. Flat-bottom 96-well microtitre polystyrene plates (Costar, Corning Incorporated, USA) were coated with 100 µl/well of murine anti-eotaxin mAb for 18 h at 25 °C and then washed with 1 M phosphate potassium buffer (pH 7.5) added with thimerosal (2 mg/ml) and 0.05% Tween 20. Non-specific binding sites were blocked with 1% BSA in PBS for 1 h at 25 °C. Plates were rinsed with wash buffer and cell-free supernatant or chemokine standards were added for 1 h at 25 °C. The plates were washed with 0.14 M NaCl, 1 M NaPO₄, 2.7 M KCl, thimerosal (2 mg/ml) and 0.05% Tween 20, and then 100 µl of the biotinylated anti-eotaxin was added for 1 h at 20 °C. After washing, neutravidin-peroxidase was applied and incubated by 1 h at 20 °C. The final wash was followed by peroxidase substrate (K-blue) incubation for 30 min and the reaction stopped with 0.19 M H₂SO₄. Plate was read at 450 nm by an ELISA reader (Molecular Devices).

2.11. Flow cytometry of pleural mast cells bearing IgE

Purified mast cells recovered from the thoracic cavity of rIL-13 or saline injected rats were added to a 96-well plate (3 × 10⁵ cells/well) and incubated overnight with IgE (1 µg/0.1 ml) or saline at 5%

CO₂ atmosphere, 37 °C. After washing once with phosphate buffered saline (PBS) containing 0.1% sodium azide, cells were incubated with anti-murine biotinylated IgE monoclonal antibody (10 µg/ml), diluted in the same buffer added with 2% bovine serum albumin, for 30 min at 4 °C. An isotype matched biotinylated mouse IgG with irrelevant specificity was used as negative control. After washing, cells were stained with streptavidin-FITC (Sigma) (10 µl/ml) for 30 min at 4 °C. After the final washing, cells were analyzed using a FACS-Calibur (Becton Dickinson, San Jose, CA). In each sample, it was analyzed to be at least five thousand mast cells to calculate the medium value of the fluorescence intensity.

2.12. Statistical analysis

Data were reported as mean ± SEM. ANOVA was used to determine the levels of difference between all groups, followed by the Newman–Keuls–Student test. Differences were considered to be statistically significant when *p* < 0.05.

3. Results

3.1. IL-13 predisposes eosinophilic response and eotaxin generation following IgE-dependent pleurisy

Confirming previous reports [18,19], it is shown here that the hypersensitivity reaction in IgE-passively sensitized rats is insufficient to induce eosinophil recruitment (Fig. 1A). However, injected into the pleural space 6 days before the passive sensitization (IgE anti-DNP, 1 µg/cavity), rIL-13 (25 and 50 ng/cavity) led to a dose-dependent increase in the eosinophil (Fig. 1A) but not in the neutrophil (Fig. 1B) numbers noted 4 h after DNP-BSA challenge (1 µg/cavity). Under this condition, rIL-13 (50 ng/cavity) also upregulated eotaxin levels in the pleural effluent as illustrated in Fig. 2.

3.2. IL-13 induces eosinophil migration in vivo but not in vitro

Recombinant IL-13 (50 ng/cavity) administered into pleural space of naïve rats induced a signifi-

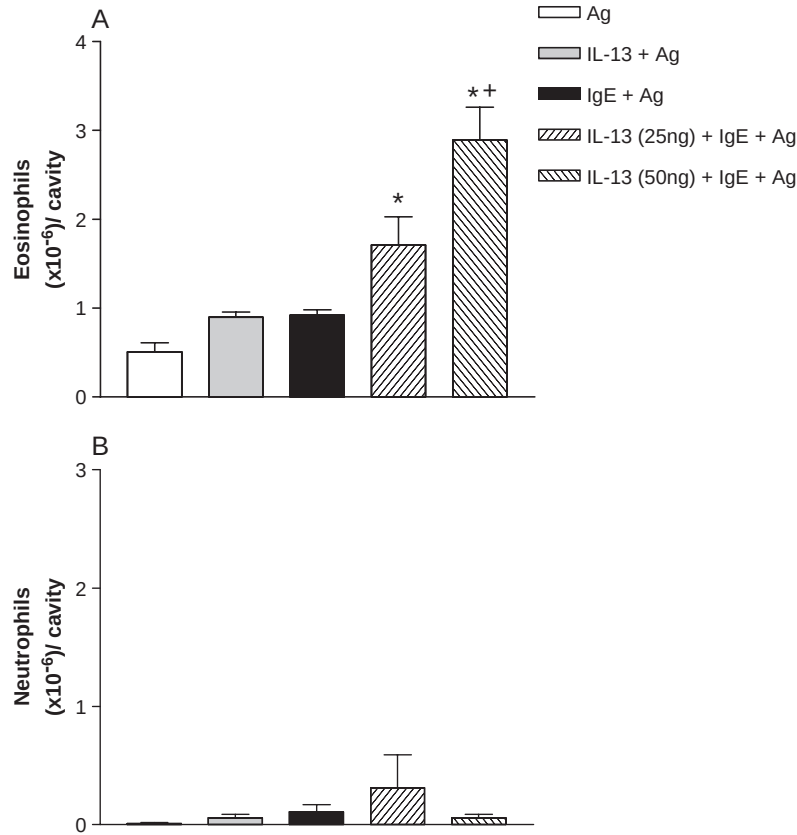


Fig. 1. Effect of rIL-13 (25–50 ng/cavity) priming on pleural eosinophil (A) and neutrophil (B) counts 4 h after antigen challenge (DNP-BSA—1 μ g/cavity) in IgE-passively sensitized (1 μ g/cavity) rats. Priming with rIL-13 was performed 7 days before antigen challenge. Columns represent the mean $\pm$ SEM from at least 6 animals. * p < 0.05 as compared to sensitized animals challenged with DNP-BSA without IL-13 injection. ** p < 0.05 as compared to sensitized and challenged animals injected with rIL-13 (25 ng/cavity).

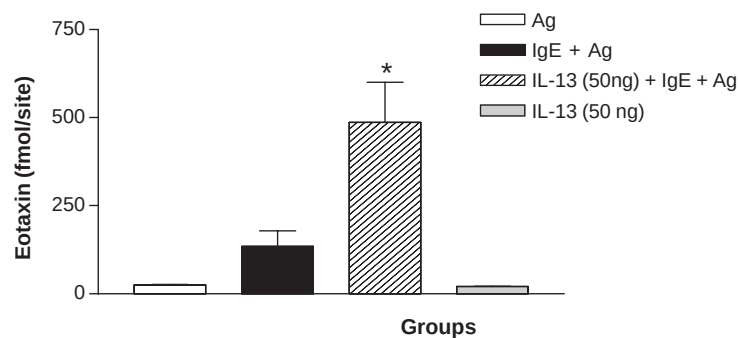


Fig. 2. Effect of rIL-13 (50 ng/cavity) priming on pleural eotaxin generation 4 h after antigen challenge (DNP-BSA—1 μ g/cavity) in IgE-passively sensitized (1 μ g/cavity) rats. Priming with rIL-13 was performed 7 days before antigen challenge. Columns represent the mean $\pm$ SEM from at least 6 animals. * p < 0.05 as compared to sensitized animals challenged with DNP-BSA without IL-13 injection.

cant increase in the number of eosinophils recovered from the pleural effluent 24 h after stimulation, returning to the baseline levels within 72 h (Fig. 3A). Using isolated rat eosinophils and the 48-well Boyden chamber system, we found that rIL-13 (5–100 ng/ml) failed to induce direct eosinophil chemotaxis (Fig. 3B), differently from what was observed following stimulation with PAF (10^{-8} – 10^{-6} M) (Fig. 3B, insert).

3.3. IL-13 induces long-term changes on mast cell numbers without altering mast cell surface IgE binding

A slight but significant increase in the mast cell numbers was detected 7 days after intrapleural injection of rIL-13 (50 ng/cavity). The number of free mast cells in the pleural effluent increased from 521.2 ± 85.3 to $825.7 \pm 64.7/\text{cavity}$ (mean $\pm$ SEM)

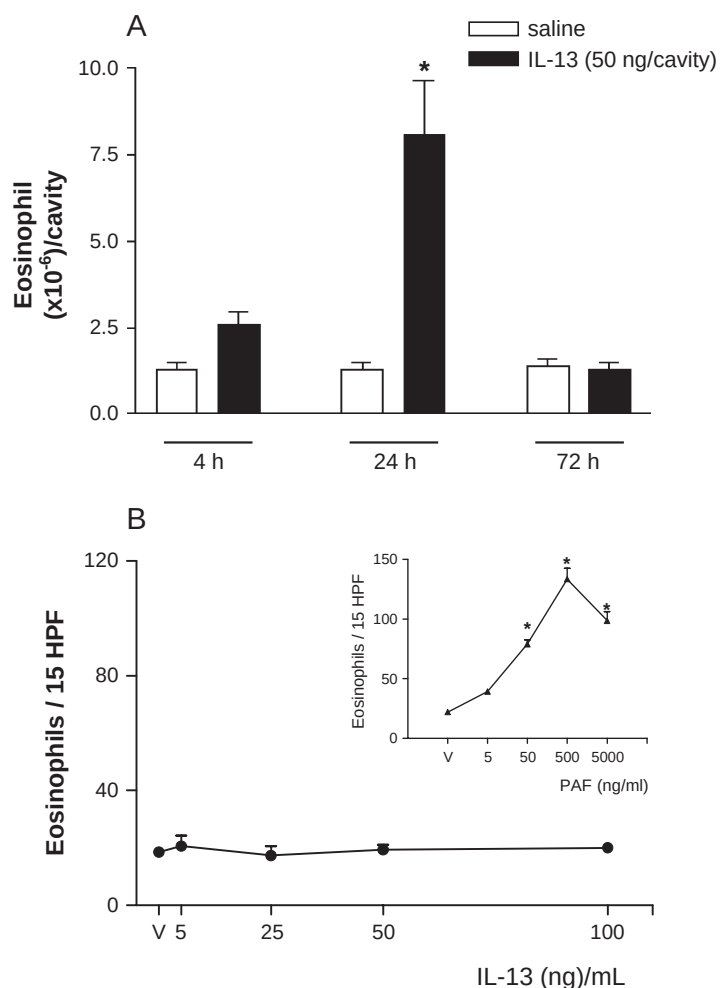


Fig. 3. (A) Kinetics of rIL-13 (50 ng/cavity) induced eosinophil migration into the pleural cavity of naïve rats. Pleural eosinophil counts were made from time 0 to 72 h after IL-13 injection. Each column represents the mean $\pm$ SEM from at least 7 animals. * $p < 0.05$ as compared to saline injected rats. (B) Eosinophil chemotaxis assay performed with isolated rat eosinophils and the 48-well Boyden chamber system. rIL-13 (5–100 ng/ml) and PAF (5–5000 ng/ml) were used as stimuli. Each point represents the mean $\pm$ SEM of two separated experiments done in duplicate. * $p < 0.05$ as compared to vehicle treated cells.

($n=8$) (Fig. 4A) and the number of adhered mast cells increased from 121.8 ± 7.4 to $178.6 \pm 16.4/\text{mm}^2$ (mean $\pm$ SEM) ($n=6$) on the pleural mesothelium (Fig. 4B). In another set of experiments, IgE binding was assessed by flow cytometry as an indicative of the Fc ϵ RI surface expression in mast cells recovered from the pleural effluents of animals injected with rIL-13 7 days before. As shown in Fig. 5, the level of IgE binding by mast cells from rIL-13 primed rats was not different from that obtained from saline treated rats.

3.4. IL-13 upregulates antigen-evoked LTC₄ generation by mast cells

Mast cells isolated from the pleural cavity of rIL-13 primed rats 7 days post-challenge and then subjected to IgE passive sensitization and antigen challenge in vitro yielded a 3-fold increase in LTC₄ production, as compared with mast cells recovered

from saline-injected rats (Fig. 6A). In contrast, comparable amounts of histamine were released from either saline or rIL-13 primed mast cells (Fig. 6B).

3.5. Inhibition of 5-LO prevents IL-13 exacerbation of the IgE-mediated eosinophilia

As shown in Fig. 7, pretreatment with zileuton (50 $\mu\text{g}/\text{cavity}$), 5 min before DNP-BSA challenge, abolished eosinophil accumulation into the pleural cavity observed in rats subjected to rIL-13 stimulation 7 days before, indicating the involvement of leukotrienes in this phenomenon.

4. Discussion

Much attention has been paid to the clarification of the pathogenic role of IL-13 on asthma and other atopic conditions. Using a model of IgE-passively sensitized rats, we demonstrated here that administration of rIL-13 predisposes for an IgE-mediated eosinophilia 7 days later and provided evidence for the involvement of mast cell-derived cys-LTs in this mechanism. These findings give to IL-13 potentially new functions on the amplification of IgE-mediated eosinophilic response.

Interaction of antigen with IgE antibody in single exposure models has been shown to be insufficient to induce eosinophil recruitment and airway hyper-responsiveness in mouse [17] and rat systems [17–19]. However, using a rat model in which a cycle of IgE-passive sensitization, followed 24 h later by antigen challenge, was repeated a second time within 7 days, we provided evidence that repeated cycles of IgE-driven inflammation is able to induce eosinophil accumulation [19,27]. Since IL-13 is generated by activation of the IgE–mast cell system [27] and shown to exacerbate systemic anaphylaxis [28], we investigated in the current study whether it could mediate the effect of the first cycle of IgE sensitization and challenge in its ability to predispose to the eosinophilic response noted in the second cycle. Our findings indicated that a local injection of rIL-13 turned the interaction of antigen with IgE antibody productive in terms of eosinophil but not neutrophil recruitment 7 days later. Of note, nothing happened when the hypersensitivity reaction

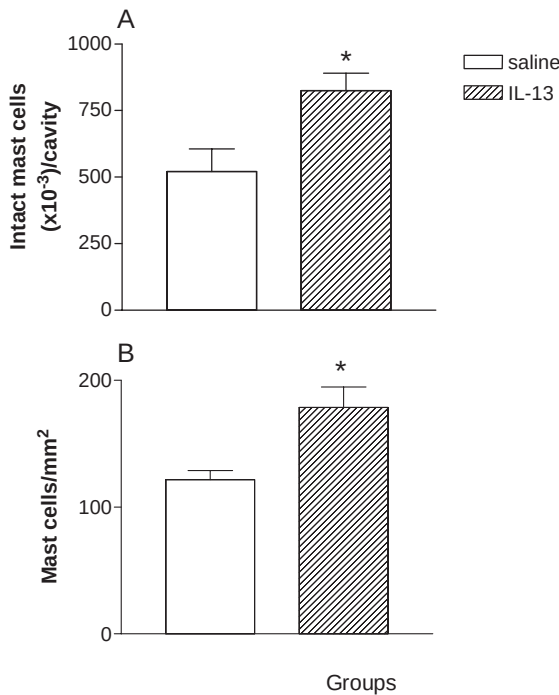


Fig. 4. Long-term effect of rIL-13 (50 ng/cavity) intrapleural injection on (A) intact mast cell number recovered from pleural fluid or (B) mesothelial mast cells, 7 days after challenge. Columns represent the mean $\pm$ SEM from at least 8 animals. * $p < 0.05$ as compared to saline-injected rats.

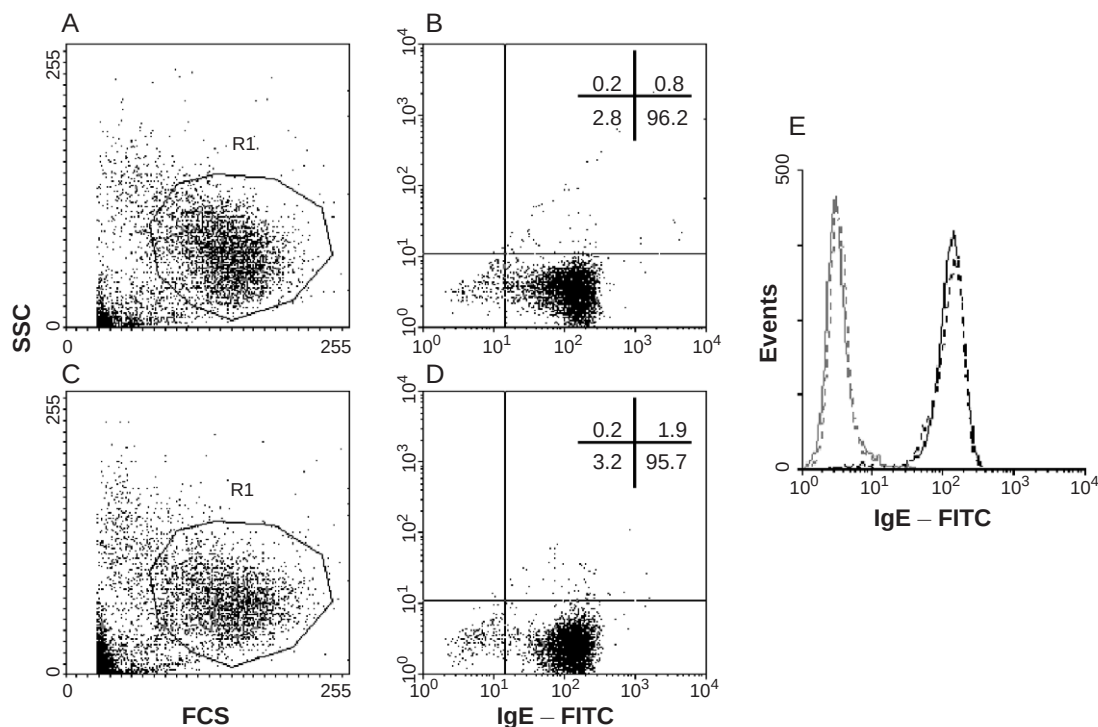


Fig. 5. Forward and side scatter profile of isolated pleural mast cells from (A) saline or (C) IL-13 primed mast cells. Dot plot showing IgE-bound mast cells from (B) saline or (D) IL-13 primed mast cells, with the percentage numbers of cells in each quadrant. Histogram (E) showing IgE-bound mast cells from saline (dashed lines), IL-13 primed (solid lines) mast cells, or background levels (only with anti-IgE and SAV-FITC, without addition of IgE) (gray lines) from 3 independent experiments.

was preceded by histamine, 5-hydroxytryptamine or PAF stimulation [19] emphasizing the distinct role of IL-13 in this process.

IL-13 has been described as a cytokine able to activate a wide variety of cell types that are relevant to the pathogenesis of allergy. In B cells for instance, it has similar effects as IL-4, promoting B-cell proliferation and inducing class switching to IgG₄ and IgE [29]. However, this does not seem to be the mechanism by which rIL-13 is upregulating IgE-mediated eosinophilic response since the immunoglobulin is passively transferred in the current study. On the other hand, IL-13 is a major cytokine involved in allergen-evoked eosinophil recruitment in different experimental models [20] and it is able to cause lung eosinophilia following airway instillations [21]. Accordingly, the intrapleural administration of rIL-13 into naïve rats led to eosinophil accumulation which peaked 24 h post-challenge. Eosinophils might be the direct targets for the effects

of rIL-13 and to rule out this possibility we evaluated the locomotory function of isolated eosinophils *in vitro*. Our findings indicated that rIL-13 failed to evoke eosinophil migration in the Boyden chamber system, differently from what was observed following stimulation with PAF and adding support to previous studies showing that IL-13-evoked eosinophilia depends on secondary mediators [30]. It is noteworthy that the pleural eosinophil accumulation triggered by rIL-13 disappeared 72 h post-challenge and could not contaminate the eosinophil counts noted after allergen provocation 7 days later. Nevertheless, the possibility that the production and release of IL-13 by eosinophils themselves are cooperating to amplify the responses cannot be discarded.

Functional IL-13 receptor has been detected on mast cells [31,32] raising about the possibility that its effects could be at least in part mediated by upregulation of mast cell functions. In this work we demonstrated that administration of rIL-13 into the pleural

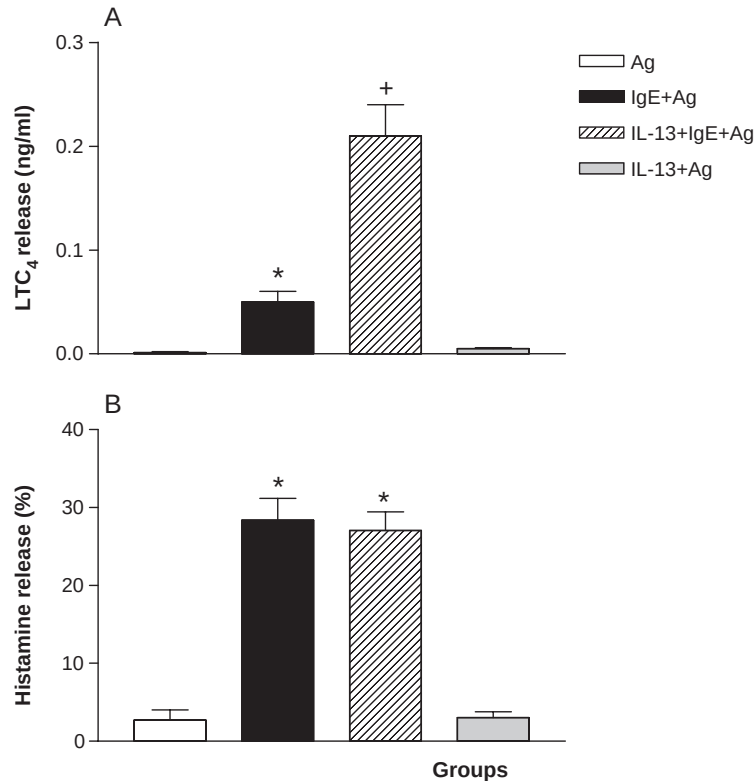


Fig. 6. Effect of rIL-13 priming (50 ng/cavity) on antigen-induced in vitro mast cell (A) LTC₄ and (B) histamine release. Pleural mast cells were purified 7 days after rIL-13 and maintained overnight in culture (1×10^5 /ml) with IgE (1 μ g/ml) followed by challenge with DNP-BSA (1 μ g/ml). Mediator quantification was performed from the supernatant 1 h post antigen challenge. Columns represent the mean $\pm$ SEM from at least 5 animals. * $p < 0.05$ as compared to non-sensitized mast cells. + $p < 0.05$ as compared to sensitized and challenged mast cells without rIL-13 priming.

cavity of naïve rats led to a slight but significant increase in the mast cell population noted in samples recovered from either the pleural effluent or mesothelium within 7 days. These findings may help to understand the pro-eosinophilic effects of IL-13 noted here since the capacity of mast cell products, including cys-LTs, to promote eosinophil recruitment is well established [30,33]. They also suggest an important role for IL-13 in the upregulation of mast cell numbers but the mechanisms responsible for that effect in the current model remain to be identified. It is noteworthy that this observation diverges from a previous study in which the number of pulmonary mast cells in sensitized IL-13 transgenic mice did not change as compared to wild-type animals [34]. This apparent inconsistency may concern the fact that different animal species and distinct read-out systems have been employed to evaluate IL-13 effects on mast cells.

Because of the involvement of IL-4 with upregulation of Fc ϵ RI expression and LTC₄ formation by mast cells [35], we compared mast cells isolated from the pleural cavity 7 days post-IL-13 and those from vehicle-injected animals for their ability to bind IgE and generate cys-LTs. We found no difference on the levels of IgE bound on those cells suggesting that the background level of Fc ϵ RI expression remained unchanged. It should be mentioned that Toru et al. (1998) have described similar effects on cord blood-derived human cultured mast cells, since the exposure to IL-13 did not upregulate Fc ϵ RI expression on these cells [36]. In contrast, mast cells pre-exposed to rIL-13 in vivo generated 3-fold more LTC₄ after antigen provocation in vitro, under conditions where the percentage of histamine released was not different between both groups. These findings are in line with those in which mast cells exposed to IL-3 or IL-5

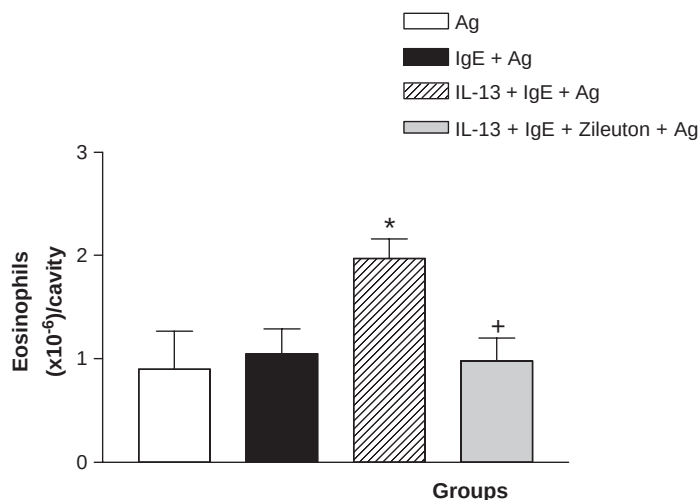


Fig. 7. Effect of 5-lipoxygenase inhibitor zileuton (50 µg/cavity) on pleural eosinophilia triggered by antigen-challenge in rIL-13 (50 ng/cavity) injected rats. Treatment with zileuton was performed 5 min before DNP-BSA challenge. Columns represent the mean $\pm$ SEM from at least 7 animals. * $p < 0.05$ as compared to sensitized and challenged rats without rIL-13 priming. † $p < 0.05$ as compared to sensitized and challenged rats primed with rIL-13 and treated with saline.

during IL-4 priming resulted in marked increase in their IgE-dependent cys-LT production, without changing either PGD₂ or histamine release [37]. Accordingly, these cytokines presented selective actions on the function of the 5-LO/LTC₄ synthase pathways, involving the redistribution of these enzymes to the mast cell nucleus [37].

Causative relationship between leukotriene generation and antigen-evoked eosinophilic inflammation is largely supported by numerous reports in animal models using cysteinyl-leukotriene receptor antagonists [38], 5-lipoxygenase and 5-lipoxygenase-activating protein inhibitors [39] and 5-lipoxygenase knockouts [40]. While trying to assess whether the enhanced production of LTC₄ by mast cells noted in animals pretreated with IL-13 may indeed account for the IgE-mediated pleural eosinophilia, we found that treatment with the 5-lipoxygenase inhibitor zileuton prior to antigen challenge abolished the eosinophilic response, strongly suggesting mediation by leukotrienes.

In conclusion, our findings show that IL-13 predisposes the microenvironment for an IgE-mediated eosinophilic reaction in a mechanism associated with heightened cysteinyl-leukotriene production by resident mast cells. It is likely that the release of IL-13 during IgE-dependent allergic reactions could exert a

long-term autocrine effect on mast cells and/or a paracrine link to other leukocytes or resident cells, upregulating the eosinophilic response.

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References

- [1] Bochner BS, Undem BJ, Lichtenstein LM. Immunological aspects of allergic asthma. *Annu Rev Immunol* 1994;12: 295–335.
- [2] Busse WW, Lemanske Jr RF. Asthma. *N Engl J Med* 2001; 344:350–62.
- [3] Umetsu DT, McIntire JJ, Akbari O, Macaubas C, DeKruyff RH. Asthma: an epidemic of dysregulated immunity. *Nat Immunol* 2002;3:715–20.
- [4] Wills-Karp M. Immunologic basis of antigen-induced airway hyperresponsiveness. *Annu Rev Immunol* 1999;17: 255–81.

- [5] Foster PS, Mould AW, Yang M, Mackenzie J, Mattes J, Hogan SP, et al. Elemental signals regulating eosinophil accumulation in the lung. *Immunol Rev* 2001;179:173–81.
- [6] Hamelmann E, Gelfand EW. IL-5-induced airway eosinophilia—the key to asthma? *Immunol Rev* 2001;179:182–91.
- [7] Black J. The role of mast cells in the pathophysiology of asthma. *N Engl J Med* 2002;346:1742–3.
- [8] Williams CM, Galli SJ. The diverse potential effector and immunoregulatory roles of mast cells in allergic disease. *J Allergy Clin Immunol* 2000;105:847–59.
- [9] Kobayashi T, Miura T, Haba T, Sato M, Serizawa I, Nagai H, et al. An essential role of mast cells in the development of airway hyperresponsiveness in a murine asthma model. *J Immunol* 2000;164:3855–61.
- [10] Williams CM, Galli SJ. Mast cells can amplify airway reactivity and features of chronic inflammation in an asthma model in mice. *J Exp Med* 2000;192:455–62.
- [11] Coyle AJ, Wagner K, Bertrand C, Tsuyuki S, Bews J, Heusser C, et al. Central role of immunoglobulin (Ig) E in the induction of lung eosinophil infiltration and T helper 2 cell cytokine production: inhibition by a non-anaphylactogenic anti-IgE antibody. *J Exp Med* 1996;183:1303–10.
- [12] Fahy JV, Fleming HE, Wong HH, Liu JT, Su JQ, Reimann J, et al. The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic subjects. *Am J Respir Crit Care Med* 1997;155:1828–1834.
- [13] Hogan SP, Mould A, Kikutani H, Ramsay AJ, Foster PS. Aeroallergen-induced eosinophilic inflammation, lung damage, and airways hyperreactivity in mice can occur independently of IL-4 and allergen-specific immunoglobulins. *J Clin Invest* 1997;99:1329–39.
- [14] Mehlhop PD, van de Rijn M, Goldberg AB, Brewer JP, Kurup VP, Martin TR, et al. Allergen-induced bronchial hyperreactivity and eosinophilic inflammation occur in the absence of IgE in a mouse model of asthma. *Proc Natl Acad Sci U S A* 1997;94:1344–9.
- [15] Korsgren M, Erjefält JS, Korsgren O, Sundler F, Persson CG. Allergic eosinophil-rich inflammation develops in lungs and airways of B cell-deficient mice. *J Exp Med* 1997;185:885–92.
- [16] Takeda K, Hamelmann E, Joetham A, Shultz LD, Larsen GL, Irvin CG, et al. Development of eosinophilic airway inflammation and airway hyperresponsiveness in mast cell-deficient mice. *J Exp Med* 1997;186:449–54.
- [17] Kaneko M, Schimming A, Gleich GJ, Kita H. Ligation of IgE receptors causes an anaphylactic response and neutrophil infiltration but does not induce eosinophilic inflammation in mice. *J Allergy Clin Immunol* 2000;105:1202–10.
- [18] Lima MC, Prouvost-Danon A, Silva PMR, Chagas MS, Calheiros AS, Martins MA, et al. Studies on the mechanisms involved in antigen-evoked pleural inflammation in rats: contribution of IgE and complement. *J Leukoc Biol* 1997;61:286–92.
- [19] Calheiros AS, Aguiar Pires AL, Pereira da Silva J, Cordeiro RS, Martins MA, Lima MCR, et al. Role of the IgE-mediated system in eosinophil recruitment triggered by two consecutive cycles of sensitisation and challenge in rats. *Int Arch Allergy Immunol* 2001;126:325–34.
- [20] Grunig G, Warnock M, Wakil AE, Venkayya R, Brombacher F, et al. Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 1998;282:2261–3.
- [21] Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, et al. Interleukin-13: central mediator of allergic asthma. *Science* 1998;282:2258–61.
- [22] Taube C, Wei X, Swasey CH, Joetham A, Zarini S, Lively T, et al. Mast cells, Fc epsilon RI, and IL-13 are required for development of airway hyperresponsiveness after aerosolized allergen exposure in the absence of adjuvant. *J Immunol* 2004;172:6398–406.
- [23] Martins MA, Etienne A, Souillard C, Domingo MT, Braquet P. Chemotactic effect of PAF-acether on peritoneal eosinophils from normal rats. *Braz J Med Biol Res* 1989;22:1151–4.
- [24] Richards KL, McCullough J. A modified microchamber method for chemotaxis and chemokinesis. *Immunol Commun* 1984;13:49–62.
- [25] de Oliveira Barreto E, de Frias Carvalho V, Diaz BL, Balduino A, Cordeiro RS, Silva PMR, et al. Adoptive transfer of mast cells abolishes the inflammatory refractoriness to allergen in diabetic rats. *Int Arch Allergy Immunol* 2003;131:212–20.
- [26] Shore PA. The chemical determination of histamine. *Methods Biochem Anal* 1971;Suppl:89–97.
- [27] Burd PR, Thompson WC, Max EE, Mills FC. Activated mast cells produce interleukin 13. *J Exp Med* 1995;181:1373–80.
- [28] Strait RT, Morris SC, Smiley K, Urban Jr JF, Finkelman FD. IL-4 exacerbates anaphylaxis. *J Immunol* 2003;170:3835–42.
- [29] Punnonen J, Aversa G, Cocks BG, de Vries JE. Role of interleukin-4 and interleukin-13 in synthesis of IgE and expression of CD23 by human B cells. *Allergy* 1994;49:576–86.
- [30] Vargaftig BB, Singer M. Leukotrienes mediate murine bronchopulmonary hyperreactivity, inflammation, and part of mucosal metaplasia and tissue injury induced by recombinant murine interleukin-13. *Am J Respir Cell Mol Biol* 2003;28:410–9.
- [31] de Vries JE. The role of IL-13 and its receptor in allergy and inflammatory responses. *J Allergy Clin Immunol* 1998;102:165–9.
- [32] Nilsson G, Nilsson K. Effects of interleukin (IL)-13 on immediate-early response gene expression, phenotype and differentiation of human mast cells. Comparison with IL-4. *Eur J Immunol* 1995;25:870–3.
- [33] Malaviya R, Chen CL, Navara C, Malaviya R, Liu XP, Keenan M, et al. Treatment of allergic asthma by targeting janus kinase 3-dependent leukotriene synthesis in mast cells with 4-(3', 5'-dibromo-4'-hydroxyphenyl)amino-6,7-dimethoxyquinazoline (WHI-P97). *J Pharmacol Exp Ther* 2000;295:912–26.
- [34] Fallon PG, Emson CL, Smith P, McKenzie AN. IL-13 overexpression predisposes to anaphylaxis following antigen sensitization. *J Immunol* 2001;166:2712–6.
- [35] Yamaguchi M, Sayama K, Yano K, Lantz CS, Noben-Trauth N, Ra C, et al. IgE enhances Fc epsilon receptor I expression and IgE-dependent release of histamine and lipid mediators from human umbilical cord blood-derived mast cells: synerg-

- gistic effect of IL-4 and IgE on human mast cell Fc epsilon receptor I expression and mediator release. *J Immunol* 1999;162:5455–65.
- [36] Toru H, Pawankar R, Ra C, Yata J, Nakahata T. Human mast cells produce IL-13 by high-affinity IgE receptor cross-linking: enhanced IL-13 production by IL-4-primed human mast cells. *J Allergy Clin Immunol* 1998;102:491–502.
- [37] Hsieh FH, Lam BK, Penrose JF, Austen KF, Boyce JA. T helper cell type 2 cytokines coordinately regulate immunoglobulin E-dependent cysteinyl leukotriene production by human cord blood-derived mast cells: profound induction of leukotriene C(4) synthase expression by interleukin 4. *J Exp Med* 2001;193:123–33.
- [38] Eum SY, Maghni K, Hamid Q, Campbell H, Eidelman DH, Martin JG, et al. Involvement of the cysteinyl-leukotrienes in allergen-induced airway eosinophilia and hyperresponsiveness in the mouse. *Am J Respir Cell Mol Biol* 2003;28:25–32.
- [39] Henderson Jr WR, Lewis DB, Albert RK, Zhang Y, Lamm WJ, Chiang GK, et al. The importance of leukotrienes in airway inflammation in a mouse model of asthma. *J Exp Med* 1996;184:1483–94.
- [40] Irvin CG, Tu YP, Sheller JR, Funk CD. 5-Lipoxygenase products are necessary for ovalbumin-induced airway responsiveness in mice. *Am J Physiol* 1997;272:L1053–8.