

Role of the IgE-Mediated System in Eosinophil Recruitment Triggered by Two Consecutive Cycles of Sensitisation and Challenge in Rats

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Key Words

IgE · Reexposure to antigen · Pleural inflammation and eosinophils

Abstract

In this study, we postulated that repeated cycles of IgE passive sensitisation and antigen challenge may play a role in up-regulating eosinophil response in allergic conditions. Antigen-mediated stimulation of the pleural cavity of rats passively sensitised with a single injection of IgE anti-DNP resulted in mast cell degranulation, increase in vascular permeability and mild neutrophilia, but no pleural eosinophilia. In contrast, a second cycle of sensitisation and challenge, performed within 7 days, showed a marked eosinophilia in parallel with a lower plasma leakage and comparable neutrophilia. The eosinophilic phenomenon was not reproduced when (1) IgE sensitisation or antigen challenge was omitted in the first cycle, or (2) the first cycle was replaced by either a histamine and 5-HT dual challenge or a PAF challenge. Furthermore, we found an increase in eotaxin levels in animals subjected to two rather than one cycle of sensitisation and challenge. Treatment with the PAF receptor antagonist BN 52021 or with the lipoxygenase inhibitor

zileuton, but not mast cell granule depletion, prevented the allergen-evoked eosinophil accumulation in rechallenged animals. Our results indicate that repeated cycles of IgE-driven inflammation may lead to eosinophil accumulation in a mechanism dependent on eotaxin, PAF and leukotrienes.

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Introduction

IgE is an essential molecule in the pathogenesis of atopic diseases including bronchial asthma. Most of the symptoms of acute-phase allergic reactions in the lung, such as bronchospasm, airway microvascular leakage and mucosal oedema are closely associated with IgE-dependent activation of mast cells [1]. This immediate response is often followed by a late-phase response which occurs 4–8 h after challenge. The phenomenon is marked by the infiltration of leucocytes, notably eosinophils and T cells, which seems to be associated with chronic changes in underlying tissues [2–6]. Despite the presence of elevated levels of circulating IgE during eosinophilic allergic inflammation [7–9], and the capacity of mast cell products to promote the traffic of leucocytes to challenged sites [10,

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11], the relative importance of the IgE-mast cell system to the installation and/or maintenance of the late-phase response remains controversial.

It has been shown that the intradermal administration of anti-IgE antibody causes immediate and late-phase cutaneous reactions in human subjects [12, 13]. More recent experiments indicated that the treatment of allergic asthmatic subjects with a non-anaphylactogenic anti-IgE antibody reduced serum IgE and both early and late-phase reactions [14]. These findings are in line with those of Coyle et al. [15], who demonstrated that IgE neutralisation 6 h before antigen challenge in sensitised mice is able to prevent lung eosinophil accumulation, and the production of IL-4 and IL-5 by pulmonary cells. On the other hand, these data are contradicted by studies with IgE knockout mice [16, 17] and B-cell-deficient mice [18], which suggest that allergen-induced eosinophilic airway inflammation can occur in the absence of allergen-specific immunoglobulin. Actually, in murine models of allergen-induced pulmonary inflammation, CD4⁺ T cells of the Th2 phenotype seem to have a critical role in the development of tissue eosinophilia [19–21]. This concept is supported by the demonstration that CD4⁺ T cells – adoptively transferred from sensitised to naive animals – can trigger pulmonary eosinophilia after specific allergen challenge [22–24] in a mechanism dependent on IL-13 and eotaxin upregulation [25, 26].

We have previously reported that pleural allergen challenge of IgE passively sensitised rats induced an intense exudatory response, comparable to that evoked in actively sensitised rats, but failed to affect the whole extent of neutrophil and eosinophil mobilisation [27]. Similar findings were obtained when the active sensitisation in rats was followed by an intrapleural challenge with the anaphylactic anti-IgE mAb MARE-1 [27], when a mild neutrophilia and no eosinophilia were found. In accordance, using a murine model of IgE-dependent inflammation, Kaneko et al. [28] demonstrated that allergen challenge caused a marked anaphylactic response and neutrophil recruitment, under conditions where eosinophil numbers remained unchanged. These findings suggest that activation of the IgE system could be implicated in the generation of the early-phase response, i.e., plasma leakage and oedema, but insufficient to trigger an absolute tissue leucocyte infiltration. It is interesting to note that in spite of delineating important acute inflammatory events related to IgE system activation, these assays do not resemble the experience of allergic patients who are constantly exposed to threshold amounts of specific allergen, establishing a persistent inflammatory response. Thus, in the present

study we investigated the possibility that repeated cycles of IgE passive sensitisation and challenge may contribute to eosinophil tissue enrichment in allergic conditions.

Materials and Methods

Animals

Wistar rats of either sex and weighing 150–200 g were obtained from the Oswaldo Cruz Foundation Breeding Unit (Rio de Janeiro, Brazil).

Antibodies and Drugs

Mouse monoclonal IgE specific for dinitrophenol [29] was kindly provided by Dr. Katz and dinitrophenylated bovine serum albumin (DNP-BSA) was prepared as reported [30]. Eotaxin, MIP-1 α and RANTES were purchased from Peprotech, biotinylated anti-mouse eotaxin, MIP-1 α and RANTES were purchased from R&D Systems. The 5-lipoxygenase inhibitor zileuton was purchased from Abbott Laboratories (North Chicago, Ill., USA), the bradykinin (B₂) receptor antagonist Hoe 140, histamine and serotonin were from Sigma, and the PAF receptor antagonist BN52021 was obtained from Institut Henri Beaufour (France).

Pleurisy Triggered by One or Two Cycles of IgE Passive Sensitisation and Challenge

Rats were sensitised with an intrapleural (i.pl.) injection of a murine IgE mAb anti-DNP (1 μ g/cavity) followed, 24 h later, by challenge with DNP-BSA (1 μ g/cavity, i.pl.). After 6 days, the animals were subjected to a second and identical protocol of IgE passive sensitisation and challenge. In control groups, IgE anti-DNP and/or DNP-BSA injections were replaced by their vehicles (sterile isotonic saline). All intrapleural injections were performed during light ether anaesthesia in a final volume of 100 μ l using a 27.5-gauge needle adjusted to 3 mm in length. Two or four hours after the first or the second cycle of passive sensitisation and challenge, the animals were killed by an excess of carbon dioxide and the thoracic cavity was rinsed with 3 ml of saline containing heparin (10 IU $\cdot$ ml⁻¹). Pleural washing was collected and its volume measured with a graduated syringe.

Pleurisy Triggered by Vasoactive Amines or PAF Followed by One Cycle of Passive Reaction

Rats were injected intrapleurally with histamine (10 μ g/cavity) immediately before serotonin (5 μ g/cavity) or with PAF (1 μ g/cavity). In the negative control groups, the agonists were replaced by 0.9% saline solution. After 1 week, they were submitted to one cycle of passive sensitisation with IgE (1 μ g/cavity) and challenge with DNP-BSA (1 μ g/cavity) and the pleurisy was evaluated within 4 h as described above.

Measurement of Total Protein Exuded

The fluid collected from the pleural cavity was centrifuged (700 g) for 10 min, and the protein content of the supernatant measured according to the biuret technique [31] by spectrophotometric analysis at 550 nm.

Leucocyte Counts

Total leucocyte from the pleural cavity and 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 from pleural fluid was performed in cytocentrifuged smears stained with May-Grünwald-Giemsa dye.

Pleural Mast Cell Depletion

The pleural cavity was selectively depleted of mast cells by local treatment with polymyxin B (10 µg/cavity). Rats received a single intrapleural injection of polymyxin B 48 h before the second cycle of passive reaction. The efficacy of pretreatment was indicated by counting the toluidine-blue-stained mast cells recovered from the pleural cavity.

Quantitation of Histamine

Histamine stored in cells recovered from the pleural fluid was spectrofluorimetrically measured by the method of Shore et al. [32]. Washing samples (1 ml) were centrifuged at 170 g for 10 min, and 0.4 N perchloric acid (0.5 ml) was added to the pellet, leading to cell lysis and protein precipitation. After centrifugation at 770 g for 10 min, the supernatants were stored at -20 °C until histamine quantification.

Quantitation of Chemokines

Pleural exudates were collected with 0.5 ml of 1% PBS containing EDTA (10 mM), 2 and 4 h after the first or the second cycle of sensitization and 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 either murine anti-eotaxin mAb, rat anti-RANTES polyclonal antibody, or rat anti-MIP-1α polyclonal antibody, 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 supernatants or chemokine standards were added for 1 h at 25 °C. The plates were then 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-chemokines 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, Neogen) incubation for 30 min and the reaction stopped with 0.19 M H₂SO₄. Plates were read at 450 nm by an ELISA reader (Molecular Devices).

Drug Treatment

PAF-receptor antagonist BN52021 (20 mg/kg) was administered intraperitoneally, 1 h before the second DNP-BSA challenge, whereas the 5-lipoxygenase inhibitor zileuton (50 µg/site) or the bradykinin receptor antagonist Hoe 140 (1 µg/site) were administered intrapleurally, 5 min before.

Statistical Analysis

Data were reported as mean ± SE of mean and statistically analysed by an analysis of variance (ANOVA) followed by the Newman-Keuls-Student test. Differences were considered to be statistically significant when $p < 0.05$.

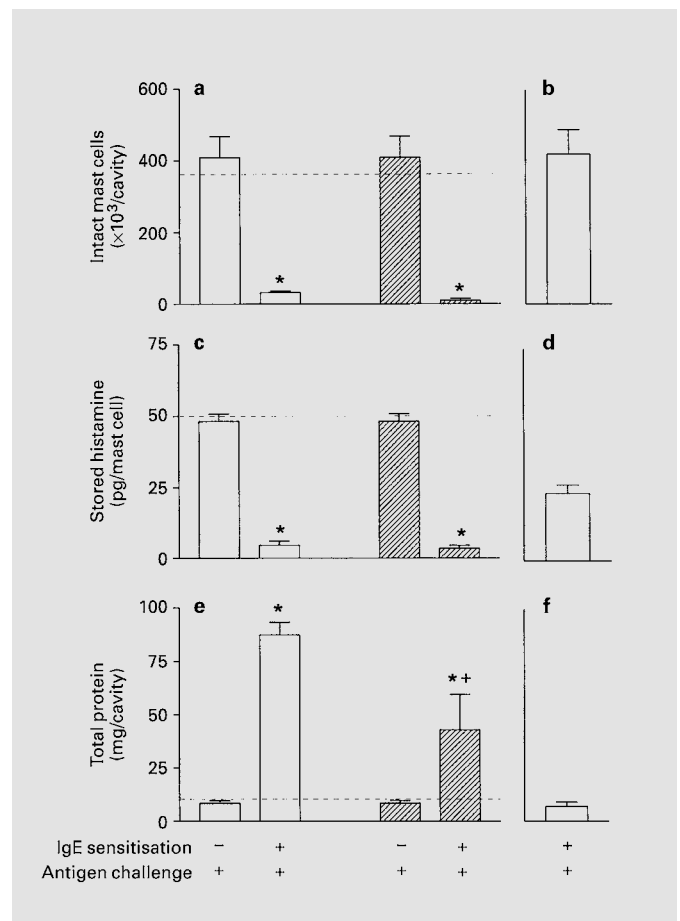


Fig. 1. Intact mast cell number (a), stored histamine (c) and total protein (e) 4 h after the first (open columns) or the second (hatched columns) DNP-BSA challenge in IgE anti-DNP passively sensitised animals. The right panels indicate the values for intact mast cells (b), stored histamine (d) and total protein (f) 7 days after one cycle of passive reaction. Dashed lines represent animals injected only with saline. Columns represent the mean ± SEM from at least 6 animals. * $p < 0.05$ as compared to animals submitted to one cycle of passive reaction; ** $p < 0.05$ as compared to the non-sensitized group.

Results

Pattern of Allergic Pleurisy after One or Two Cycles of IgE Passive Sensitisation and Challenge

In line with our previous report [27], we show here that passive sensitisation of the rat pleural cavity with IgE (1 µg/cavity) followed by DNP-BSA challenge (1 µg/cavity) led to a reduction in the number of intact mast cells (fig. 1a) and their histamine content (fig. 1c), in parallel with intense protein extravasation (fig. 1e). However, the cellular response evoked by a single cycle of IgE sensitisa-

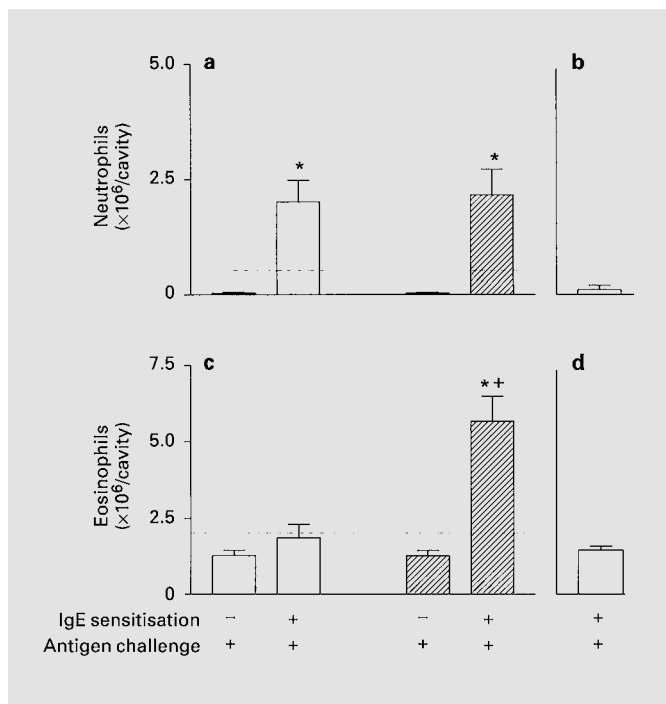


Fig. 2. Neutrophil (a) and eosinophil (c) infiltration 4 h after the first (open columns) or the second (hatched columns) DNP-BSA challenge in IgE anti-DNP passively sensitised animals. The right panels indicate the number of neutrophils (b) and eosinophils (d) 7 days after one cycle of passive reaction. Dashed lines represent animals injected only with saline. Columns represent the mean $\pm$ SEM from at least 6 animals. * $p < 0.05$ as compared to non-sensitised animals. ** $p < 0.05$ as compared to animals submitted to one cycle of passive reaction.

tion and challenge was very mild, marked by weak neutrophilia (fig. 2a) and absence of eosinophil recruitment (fig. 2c). It is noteworthy that the pleural space remained free of eosinophil infiltration 24 and 48 h after challenge [27].

The same scheme of sensitisation and challenge was then repeated within 7 days when the changes noted following the first cycle were over (fig. 1b, f, 2b, d), except for the amount of stored histamine which remained about 50% reduced, as compared to controls (sham-sensitised animals challenged once or twice with DNP-BSA) (fig. 1d). Under these conditions, reduction in the number of intact mast cells, depletion of stored histamine content and neutrophil influx triggered by the second challenge were comparable with those noted in single-sensitised and challenged rats (fig. 1a, c and 2a). However, a lesser plasma exudatory response and a 3-fold increase in the pleural

eosinophil numbers were observed within 4 h in double-sensitised and challenged rats, as shown in figures 1e and 2c, respectively. Elevated eosinophil numbers were also noted within 24 h. At that time point, the values increased from 2.22 ± 0.39 (mean $\pm$ SEM) ($n = 7$) in the negative controls to 5.56 ± 0.93 ($n = 7$) ($p < 0.01$) in double-sensitised and challenged rats.

It is noteworthy that repeated antigen challenges in sham-sensitised rats failed to evoke inflammation (fig. 1a, c, e, 2a, c) emphasising the importance of IgE passive sensitisation for the eosinophilic response in the current system.

Outcomes of a Dual Histamine and 5-HT Stimulation or PAF Stimulation Replacing the First Cycle of IgE Sensitisation and Challenge

Confirming previous data [33], a dual histamine and 5-HT challenge in the rat pleural cavity led to an exudatory response similar to that elicited by DNP-BSA in IgE-sensitised rats but failed to evoke leucocyte recruitment (table 1). On the other hand, we knew from previous reports that PAF-induced pleurisy in rats is marked by plasma leakage followed by neutrophil and eosinophil accumulation [34], in a pattern quite similar to that noted after allergen challenge in actively sensitised rats [27, 41]. While trying to evaluate the putative implication of vasoactive amines and PAF – released in the first cycle of sensitisation and challenge – in the eosinophilic response noted in the second cycle, we replaced the first cycle by either a dual histamine and 5-HT challenge or a PAF challenge. Our findings indicated that the values for plasma exudation and neutrophil and eosinophil counts following IgE-mediated pleurisy were not influenced by either the histamine and 5-HT dual challenge (fig. 3) or PAF challenge (fig. 4) performed 7 days before.

Eotaxin, MIP-1 α and RANTES Pleural Levels following the First and Second Cycle of IgE Passive Reactions

Analysis of pleural washing by specific ELISA revealed that the eotaxin level was not altered 2 or 4 h after the first cycle of the passive reaction, when compared to non-sensitized but challenged animals (fig. 5a). However, we found a significant increase in the levels of eotaxin recovered from the pleural fluid at 2 h and notably at 4 h after challenge, as shown in figure 5a. As illustrated in figure 5b, a modest and indistinct augmentation in the pleural fluid levels of MIP-1 α was observed after one or two cycles of sensitisation and challenge, whereas RANTES levels remained unchanged (fig. 5c).

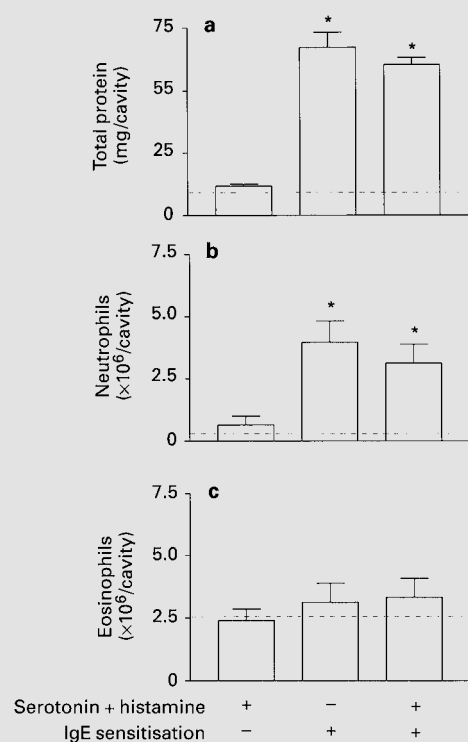


Fig. 3. Effect of an intrapleural injection of histamine (10 µg/cavity) immediately before serotonin (5 µg/cavity), 7 days before one cycle of IgE-dependent reaction. Dashed lines represent animals injected only with saline. Columns represent the mean $\pm$ SEM from at least 6 animals. * $p < 0.05$ as compared to non-sensitised animals. **a** Total protein. **b** Neutrophils. **c** Eosinophils.

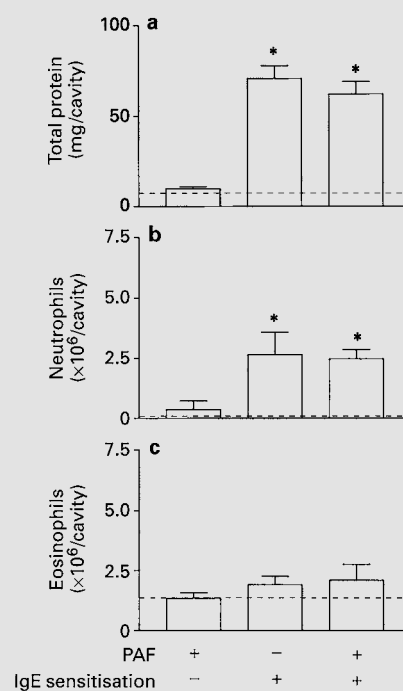


Fig. 4. Effect of an intrapleural injection of PAF (1 µg/cavity) 7 days before one cycle of IgE-dependent reaction. Dashed lines represent animals injected only with saline. Columns represent the mean $\pm$ SEM from at least 6 animals. * $p < 0.05$ as compared to non-sensitised animals. **a** Total protein. **b** Neutrophils. **c** Eosinophils.

Table 1. Evaluation of pleural total protein and leukocyte, neutrophil and eosinophil counts 4 h after the combined intrapleural injection of vasoactive amines in normal rats or antigen in IgE passively sensitised rats

Condition	Total protein mg/cavity	Leukocytes $\times 10^6$ /cavity	Neutrophils $\times 10^6$ /cavity	Eosinophils $\times 10^6$ /cavity
Saline	3.56 ± 0.3	10.0 ± 1.7	0 ± 0	2.5 ± 0.3
Hist + 5-HT	34.6 ± 3.1^1	9.9 ± 1.7	0.3 ± 0.4	2.7 ± 1.1
IgE + antigen	39.8 ± 5.3^1	12.6 ± 2.0	3.9 ± 0.8^1	3.1 ± 0.7

¹ Statistically significantly different from saline-injected animals.

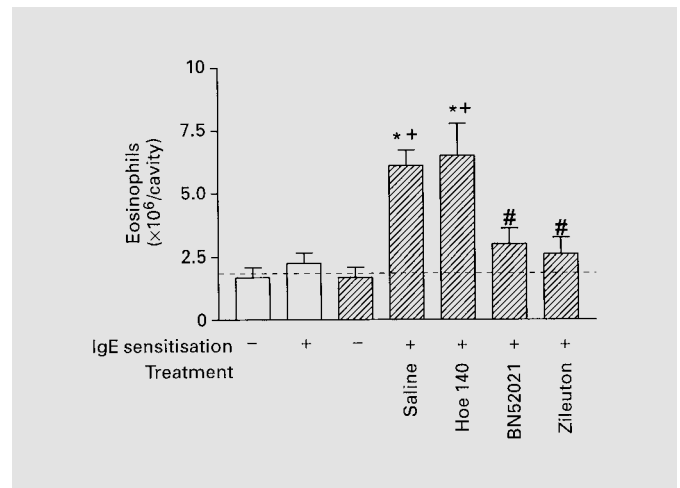
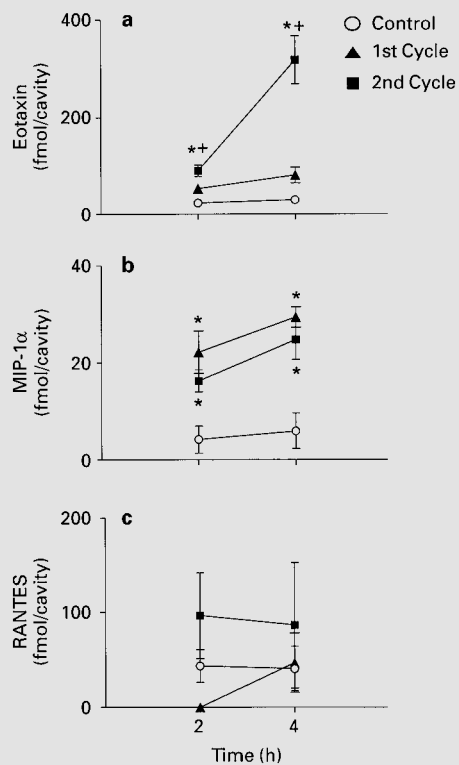


Fig. 5. Eotaxin, MIP-1α and RANTES generation after antigen challenge in non-sensitised (open symbols) or sensitised animals (closed symbols). Each point represents the mean ± SEM from at least 6 animals. * $p < 0.05$ as compared to non-sensitised animals. + $p < 0.05$ as compared to animals submitted to one cycle of passive reaction.

Fig. 6. Effect of local treatment (5 min before) with Hoe 140 (1 μg/cavity) or zileuton (50 μg/cavity), or systemic treatment (1 h before) with BN 52021 (20 mg/kg) on pleural eosinophilia triggered 4 h after the second cycle of passive reaction. Open and hatched columns represent one and two cycles, respectively. Dashed lines represent animals injected only with saline. Columns represent the mean ± SEM from at least 6 animals. * $p < 0.05$ as compared to non-sensitised animals. + $p < 0.05$ as compared to animals submitted to one cycle of passive reaction. # $p < 0.05$ as compared to animals submitted to two cycles of passive reaction and treated with saline.

Involvement of PAF, Bradykinin and 5-Lipoxygenase Products on IgE-Mediated Eosinophilia in Double-Challenged Rats

A 5-lipoxygenase inhibitor, B₂-subtype receptor and PAF receptor antagonists were used to explore a possible involvement of chemical mediators other than chemokines in the current model of IgE-mediated pleural eosinophilia. We have reported that distinct bradykinin B₂ receptor blockers, including Hoe 140, significantly inhibited antigen-mediated eosinophilia in actively sensitised rats [35]. As illustrated in figure 6, pretreatment with Hoe 140 (1 μg/cavity), 5 min before the second antigen challenge, failed to modify antigen-evoked eosinophilia in double-sensitised rats. However, the phenomenon was clearly sensitive to treatment with either PAF receptor antagonist BN 52021 (20 mg/kg, i.p.) or the 5-lipoxygenase inhibitor zileuton (50 μg/cavity, i.pl.), administered 1 h and 5 min before the second challenge, respectively.

Influence of Mast Cell Granule Depletion on Allergen-Mediated Eosinophilia Noted in Double-Challenged Rats

Polymyxin B has been shown to induce mast cell degranulation and release of biogenic amines [36, 37]. We reported here that a single intrapleural injection of polymyxin B (10 μg/cavity) led to a massive pleural mast cell degranulation within 48 h, while the amount of total protein and neutrophil and eosinophil counts remained unchanged (table 2). Polymyxin-B-induced mast cell granule depletion performed 48 h before the second cycle of IgE passive reaction failed to interfere with the subsequent eosinophil accumulation noted 4 h after rechallenge (fig. 7).

Table 2. Effect of polymyxin B (10 µg/cavity) on pleural mast cell population, protein content and neutrophil and eosinophil counts 48 h after its injection in normal rats.

Stimulus	Total protein mg/cavity	Mast cells × 10 ³ /cavity	Neutrophils × 10 ⁶ /cavity	Eosinophils × 10 ⁶ /cavity
Saline	5.0 ± 0.5	560 ± 19	0 ± 0	2.2 ± 0.2
Polymyxin B	4.5 ± 0.3	9 ± 2 ¹	0.3 ± 0.2	2.5 ± 0.8

¹ Statistically significantly different from saline-injected animals.

Discussion

The present study investigates the potential of the IgE-mediated system in triggering eosinophil recruitment, using a model of pleural antigen re-exposure in IgE-passively sensitised rats. Basically, a cycle of IgE passive sensitisation followed 24 h later, by an antigen challenge was repeated a second time after an interval of 7 days. Under these conditions, eosinophil pleural numbers were increased only after the second cycle of sensitisation and challenge, indicating that reactivation of the IgE-mediated system could play an important role in the induction of tissue eosinophilia during chronic allergies.

In an attempt to reproduce the characteristics of the allergic inflammatory response associated with the persistent contact with offending antigens – a current phenomenon in the routine of atopic subjects – some investigators have established experimental models in which successive allergen challenges were employed. For example, Campbell et al. [38] documented the characteristics of a pulmonary inflammatory response after one or two consecutive antigen challenges in actively sensitised mice. They found a clear exacerbation of the inflammatory response, including tissue eosinophilia, when lungs still inflamed were re-stimulated within 48 h. The report by Ciprandi et al. [39] corroborates these findings by demonstrating that the intranasal antigen re-stimulation of individuals with allergic rhinitis, at an interval of 3 days (but not 1 week), led to an exacerbation of neutrophil and eosinophil influx. Despite the evidence that consecutive antigen exposures could lead to exacerbation of inflammatory mechanisms, the potential roles of IgE or IgE-induced mast cell activation in this phenomenon had not been assessed.

The data obtained in the present study revealed that rats subjected to consecutive cycles of IgE passive sensitisation and challenge, in the pleural space, responded with mast cell degranulation and histamine release as should be expected. Pleural exudation appeared significantly attenuated after the second cycle of passive reaction, corre-

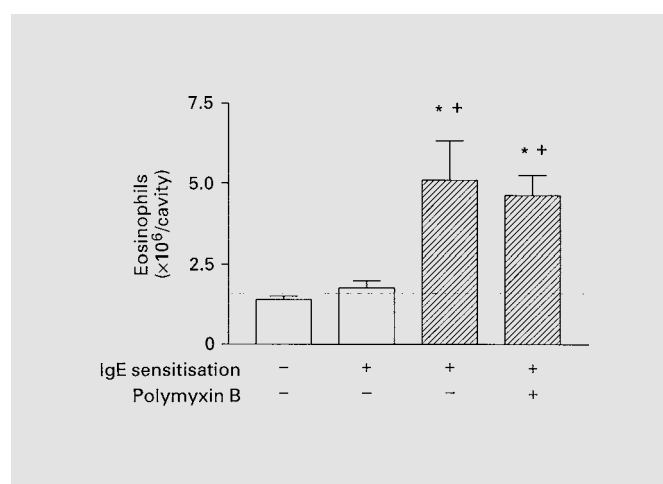


Fig. 7. Effect of pleural mast cell granule depletion on eosinophil migration noted 4 h after the second cycle of passive reaction. Open and hatched columns represent one and two cycles, respectively. Dashed lines represent animals injected only with saline. Columns represent the mean ± SEM from at least six animals. * $p < 0.05$ as compared to non-sensitised animals. + $p < 0.05$ as compared to animals submitted to one cycle of passive reaction.

sponding to approximately 50% of that observed after a single cycle. These findings are in line with the incomplete reposition of the stored histamine content in cells recovered from the pleural cavity 7 days after the first cycle of sensitisation and challenge, which was of about 50% of the controls. A reduction in immediate phase reactivity in sites of repeated antigen challenge has also been previously reported [40].

The anaphylactic phase following both single- or double-challenged animals were accompanied by a very mild neutrophil infiltration. It is noteworthy that there is no eosinophil recruitment following a single cycle of IgE sensitisation and challenge, which is in line with previous reports from our group [27] and others [28]. In contrast, a significant increase in the number of eosinophils was

noted at 4 and 24 h after re-exposure to antigen in the second cycle of IgE passive sensitisation. It should be emphasised that when IgE sensitisation was omitted during the second cycle of the allergic reaction, the pleural cavity was absolutely refractory to antigen challenge, indicating that the re-sensitisation procedure is absolutely crucial to the development of the secondary eosinophilic inflammatory response. The involvement of vasoactive amines and lipid mediators, including PAF, in the vascular and cellular changes triggered by antigen challenge is well established [41]. We reported here that similar to what was observed following a single allergen challenge in IgE passively sensitised rats, a combined stimulation with histamine and serotonin caused plasma leakage but failed to recruit eosinophils. On the other hand, pleurisy by PAF was marked by exudation followed by neutrophil and eosinophil infiltration into the pleural space [34]. In order to investigate whether the release of such mediators in the first cycle of sensitisation and challenge could favour the eosinophilic response in the second cycle, rats were sensitised and challenged 7 days after stimulation with either vasoactive amines or PAF. Our results indicated that the previous inflammatory response evoked by these mediators is not enough to trigger the subsequent cycle of sensitisation and challenge productive in terms of eosinophil recruitment.

Admitting that mast cell re-sensitization is an important event in tissue eosinophilia after the second cycle of passive reaction, one can assume the existence of alterations in the reactivity of these cells following the first antigen challenge. One possibility is that mast cells could be enhancing the expression of the high-affinity IgE receptors (FcεRI) leading to modifications and/or increasing the production of chemotactic mediators. In fact, Rajakulasingan et al. [42] have demonstrated that patients with allergic rhinitis, re-exposed to antigen, presented increased expression of FcεRI receptor. On the other hand, it is important to mention that IgE and IgE aggregates are also able to bind to other cell types than mast cells, via specific receptors [43, 44]. While trying to address the role of mast cells in the eosinophilia following the second cycle, we demonstrated that the previous local mast cell granule depletion failed to alter the eosinophil recruitment, despite being able to prevent plasma leakage. These data are consistent with the interpretation that mast cell granules and probably their content of stored mediators are implicated in protein exudation but not eosinophil accumulation. It has also been reported that the magnitude of leukocyte infiltration noted during a hypersensitivity cutaneous reaction in mast-cell-deficient mice is

equivalent or superior to that observed in wild-type animals [45]. Moreover, Ha and Heed [46] have shown that the absence of mast cells does not interfere with the development of harmful systemic IgE-dependent anaphylaxis supporting the interpretation that other cell types potentially have the ability to be activated by IgE. It is noteworthy that IgE receptors can also be expressed in alternative cell targets, including macrophages, dendritic cells and eosinophils [43, 44, 47, 48]. Therefore, the possibility does exist that at least one of these targets has been sensitised after repeated IgE administration, generating chemical mediators and effectively favouring pleural eosinophilia. This hypothesis is now under investigation in our laboratory.

It has been described that eosinophil accumulation in the sites of allergic inflammation is tightly regulated by specific adhesion molecules [49] and by chemical mediators released from activated inflammatory cells, notably cytokines and chemotactic factors [50]. Our results demonstrate that the anti-PAF receptor antagonist BN52021 and 5-lipoxygenase inhibitor zileuton abolished the eosinophilia triggered after antigen re-exposure, strongly suggesting the contribution of PAF and 5-lipoxygenase derivatives as eosinophil chemoattractants in this model. Similarly, Silva et al. [51] have shown that PAF antagonists and lipoxygenase inhibitor were able to inhibit antigen-induced pleural eosinophilia in actively sensitised rats. Our results also revealed that the peptide bradykinin does not appear to be involved in the current model of pleural eosinophilia, since pretreatment with the B₂ receptor antagonist Hoe 140 did not modify the number of these cells after rechallenge. These findings are in contrast with a previous study [35] which demonstrated that distinct bradykinin B₂ receptor antagonists suppressed late pleural eosinophilia evoked by antigen challenge in actively sensitised rats.

Eotaxin has been extensively studied in association with allergic inflammation by virtue of its preferential recruitment of eosinophils [52]. We have detected increased levels of eotaxin in the pleural effluent recovered from double-sensitised and challenged animals but not from those subjected to a single allergen challenge, pointing out a close correlation between eotaxin generation and eosinophil influx in that system. We have also investigated the role of MIP-1α and RANTES. While no significant changes could be detected concerning RANTES, a slight increase in the levels of MIP-1α detected following allergen challenge in the first and second cycles, without a difference between these increases. Thus, in contrast to what was observed with eotaxin, there were no differences

in the levels of MIP-1 α detected following one or two cycles of sensitisation and challenge. These results are in line with those of [36] which revealed a significant increase in eosinophil numbers and pulmonary eotaxin levels, but not MIP-1 α levels, during the secondary allergen airway rechallenge in actively sensitised mice.

In conclusion, our findings demonstrate that IgE passive sensitisation is able to promote allergen-mediated eosinophil recruitment into the rat pleural space. A single

cycle of sensitisation and challenge is insufficient, but two consecutive cycles allow the eosinophil infiltration to be established in a mechanism which seems to be dependent on eotaxin, PAF and 5-lipoxygenase derivatives.

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