

Revisiting the Roles of Mast Cells in Allergic Rhinitis and its Relation to Local IgE Synthesis

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ABSTRACT

Mast cells are important effector cells in the immediate-phase allergic reaction. However, in recent years much evidence has accumulated on the versatile role of mast cells in allergic inflammation. The present article is an overview of the roles of mast cells in allergic inflammation, especially in light of the local production of IgE and the IgE-IgE receptor network. Although both nasal mast cells (NMC) and T cells in allergic rhinitis are important sources of Th2-type cytokines like IL-4 and IL-13, and can induce IgE synthesis, we report here that antigen-activated NMC can secrete greater levels of IL-4/IL-13 and induce increased levels of IgE synthesis than antigen-activated nasal T cells. Furthermore, IgE production can occur locally in the nasal mucosa (target organ) and IgE itself can enhance the FcεRI expression and subsequent mediator release from NMC, thus contributing to the perpetuation of on-going allergic inflammation. Again, mast cells can contribute to the late-phase allergic reaction not only via the upregulation of adhesion molecules like VCAM-1, but also through the interactions of NMC with the extracellular matrix proteins, and interaction of NMC with nasal epithelial cells (NEC). Thus, it is increasingly evident that mast cells are not only important for the genesis of the allergic reaction, but also contribute to the late-phase allergic reaction and on-going allergic inflammation. (American Journal of Rhinology 14, 309–317, 2000)

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Allergic diseases like atopic rhinitis, bronchial asthma, and urticaria affect millions of people world-wide and its incidence is dramatically rising in both developed and developing nations. The reason why only a proportion of individuals develop this allergic reaction while others do not, even if they are all exposed to the same allergens, is not yet completely understood. A genetic basis, an abnormal T cell response, elevated levels of allergen-specific IgE, and perhaps environmental factors are among the factors responsible for inducing this allergic phenomenon. The need to better understand the pathogenesis of these diseases is a key factor in developing newer and more effective modes of treatment of these diseases.

It is well known that the allergic reaction is comprised of two phases, the immediate-phase reaction and the late-phase reaction. The immediate-phase reaction occurs as a result of crosslinking of the IgE receptor on the surface of mast cells by allergen and allergen-specific IgE, resulting in the release of chemical mediators like histamine, leukotrienes, and prostaglandin. The late-phase reaction is primarily due to the infiltration of a variety of inflammatory cells like eosinophils, neutrophils, basophils, macrophages, and lymphocytes, and the release of a number of soluble products like prostaglandins, leukotrienes, and PAF. Mast cells have been considered to play a central role in the immediate-phase allergic reaction. However, recent studies demonstrate that mast cells are an important source of various cytokines,^{1–6} indicating that mast cell activation and cytokine secretion may contribute to the leukocyte infiltration associated with the late-phase reaction. Still, in situations where the functions of different cells overlap, the question remains as to what is the relative contribution of each cell type to these allergic inflammatory responses. Here, we have reviewed the roles of nasal mast cells (NMC) in airway allergic inflammation compared the relative contribution of NMC and nasal T cells in IgE synthesis, demonstrated evi-

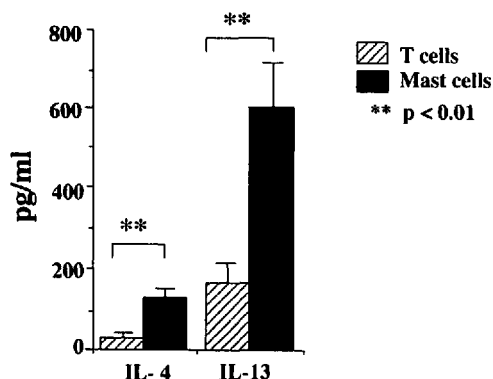


Figure 1. IL-4 and IL-13 production by nasal T cells and mast cells of PAR patients; 1×10^5 nasal T cells or mast cells from PAR patients were cultured in 100 μ L of RPMI with 10% FCS, in the presence or absence of 10 μ g/mL rDer fII^{19,20} for 48 hours. Levels of IL-4 and IL-13 in culture supernatants were estimated by sandwich ELISA using IL-4, (Genzyme, Cambridge, MA), and IL-13 specific (Chemicon, Temecula, CA) ELISA kits following the manufacturer's instructions. ($n = 10$) ** $P < 0.01$.

dence for local IgE synthesis, and also discussed the clinical significance of the mast cell-IgE-IgE receptor network.

MATERIALS AND METHODS

Patient Profile

Thirty patients with PAR (M/F = 16:14, mean age 22.8 ± 6.2 years), and 15 non-atopics with CIR (M/F = 9:6, mean age 35.2 ± 8.6 years) were selected after careful screening. All PAR patients were symptomatic, had positive radioallergosorbent tests (RAST: grades 3–6) to house dust mite (negative RAST to 16 other inhaled allergens tested), had no associated atopic disease, had not received immunotherapy, and were not taking steroids for at least a month before the study. Levels of serum total and specific IgE Abs were estimated by an ELISA (PRIST) and CAP RAST, respectively (Pharmacia, Uppsala, Sweden).

Reagents and mAbs

Collagenase, hyaluronidase, DNase, saponin (Sigma Chemical Co., St. Louis, MO); RPMI 1640, HBSS, HEPES, FCS, penicillin-streptomycin (GIBCO-BRL, Gaithersburg, MD), human rIL-4/rIL-13 (DNAX, Palo Alto, CA), and recombinant *Dermatophagoides farinae* II (rDer fII, mite allergen; Asahi Breweries Ltd., Tokyo, Japan)^{19,20} were obtained as indicated. The anti-c-kit (Nichirei, Tokyo, Japan), anti-tryptase (Chemicon, Temecula, CA), anti-CD40L (Pharmingen, San Diego, CA), anti-CD3 (Ortho Diag, Raritan, NJ), anti-IgE, anti-CD20 (Dako, Palo Alto, CA), anti-IFN- γ (Genzyme, Cambridge, MA), anti-IL-4/IL-13 (DNAX, Palo Alto, CA), and anti-Fc ϵ RI α chain (CRA1, non-competitive with IgE Kyokuto Pharmaceutical, Tokyo, Japan) mAbs, mouse IgG2b (Coulter Immunology, Hialeah, FL), and the anti-

CD19/anti-mouse IgG₁ coated magnetic beads (Miltenyi Biotech, Gladbach, Germany) were obtained as indicated.

Isolation and Purification of Mast Cells and T Cells

Nasal inferior turbinate mucosa obtained at surgery (conchotomy), was minced into 2–3-mm pieces and subjected to gentle agitation in two changes of Ca^{++} - and Mg^{++} -free HEPES-HBSS containing 1 mg/mL collagenase, 1 mg/mL hyaluronidase, 500 U DNase, 10% FCS, and 1% penicillin-streptomycin at 37°C for 60 minutes each, and mast cells were isolated and purified as previously described.⁵ Briefly, dissociated cells were passed through a 150- μ pore Nytex Cloth, resuspended in HEPES-HBSS with 500 U of DNase, 10% FCS, and centrifuged at $500 \times g$ for 5 minutes. After removal of monocytes by plastic adherence at 37°C for 90 minutes, the T cells were isolated by positive immunomagnetic selection of the CD3⁺ cells using MACS (purity >98%). Subsequently, the mast cells were purified by positive selection of CD117⁺ (c-kit⁺) cells⁵ using MACS (purity >98%). Purity of NMC was assessed by staining with the anti-tryptase mAb and the viability by the trypan blue dye exclusion test. Average numbers of isolated T cells were 1.2×10^6 /g wet tissue (net wet tissue weight 4–5 g; viability >95%), and average number of NMC was 1.6×10^5 /g wet tissue (net wet tissue weight 4–5 g; viability >95%). For flow cytometric analysis mast cells were semi-purified by density-gradient centrifugation with Percoll, as previously described.²⁴

Purification of B Cells

B cells were purified from PBMC by positive immunomagnetic selection of CD19⁺ cells using MACS, after Ficoll-Hypaque purification of the mononuclear cells (purity >98%).

Induction of Cytokine Production in NMC and Nasal T Cells

Nasal T cells (1×10^5) or mast cells from PAR patients were cultured in 100 μ L of RPMI with 10% FCS, in the presence or absence of 10 μ g/mL rDerf II^{19,20} for 48 hours. Next, to elucidate the time-kinetics of cytokine secretion by NMC, we stimulated NMC with rDerf II for 2, 4, 12, and 24 hours, and collected the supernatants at these different time points. Levels of IL-4 and IL-13 in culture supernatants were estimated by sandwich ELISA using IL-4 (Genzyme, Cambridge, MA), and IL-13-specific (Chemicon, Temecula, CA) ELISA kits following the manufacturer's instructions. Sensitivity of IL-4 and IL-13 assay systems were 0.045–3 and 0.195–200 ng/mL, respectively.

Induction of IgE Synthesis and Measurement of Secreted IgE

Nasal T cells (1×10^5) or mast cells (purity >98%) from PAR patients were cultured in round-bottom 96-well plates (Corning, New York, NY) with 1×10^5 autologous peripheral blood B cells (purity >98%) at a

ratio of 1:1 in 200 μ L of RPMI with 10% FCS for 12 days in the presence of rDerf II (10 μ g/mL). In some experiments, neutralizing anti-IL-4/IL-13 or anti-CD40L mAbs were added to the cultures at 10 μ g/mL each. Secreted IgE in the culture supernatants was measured using an ELSIA-IgE kit (Int. Reagents Corp., Kobe, Japan) following the manufacturer's instructions.

Immunohistochemical Analysis for Detection of IgE+ B Cells

Briefly, 5- μ m-thick, periodate-lysine paraformaldehyde (PLP) fixed frozen sections were incubated overnight at 4°C with the anti-IgE mAb, and stained by the alkaline phosphatase anti-alkaline phosphatase (APAAP; Dako, Palo Alto, CA) method. Sections were then treated with 10% normal goat serum, incubated in the anti-CD20 mAb and the FITC-conjugated anti-mouse IgG, rinsed in distilled water (DW) and mounted in Dako gel, as previously described.⁵ Negative controls were performed using isotype-matched unrelated mAbs. Results were examined with a Nikon fluorescent microscope (Tokyo, Japan).

Analysis of IL-4, IL-13, and IgE C ϵ Gene Expression by RT-PCR

Specific sense and anti-sense primers for IL-4, IL-13, and IgE C ϵ were constructed according to the published sequences. Total RNA was extracted from the homogenized nasal biopsies and reverse-transcribed as previously described.^{2,7} IL-4, IL-13 and IgE C ϵ gene segments from the resultant cDNA samples were PCR-amplified in the presence

of specific sense and anti-sense primers (1 mM each), 200 μ M dNTP, 0.5U/mL Ampli Taq (Cetus Corp, Emeryville, CA), 1U Perfect Match (Stratagene, La Jolla, CA), and PCR buffer (2.5 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 0.001% gelatin) in a final reaction volume of 20 μ L. PCR was performed for 30 cycles, each cycle including denaturation (94°C, 1 minute) annealing (60°C, 2 minutes) and extension (72°C, 3 minutes), and a final incubation (72°C, 10 minutes) after the last cycle. β -Actin cDNA was amplified as internal control^{2,7} and cDNA was substituted with DW as negative control. PCR products were electrophoresed, blotted onto nylon membranes, and detected using an alkaline phosphatase-labeled anti-digoxigenin Ab, as previously described.^{2,5}

Flow Cytometric Analysis

NMC (1×10^5) were incubated with saturating concentrations of FITC/PE-conjugated mAbs for 30 minutes on ice, washed twice in PBS with 0.2% BSA and 0.1% NaN₃, and analyzed using a FACScan (Becton Dickinson). Since in previous studies we have confirmed that all c-kit⁺ cells are tryptase⁺ (mast cells), correct gating for mast cells was performed by gating on CD117⁺ (c-kit⁺) cells, as previously described.⁵ As controls, isotype-matched unrelated mAbs were used in place of the primary Abs.

Effect of IL-4 and IgE on IgE-Mediated Histamine Release from NMC

To examine the effect of IL-4 and IgE on the IgE-mediated secretory capacity of NMC, NMC (1×10^5 cells/100 μ L, purity >98%) from CIR patients were either

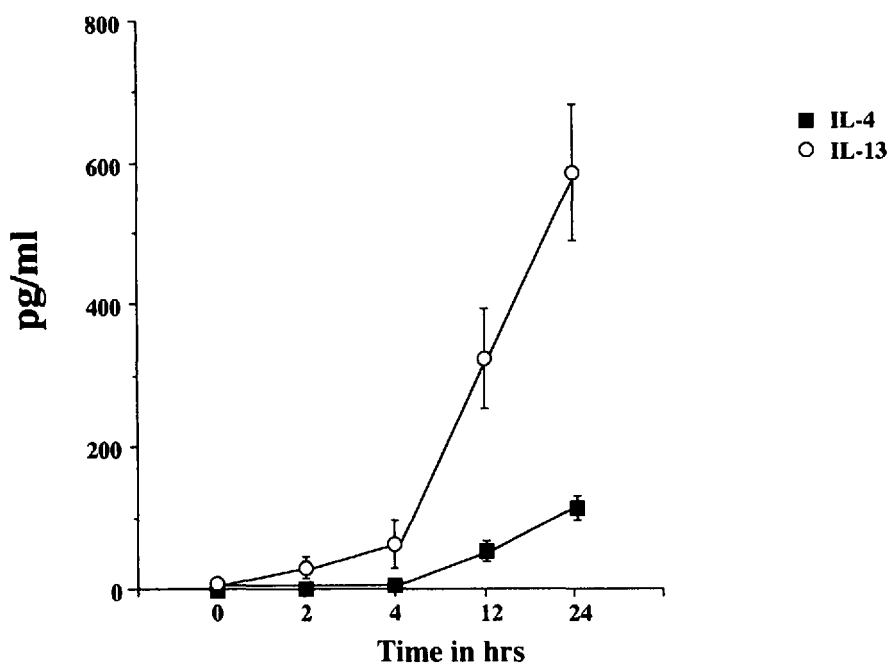


Figure 2. Time kinetics of IL-4 and IL-13 secretion by nasal mast cells (NMC). 1×10^5 nasal mast cells from PAR patients were cultured in 100 μ L of RPMI with 10% FCS, in the presence or absence of 10 μ g/mL rDerf II^{19,20} for 2, 4, 12, and 24 hours. Levels of IL-4 and IL-13 in culture supernatants at different time points were estimated by sandwich ELISA using IL-4, and IL-13 specific ELISA kits following the manufacturer's instructions. $n = 5$.

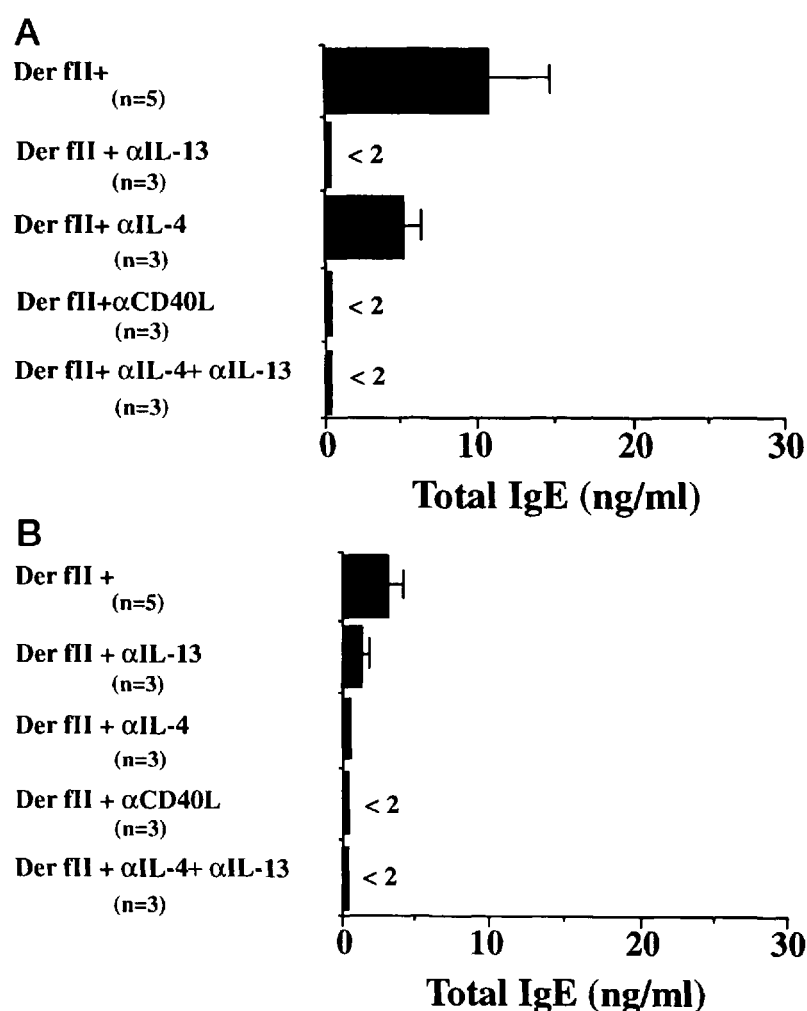


Figure 3. Nasal mast cell (NMC) and T cell induced IgE synthesis. 1×10^5 nasal T cells or mast cells (purity >98%) from PAR patients were cultured in round-bottom 96-well plates (Corning, USA) with 1×10^5 autologous peripheral blood B cells (purity >98%) at a ratio of 1:1 in 200 μ L of RPMI with 10% FCS for 12 days, in the presence of rDerfII (10 μ g/mL). In some experiments, neutralizing anti-IL-4/IL-13, or anti-CD40L mAbs were added to the cultures at 10 μ g/mL each. Secreted IgE in the culture supernatants was measured using an ELSIA-IgE kit (Int. Reagents Corp., Kobe, Japan) following the manufacturer's instructions. A) NMC-induced IgE synthesis; B) nasal-T cell induced IgE synthesis.

freshly isolated or isolated and passively sensitized with human myeloma IgE (10 μ g/mL), or isolated and treated for 72 hours with IL-4 and IgE, and then passively sensitized with human myeloma IgE (10 μ g/mL). Subsequently, these cells were washed twice, incubated in Ca^{++} and Mg^{++} free Tris ACM buffer at 37° C for 5 minutes, and then incubated with 200 μ L goat antihuman IgE (10 μ g/mL) in Tris ACM buffer containing Ca^{++} and Mg^{++} for 30 minutes at 37° C. The reaction was stopped by adding ice-cold Ca^{++} and Mg^{++} free Tris ACM. Controls were performed to calculate the spontaneous release by incubating the cells in Tris ACM buffer without the Ab. Samples were then centrifuged at $450 \times g$ for 10 minutes at 4° C and the cell pellets and supernatants were collected and stored at -80° C. Histamine was extracted from the cells and supernatants, and measured by ELISA using a Histamine EIA kit (Immuno-

tech, Marseille, France), according to the manufacturer's instructions.

For the cytokine secretion, NMC (1×10^5 cells/100 μ L, purity >98%) from CIR patients were either freshly isolated or isolated and passively sensitized with human myeloma IgE (10 μ g/mL), or isolated and treated for 72 hours with IL-4 and IgE and then passively sensitized with human myeloma IgE (10 μ g/mL). These cells were then cultured in vitro in 96-well culture plates and stimulated with anti-IgE (10 μ g/mL) for 24 hours. The levels of IL-4, IL-6, and IL-13 in culture supernatants were measured by ELISA using IL-4-, IL-6-, and IL-13-specific ELISA kits (Genzyme and R&D Systems, Minneapolis, MN). Sensitivity of IL-4, IL-13, and IL-6 assay systems were 0.045–3 ng/mL, 0.195–200 ng/mL, and 0.70 pg/mL, respectively.

Statistical Analysis

Results are expressed as mean $\pm$ SD. Statistical significance was determined by the Mann-Whitney U test (STAX, Computer Medical Lab, Tokyo, Japan).

RESULTS AND DISCUSSION

The levels of serum total and specific IgE antibodies for house dust mite ranged from 120 to 1,600 IU/mL (normal limit <250 IU/mL) and 32.8–134 kU/L (normal limit <0.35 kU/L) in PAR patients, and 8.1–30 IU/mL and <0.35 kU/L in CIR patients.

IL-4 and IL-13 Production from Nasal T Cells and Mast Cells of PAR Patients

It is well known that Th2-type cells are increased at sites of allergic inflammation.^{2,8,9} It is also well known that mast cells are an important source of Th2-type cytokines.^{1–6} Recently, we also demonstrated the protein production of IL-4, IL-6, and IL-13 from rDerfII-stimulated NMC of PAR patients.^{5,6} Here, we have compared the relative production of IL-4 and IL-13 from nasal T cells and mast cells. By comparison, NMC released greater levels of IL-4 and IL-13 as compared to nasal T cells. Interestingly, the level of IL-13 production was significantly greater than that of IL-4 (Fig. 1). Next, to verify the possible role of NMC in the late-phase allergic reaction, we examined the time-kinetics of IL-4 and IL-13 secretion from NMC. IL-4 and IL-13 were released as early as 2–4 hours after stimulation with specific antigen and peaked at 24 hours (Fig. 2).

Induction of IgE Synthesis by NMC and T Cells of PAR Patients

Recently, we demonstrated that NMC in PAR patients can express the CD40L and can induce IgE synthesis in B cells.⁵ While it is well established that activated T cells provide help to B cells in IgE synthesis, and this induction is believed to occur within the organ-associated lymphoid tissues like the GALT, NALT or BALT, our results suggest a novel and critical role for mast cells in perpetuating chronic allergic inflammation, by amplifying and maintaining the IgE synthesis, within the local microenvironment.

Here, we compared the IgE synthesis induced by nasal T cells and mast cells. Highly purified NMC and T cells from PAR patients induced IgE synthesis in autologous peripheral blood B cells in the presence of rDerf II (10 μ g/mL) (Fig. 3). Anti-IL-13 and anti-CD40L mAbs completely inhibited the NMC-induced IgE synthesis, whereas anti-IL-4 mAb only partially inhibited it (Fig. 3). This could be explained in part by the observations that the levels of IL-13 secreted by antigen activated NMC are 8- to 10-fold higher as compared to IL-4. By contrast, anti-IL-4 and anti-CD40L mAbs completely inhibited the nasal T cell-induced IgE synthesis, whereas anti-IL-13 only partially inhibited it. In a comparative study of *in vitro* IgE synthesis by lymphocytes

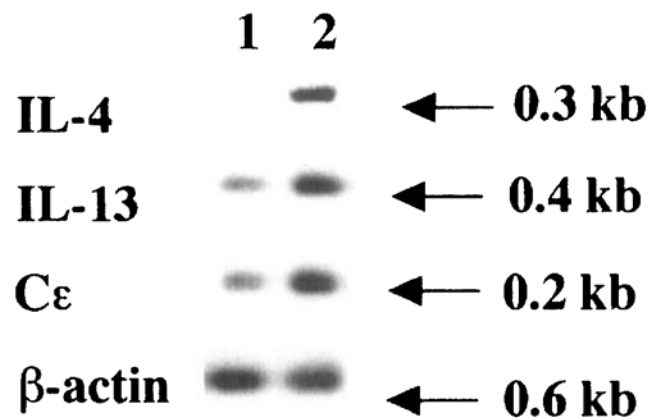


Figure 4. IL-4, IL-13 and IgE C ϵ mRNA expression in the nasal biopsy of a representative PAR patient. IL-4, IL-13 and IgE C ϵ mRNA expression in PAR patients was analyzed by RT-PCR after *in vitro* challenge of nasal biopsies with rDerfII. Lane 1: unstimulated; lane 2: stimulated with rDerf II.

obtained from allergic asthmatics and controls, it is reported that IgE synthesis *in vitro* was more IL-13-dependent. However, when comparing within the allergic asthmatic group, anti-IL-4 appeared to be more potent in inhibiting IgE synthesis.¹⁰

The relatively lower levels of secreted IL-4/IL-13 and the lower levels of synthesized IgE by nasal T cells as compared to NMC may be explained in part by the fact that we used mite antigen to stimulate the T cells and not anti-CD3 or PMA. In this context, it is worthwhile to add that within the nasal T lymphocyte population only a proportion of T cells are allergen-specific, whereas almost all NMC can respond to the mite allergen.¹¹ More importantly, in polysensitized individuals, unlike T cells that are specifically sensitized to a single allergen, the same mast cell can be activated by different allergens and thus induce the secretion of increased levels of IL-4/IL-13 and contribute to increased IgE synthesis. Furthermore, mast cells generally reside in the tissues and can therefore be activated by different inhalant allergens at the same time to induce IgE synthesis in an MHC-unrestricted manner. Thus, although T cells undoubtedly play a crucial role in IgE synthesis, at least within the local microenvironment, mast cells may play an important role in the induction of IgE synthesis. By contrast, circulating cells like T cells may be more important in the systemic synthesis of IgE.

Local IgE Synthesis in the Nasal Mucosa of PAR Patients

In 1970, Tada and colleagues first reported the existence of a significant number of IgE positive cells in the bronchial mucosa and nasal polyps of atopics¹² and proposed the concept of local IgE synthesis. We previously reported that B cells comprise ~20% of the lymphocytes in the nasal mucosa of allergic rhinitis.¹³ Based on the evidence that NMC can induce IgE synthesis in B cells, we

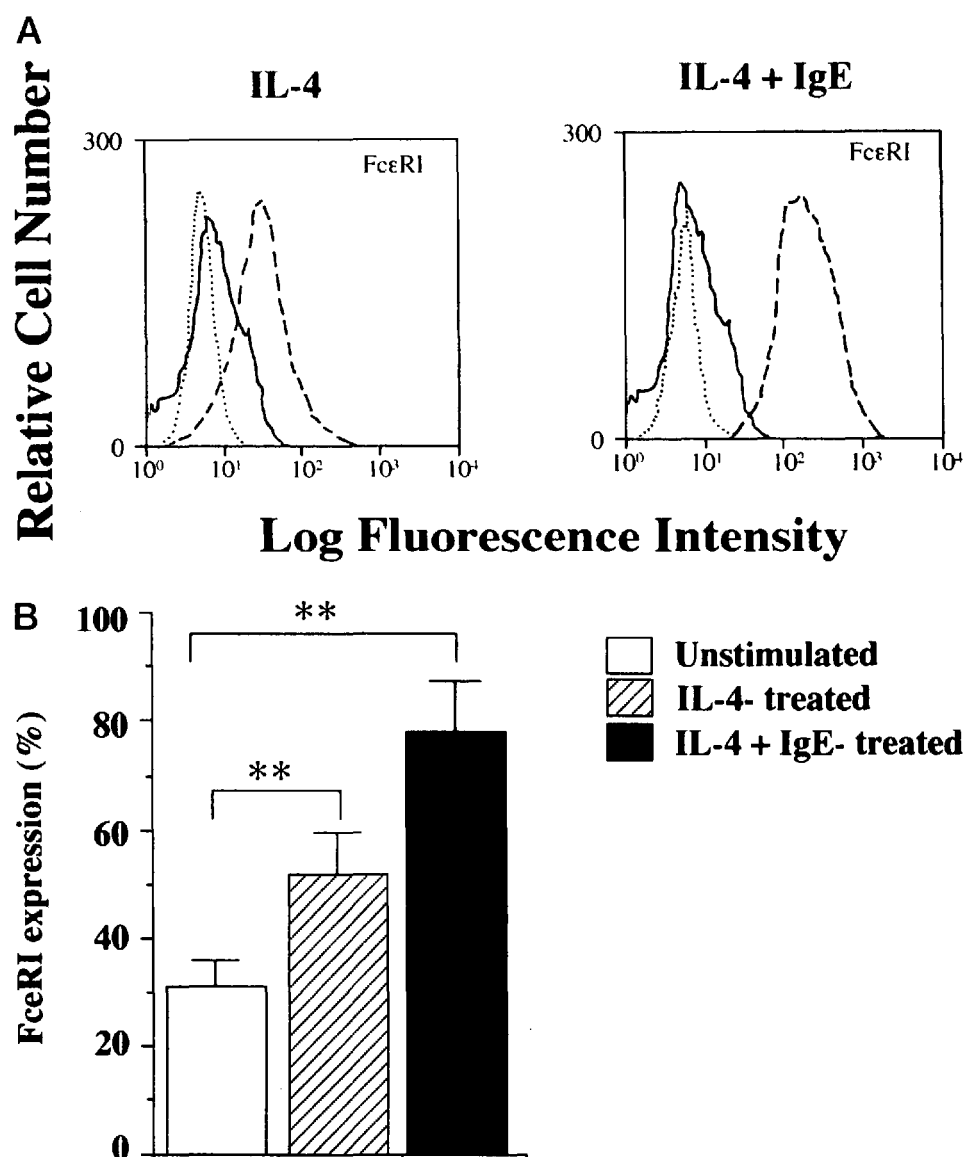


Figure 5. Upregulation of FcεRI expression in NMC with IL-4 and IgE. NMC from CIR patients were treated with IL-4 or IL-4 + IgE and the FcεRI expression in NMC was analyzed by flow cytometry as described in the text. **A**) FcεRI expression in NMC with or without treatment with IL-4 or IL-4 + IgE, a representative case. (·····), Control; (—), Unstimulated; (---), Stimulated. **B**) FcεRI expression in NMC ($n = 6$) was analyzed by flow cytometry as described in the text with and without treatment with IL-4, or IL-4 + IgE. Stimulation with IL-4 + IgE upregulated the FcεRI expression in NMC to a significantly greater extent than that with IL-4 alone.

hypothesized that IgE synthesis may occur locally in the target organ itself. In fact, recently, Durham et al. reported about the *in vivo* expression of mRNA for IgE in patients with seasonal allergic rhinitis after allergen challenge.¹⁴

Here, we report that the number of IgE+ B cells are significantly greater in the nasal mucosa of PAR patients than CIR patients (PAR: 4.6 ± 2.2 ; CIR: 0.5 ± 0.3 in an area of $0.202 \text{ mm}^2 \times 200 \text{ HPF}$) ($P < 0.01$). Although we could detect the expression of mRNA for the IgE Cε chain in nasal biopsies obtained from patients with PAR even under natural allergen exposure, it was interesting to note that mRNA expression for the IgE Cε chain was upregulated after *in vitro* challenge of nasal biopsies with

the purified mite antigen (rDerfII) (Fig. 4). The levels of protein for IgE were also increased after *in vitro* antigen challenge (data not shown). Similar observations on the synthesis of IgE have been reported in the lower airways of patients with bronchopulmonary aspergillosis and asthma.^{15,16}

Regulation of the FcεRI Expression in NMC

The FcεRI is a tetrameric structure comprising of an α-subunit, a β-subunit, and two disulfide-linked γ-subunits.^{17–19} The extracellular portion of the FcεRIα chain contains the entire IgE-binding site^{20,21} and studies in FcεRIα chain-deficient mice showed the inability of these

mice to exert allergen-induced anaphylaxis even with a normal number of mast cells.²² Several lines of evidence indicate that mast cells/basophils must display the high-affinity IgE receptors on their surface to be able to have significant IgE-antigen specific effector function.

Recently, we reported about the increased expression of the FcεRI in NMC of PAR patients in good correlation with the levels of serum-specific IgE.⁵ We and others also demonstrated that IL-4 upregulated the FcεRI in NMC.⁵ In this context, it is also reported that IgE itself can markedly upregulate the expression of the FcεRI in mast cells²³ and that anti-IgE mAbs can downregulate the expression of the FcεRI in basophils from atopics.^{24,25} Here we examined the effect of IgE on IL-4-mediated upregulation of FcεRI expression in NMC. IgE upregulated the IL-4-induced upregulation of FcεRI expression in NMC (Fig. 5). This was also associated with an increase in IgE-mediated mediator release (Fig. 6).

Versatile Roles of the Mast Cells in the Allergy and its Relation to IgE

It is well known that mast cells are increased in the epithelial compartment of the nasal mucosa of patients with allergic rhinitis and play a crucial role in the immediate-phase reaction through the allergen- and IgE-dependent release of inflammatory mediators. After crosslinking of allergen specific IgE on the surface of mast cells with multivalent antigen, several morphologic changes occur in the mast cells, including swelling of the cytoplasmic gran-

ules and subsequent solubilization of its granule contents. Histamine, tryptase, PGD₂, and LTC₄ are among the mast cell products that can be detected immediately after exposure to allergens.^{26,27} Histamine induces vasodilatation, increased vascular permeability, and increased glandular secretion in the ipsilateral and contralateral sides through neural reflexes. Sneezing results from the action of histamine on H₁ receptors present on the sensory nerve endings of the trigeminal nerve. Of particular importance is the action of histamine on the subepithelial blood vessels, causing vasodilatation, hyperemia, and mucosal edema. Prostaglandins like PGD₂ also cause edema by vasodilatation, and increased vascular permeability. The precise role of tryptase is not yet known. The physiologic effects of the newly synthesized leukotrienes like LTC₄, LTD₄, and LTE₄ are mediated by increasing vascular permeability, vasodilatation, and inducing mucous secretion.

Thus, until recently much emphasis was given to the role of mast cells in the genesis of the allergic reaction. At the same time, recent studies demonstrate that mast cells are an important source of Th2-type cytokines.^{1-6,8,9} This suggests that these cells may play more important roles in the allergic inflammation than as yet considered. For instance, mast cells can induce basophil, eosinophil, and T cell recruitment through the upregulation of adhesion molecules like VCAM-1 on endothelial cells (IL-4, IL-13, TNF-α). Furthermore, via the histamine-induced upregulation of RANTES/GM-CSF production from nasal epithelial cells, and IL-4/IL-13/TNF-α-induced up-

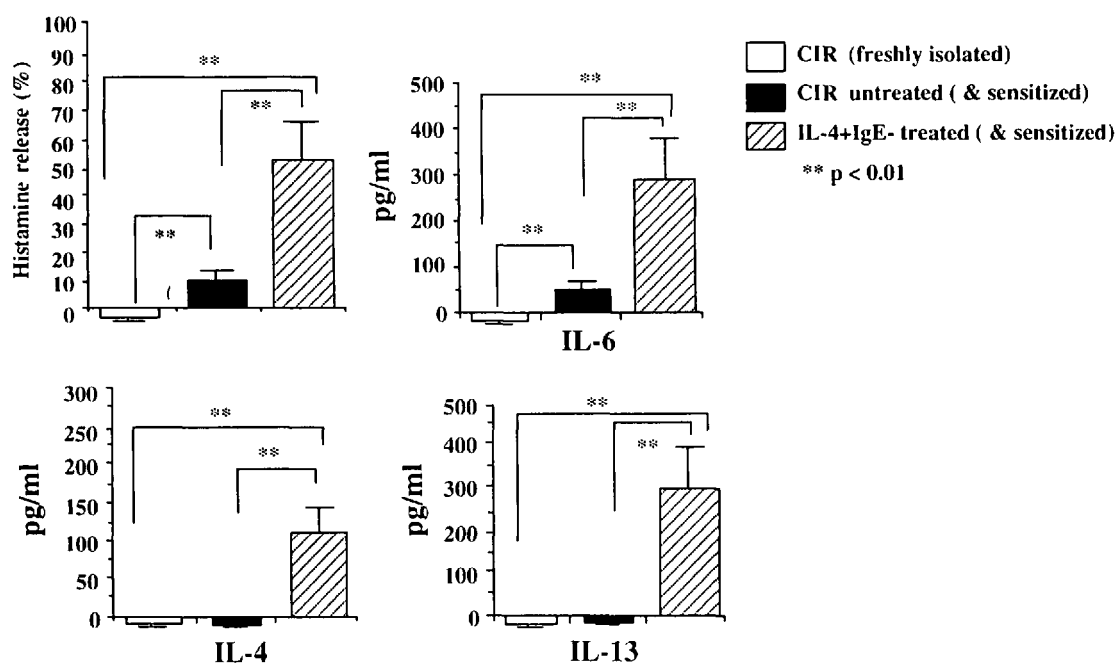
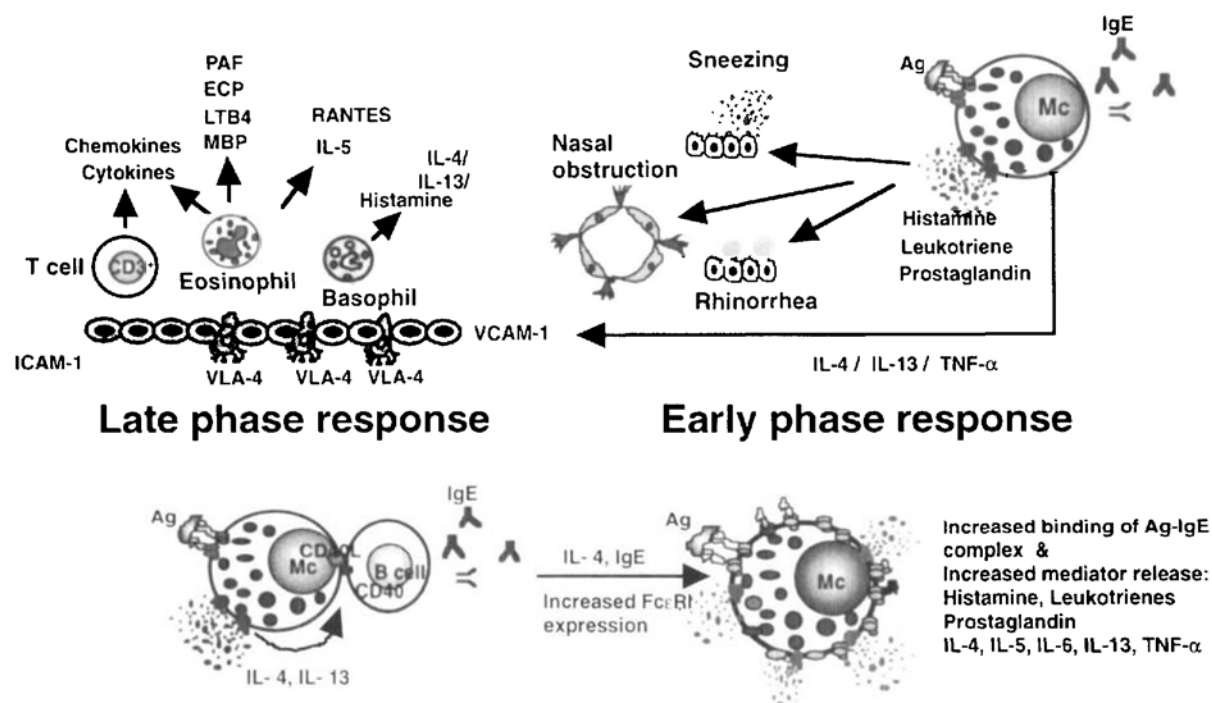


Figure 6. Effect of IL-4 and IgE on the release of histamine, IL-4, IL-6 and IL-13 from NMC. NMC (1×10^5 cells/100 μ L, purity: >98%) from CIR patients were either freshly isolated, or isolated and passively sensitized with human myeloma IgE (10 μ g/mL), or isolated and treated for 72 hours with IL-4 + IgE and then passively sensitized with human myeloma IgE (10 μ g/mL). Subsequently, these cells were stimulated with anti-IgE (10 μ g/mL) and the levels of histamine, IL-4, IL-6 and IL-13 were analyzed, as described in the text. ** $p < 0.01$. ($n = 6$).



On-going allergic inflammation

Figure 7. Integrated roles of nasal mast cells in the pathogenesis of allergic rhinitis.

regulation of the TARC production from nasal epithelial cells and fibroblasts, NMC can further contribute to the late-phase allergic reaction (manuscript in preparation). Indeed, time-kinetics of IL-4 and IL-13 secretion from NMC showed that allergen-activated NMC released cytokines as early as 2–4 hours and peaked at 24 hours, thus supporting their role in the late-phase allergic reaction. Thus, in addition to the well established role of T cells in the late-phase allergic reaction, mast cells can also be considered as key effector cells in the late-phase allergic reaction.

Although T cells are important players in the allergic inflammation, mast cells can also contribute to the on-going allergic inflammation by inducing IgE synthesis in B cells, promoting eosinophil development and survival (IL-5), and by inducing the Th0- to Th2-type switch. Furthermore, the synthesized IgE can further upregulate the FcεRI expression in NMC and the subsequent mediator release. These observations are of clinical significance since mast cells are an important source of IL-4, and in comparison to T cells, NMC produces higher levels of IL-4 or IL-13. In fact, the augmented FcεRI in NMC may then contribute to the binding of increased numbers of IgE-Ag complexes and thereby enhance the allergen- and IgE-mediated production of inflammatory mediators, forming an important positive feedback amplification loop (Fig. 7). Furthermore, interactions between mast cells and ECM via β1 integrins increases the cytokine release from mast cells, indicating the possibility of potentiating the allergic reaction (manuscript submitted).

More recently, we also observed that IL-4 upregulates the production of SCF in nasal epithelial cells.²⁸ Since SCF is an important cytokine necessary for the survival, growth and activation of mast cells, it may be presumed that mast cells can play a role in regulating its own survival.

Taken together, mast cells are not only important effector cells in the immediate phase of the allergic reaction, but rather play an integrated role in regulating allergic inflammation.

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