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### Research Article

Cross-linking of allergen specific IgE bound to the high affinity IgE receptor (Fc epsilonRI) on the surface of mast cells with multivalent allergens results in the release of both pre-formed and newly generated mediators, and in the manifestation of allergic symptoms. The expression of Fc epsilonRI, and the synthesis of IgE are therefore critical for the development of allergic diseases. In this study, we report that nasal mast cells (NMC) from patients with perennial allergic rhinitis (PAR) expressed significantly greater levels of the Fc epsilonRI, CD40L, IL-4, and IL-13 as compared to NMC from patients with chronic infective rhinitis (CIR). The level of Fc epsilonRI expression in NMC of PAR patients strongly correlated with the levels of serum total ( $r = 0.8$ ,  $P < 0.003$ ) and specific IgE ( $r = 0.89$ ,  $P < 0.0004$ ) antibodies. In addition, stimulation of NMC with IL-4, upregulated the Fc epsilonRIalpha chain expression both at the protein and mRNA levels, as detected by flow cytometry and reverse transcriptase-polymerase chain reaction. Furthermore, NMC from PAR, but not CIR, patients induced IgE synthesis by purified B cells in the presence of Der fII (mite antigen). These results suggest novel and critical roles for mast cells in promoting the allergic reaction through the increased expression of Fc epsilonRI and by enhancing and amplifying the IgE production, within the [...]

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## Nasal Mast Cells in Perennial Allergic Rhinitis Exhibit Increased Expression of the FcεRI, CD40L, IL-4, and IL-13, and Can Induce IgE Synthesis in B Cells

Ruby Pawankar,<sup>\*,†</sup> Minoru Okuda,<sup>‡</sup> Hans Yssel,<sup>§</sup> Ko Okumura,<sup>\*</sup> Chisei Ra<sup>\*</sup>

<sup>\*</sup>Department of Immunology, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113, Japan; <sup>†</sup>Department of Otolaryngology, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113, Japan; and <sup>§</sup>Department of Human Immunology, DNAX Research Institute, Palo Alto, California 94304-1104

### Abstract

Cross-linking of allergen specific IgE bound to the high affinity IgE receptor (FcεRI) on the surface of mast cells with multivalent allergens results in the release of both preformed and newly generated mediators, and in the manifestation of allergic symptoms. The expression of FcεRI, and the synthesis of IgE are therefore critical for the development of allergic diseases. In this study, we report that nasal mast cells (NMC) from patients with perennial allergic rhinitis (PAR) expressed significantly greater levels of the FcεRI, CD40L, IL-4, and IL-13 as compared to NMC from patients with chronic infective rhinitis (CIR). The level of FcεRI expression in NMC of PAR patients strongly correlated with the levels of serum total ( $r = 0.8, P < 0.003$ ) and specific IgE ( $r = 0.89, P < 0.0004$ ) antibodies. In addition, stimulation of NMC with IL-4, upregulated the FcεRIα chain expression both at the protein and mRNA levels, as detected by flow cytometry and reverse transcriptase-polymerase chain reaction. Furthermore, NMC from PAR, but not CIR, patients induced IgE synthesis by purified B cells in the presence of Der fII (mite antigen). These results suggest novel and critical roles for mast cells in promoting the allergic reaction through the increased expression of FcεRI and by enhancing and amplifying the IgE production, within the local microenvironment. (*J. Clin. Invest.* 1997; 99:1492–1499.) Key words: Fc receptor • allergy • mast cells • IgE • FcεRI

### Introduction

Mast cells and basophils are critical effector cells in IgE-mediated allergic diseases like atopic asthma, allergic rhinitis, and atopic dermatitis, but also participate in a variety of IgE-independent biological responses (1–4). Mast cells are derived from hematopoietic precursors that leave the bone marrow and complete their differentiation in the microenvironment of

peripheral tissues under the influence of stem cell factor (SCF) and other cytokines derived from resident cells like fibroblasts and epithelial cells (5). Even under normal physiological conditions, vascularized tissues have a resident population of mast cells whose numbers and phenotypes vary according to the anatomic site (6). Unlike mast cells, basophils circulate in the blood and normally do not reside in the peripheral tissues.

It is well-known that mast cells play a central role in allergic diseases through their capacity to bind IgE-Ag complexes via the high affinity IgE receptor (FcεRI),<sup>1</sup> resulting in the release of various chemical mediators and cytokines. However, the relationship between the level of FcεRI expression in nasal mast cells (NMC) and atopy is not well-defined. Studies using anti-CD40 mAbs (7) and CD40 L transfectants (8) indicated that the CD40/CD40L interaction is a key event in the immunoglobulin class switching for IgE synthesis. In addition, the synthesis of IgE by B cells requires the presence of soluble factors like IL-4 or IL-13 (9–11).

The nose is a potential site for chronic inflammatory diseases, like perennial allergic rhinitis (PAR), an IgE-mediated atopic disease characterized by elevated levels of serum-specific IgE and nasal eosinophilia, and chronic infective rhinitis (CIR), a bacteria or viral-induced disease. Previous studies on NMC demonstrated heterogeneity in granule protease expression, namely MC<sub>T</sub> and MC<sub>TC</sub>, and in their responses to various agonists (12–14), and recently, we and others demonstrated that NMC are an important source of multifunctional cytokines (IL-4, IL-5, IL-6, TNF-α, IL-13) (15, 16). However, the direct contribution of mast cell-derived cytokines in perpetuating chronic allergic inflammation is not yet defined. In this study, we investigated the capacity of NMC from PAR and CIR patients to express FcεRI, CD40L, IL-13, and IL-4, and induce IgE synthesis in B cells.

### Methods

**Patient profile.** 40 patients with PAR (M/F = 25:15; mean age: 23.2±8.2 yr), and 25 non-atopics with CIR (M/F = 12:13; mean age: 33.2±11.8 yr) 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 1 mo prior

Address correspondence to Dr. C. Ra, Department of Immunology, Juntendo University School of Medicine, 2-1-1, Hongo, Bunkyo-ku, Tokyo 113, Japan. Phone: 81-3-5802-1045; FAX: 81-3-3813-0421.

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1. **Abbreviations used in this paper:** CIR, chronic infective rhinitis; Cry j1, *Cryptomeria japonica*; FcεRI, high affinity IgE receptor; NMC, nasal mast cells; PAR, perennial allergic rhinitis; RAST, Radioallergosorbent tests; rDer fII, recombinant *Dermatophagoides farinae* II; RT-PCR, reverse transcriptase-PCR; TT, tetanus toxoid.

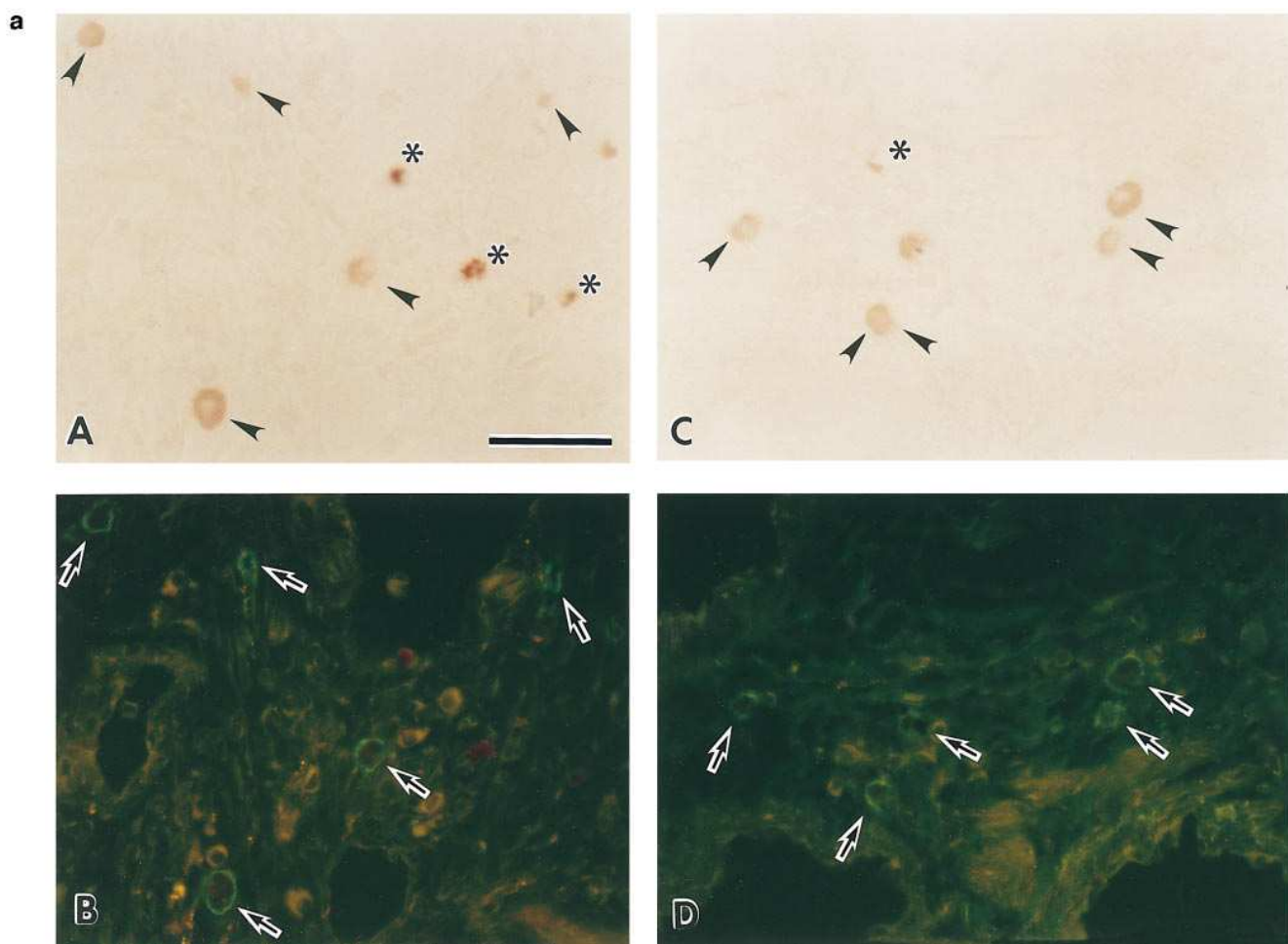
to the study. Levels of serum total and specific IgE Abs were estimated by an ELISA (PRIST) and CAP RAST, respectively (Pharmacia, Uppsala, Sweden).

**Reagents.** Collagenase, hyaluronidase, DNase, saponin (Sigma Chemical Co., St. Louis, MO); tetanus toxoid (TT; LBL, Campbell, CA), RPMI 1640, HBSS, Hepes, FCS, penicillin-streptomycin, (GIBCO BRL, Gaithersburg, MD), human rIL-4/IL-13 (DNAX, Palo Alto, CA), recombinant Dermatophagoides farinae II (rDer fII, mite allergen; Asahi Breweries Ltd., Tokyo, Japan) (19, 20) and purified major allergen of Cryptomeria japonica (Cry j1, Japanese cedar pollen; Hayashibara Biochemicals Ltd., Tokyo, Japan) (21) were obtained as indicated.

**mAbs.** The anti-c-kit (Nichirei, Tokyo, Japan); anti-tryptase (Chemicon International Inc., Temecula, CA); anti-CD40L/anti-IL-12 (PharMingen, San Diego, CA); anti-CD3 (Ortho Diagnostic Systems, Inc., Raritan, NJ); anti-IFN- $\gamma$  (Genzyme Corp., Cambridge, MA) anti-IL-4/IL-13 (DNAX); and anti-Fc $\epsilon$ RI $\alpha$  chain (CRA1, non-competitive with IgE) (22, 23) mAbs and the anti-CD19/anti-mouse IgG, coated magnetic beads (Miltenyi Biotech, Gladbach, Germany) were obtained as indicated.

**Isolation and purification of NMC.** 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<sup>++</sup> and Mg<sup>++</sup> 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 min each. Dissociated cells were passed through a 150  $\mu$  pore Nytex Cloth, washed, resuspended in Hepes-HBSS with 500 U of DNase, 10% FCS, and centrifuged at 500 g for 5 min at 22°C. Cells were then resuspended in Hepes-HBSS at a density of 10–50  $\times$  10<sup>6</sup> per ml, layered onto Percoll gradients (1.1–1.051 g/ml), and mast cells were isolated as previously described (24) (purity: 50–60%). In some experiments, NMC were purified by removal of monocytes by plastic adherence at 37°C for 90 min, immunomagnetic removal of CD3<sup>+</sup> cells, and positive selection of CD117<sup>+</sup> (c-kit<sup>+</sup>) cells (18) using magnetic activated cell sorting (MACS) (purity > 99%). Purity of NMC was assessed by staining with the anti-tryptase mAb and viability by the trypan blue dye exclusion test. Average number of isolated NMC was 1.6  $\times$  10<sup>5</sup> per g wet tissue (net wet tissue weight: 4–5 g) (viability > 95%).

**Purification of B cells.** Tonsillar tissue, obtained at surgery from nonatopics with chronic tonsillitis/sleep apnea syndrome, was finely



**Figure 1.** (a) IL-4 and IL-13 expression in NMC of a PAR and (b) CIR patient. Double staining of 5  $\mu$  thick frozen sections of the nasal mucosa with anti-IL-4/IL-13, and anti-c-kit mAbs, was performed as described in Methods. Bar = 50  $\mu$ m. Immunoreactivity for IL-13 (A) (arrow heads) and IL-4 (C) (arrow heads) was localized to c-kit receptor<sup>+</sup> cells (B and D) (arrows). Some c-kit receptor negative cells also expressed IL-4/IL-13 (\*). B and D represent the same sections as A and C, respectively. IL-4/IL-13 expression was detected in NMC of the PAR, but not CIR patient. (c) Cell surface expression of CD40L in isolated NMC. Percoll-purified NMC from PAR and CIR patients were analyzed by FACS after staining with FITC/PE-conjugated anti-CD40L and anti-c-kit mAbs. Initial purity of NMC ranged 50–60%. Correct gating for mast cells was performed by gating on the c-kit receptor<sup>+</sup> cells (18). Results shown are from a single experiment representative of fifteen with similar results. (----) Isotype control; (—) CD40L<sup>+</sup> cells. (d) Comparison of CD40L expression in NMC of PAR and CIR patients. Results are expressed as mean  $\pm$  SD. \*\**P* < 0.01.

minced, and subjected to gentle agitation in Hepes-HBSS with 1 mg/ml collagenase, 10% FCS, and 1% penicillin-streptomycin, at 37°C for 90 min. B cells were purified by positive immunomagnetic selection of CD19<sup>+</sup> cells using MACS, from the Ficoll-Hypaque purified mononuclear fraction of isolated cells (purity > 98%).

*Immunohistochemical analysis for cytokine expression in NMC.* Briefly, 5 μm thick periodate-lysine paraformaldehyde (PLP) fixed frozen sections were incubated overnight at 4°C with anti-IL-4/IL-13 mAb, and stained by the biotin-streptavidin horseradish peroxidase method (ABC kit; Vector Laboratories, Inc., Burlingame, CA) as

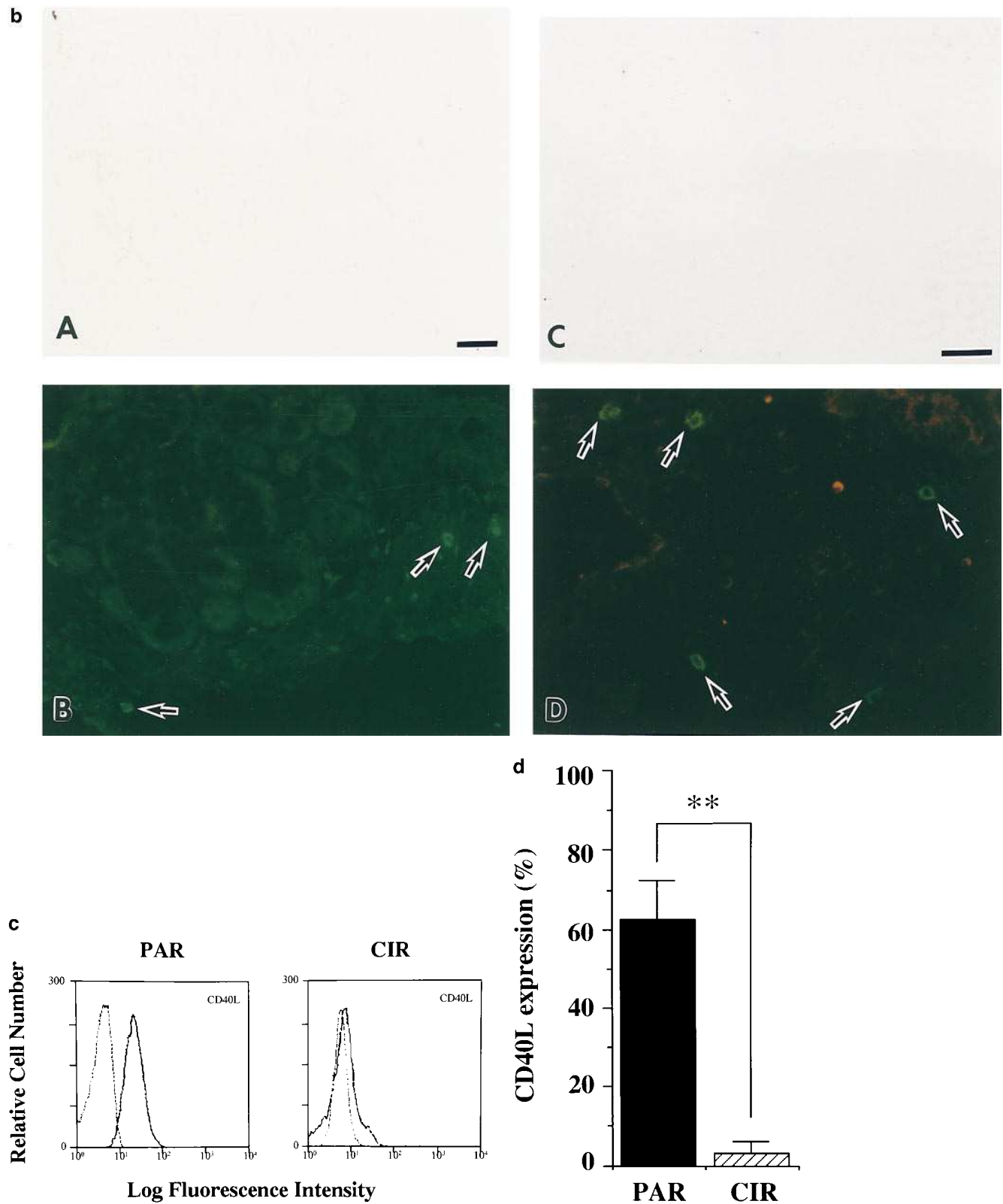


Figure 1 (Continued)

previously described (16). Sections were then treated with 10% normal goat serum, the anti-c-kit mAb, and FITC-conjugated anti-mouse IgG, rinsed in distilled water (DW) and mounted in Dako gel. IFN- $\gamma$ /IL-12 expression in NMC were analyzed by double staining with relevant anti-cytokine and anti-c-kit mAbs using the alkaline phosphatase anti-alkaline phosphatase (APAAP; Dako, Palo Alto, CA) and immunofluorescence methods. Negative controls were performed using isotype-matched unrelated mAbs. Results were examined with a fluorescent microscope (Nikon, Tokyo, Japan).

**Induction of cytokine production in NMC.**  $1 \times 10^5$  NMC (purity > 99%) from PAR or CIR patients were cultured in 100  $\mu$ l of RPMI with 10% FCS, in the presence or absence of 10  $\mu$ g/ml rDer fII (19, 20) for 48 h. Levels of IL-4, IFN- $\gamma$ , IL-12, and IL-13, in culture supernatants were estimated by sandwich ELISA using IL-4, IFN- $\gamma$ , IL-12 (Genzyme Corp.), and IL-13 specific (Chemicon International, Inc.) ELISA kits following the manufacturer's instructions. Sensitivity of IL-4, IFN- $\gamma$ , IL-12 and IL-13 assay systems were 0.045–3, 0.03–2, 0.01–2, and 0.195–200 ng/ml, respectively.

**Flow cytometric analysis of Fc $\epsilon$ RI, CD40L, and tryptase expression in NMC.**  $1 \times 10^5$  NMC were incubated with saturating concentrations of FITC/PE-conjugated mAbs for 30 min on ice, washed twice in PBS with 0.2% BSA and 0.1% NaN<sub>3</sub>, and analyzed using a FACScan (Becton Dickinson, Fullerton, CA). For staining of intracytoplasmic tryptase, NMC were first stained with the FITC-conjugated anti-c-kit mAb, fixed in 4% paraformaldehyde, permeabilized with 0.1% saponin in PBS, and then stained with the anti-tryptase mAb. Initial purity of NMC was 50–60%, and correct gating for mast cells was performed by gating on CD117<sup>+</sup> (c-kit<sup>+</sup>) cells. As controls, isotype matched unrelated mAbs were used in place of primary Abs.

**Induction of IgE synthesis and measurement of secreted IgE.**  $1 \times 10^5$  NMC (purity > 99%) from PAR or CIR patients were cultured in round-bottom 96-well plates (Corning Glass Works, Corning, NY) with  $1 \times 10^5$  tonsillar B cells (purity > 98%) at a ratio of 1:1 in 200  $\mu$ l of RPMI with 10% FCS for 12 d, with or without rDer fII (10  $\mu$ g/ml), and with or without rIL-4 (200 U/ml) or rIL-13 (250 U/ml). Alternatively, NMC from PAR patients were stimulated with unrelated antigens like TT or Cry j1 (1–10  $\mu$ g/ml each). In some experiments, neutralizing anti-IL-4/IL-13, or anti-CD40L mAbs were added to the cultures at 10  $\mu$ g/ml each (11). As controls, only B cells were cultured with rDer fII, in the presence or absence of rIL-13/IL-4. Secreted IgE in the culture supernatants was measured using an ELSIA-<sup>®</sup>IgE kit (Int. Regent Corp., Kobe, Japan) following the manufacturer's instructions.

**Stimulation of NMC with IL-4/IL-13.** Based on preliminary experiments performed to estimate the optimal dose and time for stimulation, Percoll-purified NMC from PAR or CIR patients were cultured at a density of  $1 \times 10^6$ /ml of RPMI with 10% FCS, in the presence or absence of rIL-4 or rIL-13 (10 ng/ml each; range 0.1–100 ng/ml) for 72 h.

**Analysis of Fc $\epsilon$ RI $\alpha$ ,  $\beta$ , and  $\gamma$  chain gene expression by RT-PCR and Southern blotting.** Specific sense and antisense primers for Fc $\epsilon$ RI $\alpha$ ,  $\beta$ , and  $\gamma$  chains were constructed according to the published sequences (24–26). Total RNA was extracted from NMC (purity > 85%) cultured with or without rIL-4 for 24 h, and reverse transcribed as previously described (16, 27). Fc $\epsilon$ RI $\alpha$ ,  $\beta$ , and  $\gamma$  chain gene segments from the resultant cDNA samples were PCR amplified in the presence of specific sense and antisense primers (1  $\mu$ M each), 200  $\mu$ M dNTP, 0.5 U/ml Ampli Taq (Cetus Corp, Emeryville, CA), 1 U Perfect Match (Stratagene Inc., La Jolla, CA) and PCR buffer (2.5  $\mu$ M MgCl<sub>2</sub>, 50  $\mu$ M KCl, 10  $\mu$ M Tris-HCl, pH 8.3, 0.001% gelatin) in a final reaction volume of 20  $\mu$ l. PCR was performed for 35 cycles, each cycle including denaturation (94°C, 1 min) annealing (55°C, 2 min) and extension (72°C, 3 min), and a final incubation (72°C, 10 min) after the last cycle.  $\beta$ -actin cDNA was amplified as internal control (16, 28) and cDNA was substituted with DW as negative control. For Southern blotting, PCR products were electrophoresed, blotted onto nylon membranes, hybridized (65°C, 15 h) with 2 ng/ml digoxigenin-labeled probes specific for Fc $\epsilon$ RI $\alpha$ ,  $\beta$ , and  $\gamma$  chains (Fc $\epsilon$ RI $\alpha$ : 783 mers, –1 to 782 bp; Fc $\epsilon$ RI $\beta$ : 738 mers, –1 to 737 bp; Fc $\epsilon$ RI $\gamma$ : 268

mers, –1 to 267 bp) and  $\beta$ -actin, and detected using an alkaline phosphatase labeled anti-digoxigenin Ab, as previously described (16, 28).

**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). Correlation coefficient was estimated by the Spearman's correlation coefficient test.

## Results

The levels of serum total and specific IgE antibodies for house dust mite ranged from 80–1,200 IU/ml (normal limit < 250 IU/ml) and 28.4–145 kU/liter (normal limit < 0.35 kU/liter) in PAR patients, and 7.1–25 IU/ml and < 0.35 kU/liter, in CIR patients.

Since IL-4, IL-13, IFN- $\gamma$ , and IL-12 are important cytokines that regulate the synthesis of IgE, we examined the expression of these cytokines in NMC of PAR and CIR patients by immunohistochemistry. Distinct IL-4 and IL-13 expression was detected in NMC of PAR patients (Fig. 1 A), and a remarkable proportion of these NMC expressed IL-4 (64.2  $\pm$  6.8%) and IL-13 (82.4  $\pm$  8.1%). In contrast, NMC in only 6 out of 15 CIR patients expressed IL-4 (2.5  $\pm$  2.0%), and none expressed IL-13 (Fig. 1 B). Stimulation of NMC from PAR patients with Der fII induced 10-fold more IL-13 secretion (0.58  $\pm$  0.02 ng/ml) than IL-4 (0.048  $\pm$  0.002 ng/ml), but IL-4 and IL-13 were not detected in the culture supernatants of unstimulated NMC from PAR patients, and Der fII-stimulated NMC from CIR patients. Neither IFN- $\gamma$  nor IL-12 was expressed/produced by NMC from either groups of patients. Flow cytometric analysis of CD40L expression in NMC revealed that > 50% of NMC from PAR patients, but < 5% of those from CIR patients expressed the CD40L (Fig. 1, C and D).

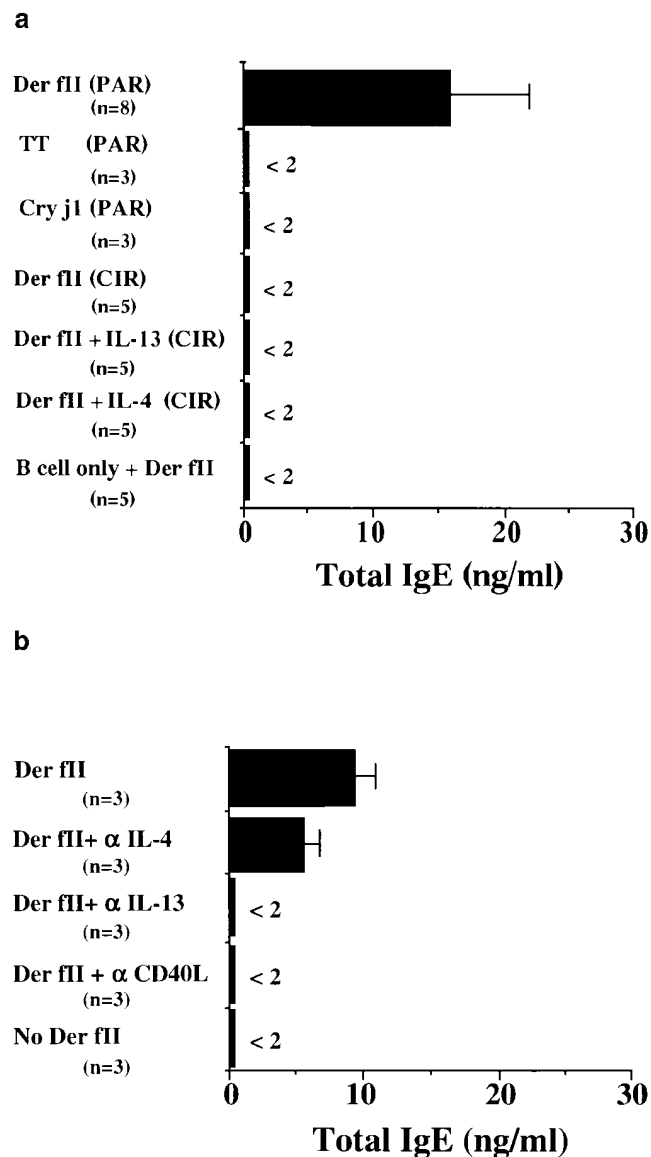
We next investigated whether NMC could support IgE production by B cells. Highly purified NMC (purity > 99%) from PAR patients induced IgE synthesis by purified tonsillar B cells (purity > 98%) in the presence of rDer fII (10  $\mu$ g/ml) even without exogenous IL-4 or IL-13 (Fig. 2 A). However, NMC from PAR patients did not induce IgE synthesis in B cells, in the absence of Der fII, or when stimulated with TT or Cry j1 (1–10  $\mu$ g/ml each) (Fig. 2 A). Similarly, culture supernatants of Der fII-stimulated NMC from CIR patients, with and without IL-4 or IL-13, or that of only B cells with rDer fII, did not contain detectable IgE (Fig. 2 A). NMC-induced IgE synthesis was partially blocked with the neutralizing anti-IL-4 mAb, but was completely blocked with anti-IL-13/CD40L mAbs (10  $\mu$ g/ml each) (Fig. 2 B).

Flow cytometric analysis of tryptase expression in NMC demonstrated that hundred percent of gated c-kit receptor<sup>+</sup> cells from both groups of patients expressed tryptase (Fig. 3 A). This indicated that all the gated c-kit<sup>+</sup> cells were mast cells. We next analyzed the Fc $\epsilon$ RI $\alpha$  chain expression in c-kit<sup>+</sup> cells, and found that > 70% of NMC from PAR patients, but < 50% of NMC from CIR patients expressed the Fc $\epsilon$ RI $\alpha$  chain (Fig. 3 B), indicating differential expression of the Fc $\epsilon$ RI $\alpha$  chain in NMC, and enhanced expression of Fc $\epsilon$ RI $\alpha$  chain in NMC from PAR patients. Differential expression of Fc $\epsilon$ RI $\alpha$  chain in NMC of PAR and CIR patients was also observed by double immunohistochemical analysis of nasal biopsies (not shown). To clarify the relationship between Fc $\epsilon$ RI expression in NMC and allergic rhinitis, we investigated the relation between the levels of Fc $\epsilon$ RI expression in NMC from PAR patients and the levels of serum total and specific IgE. Interestingly, we found

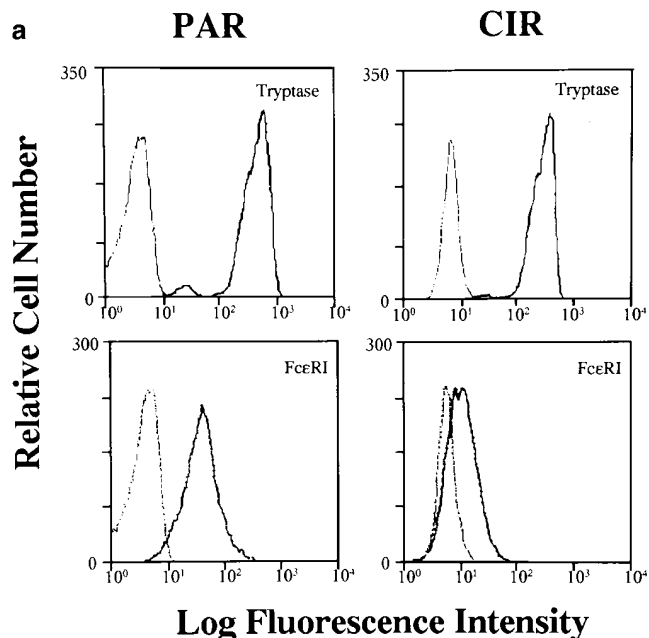
that the level of FcεRI expression in NMC of PAR patients strongly correlated with the levels of serum total ( $r = 0.8, P < 0.003$ ) and specific IgE ( $r = 0.89, P < 0.0004$ ) antibodies.

In light of the above mentioned observations, we asked whether Th2 type cytokines like IL-4 or IL-13 could upregulate FcεRI and CD40L expression in NMC. Taking NMC from CIR patients as representative of NMC that express lower levels of the FcεRI and CD40L, we demonstrated that IL-4 (10 ng/ml), but not IL-13, markedly upregulated FcεRI expression

in NMC ( $P < 0.01$ ) (Fig 4, A and B). FcεRIα chain expression in NMC of PAR patients was also upregulated with IL-4 (not shown). Furthermore, the IL-4-induced upregulation of FcεRI expression was inhibited with the neutralizing anti-IL-4 mAb (not shown). Neither cytokines examined had any effect on the CD40L expression in NMC (Fig. 4 B). We next investigated the effect of IL-4 on the gene expression of the α, β, and γ chains of the FcεRI in NMC, by RT-PCR and Southern blotting. When NMC were cultured with rIL-4, the FcεRIα chain gene expression was markedly upregulated but no difference was detected in the FcεRI β and γ chain, or in the β-actin gene



**Figure 2.** Induction of IgE synthesis in B cells by NMC. (a) Highly purified NMC (purity > 99%) from PAR or CIR patients were cultured with highly purified B cells (purity > 98%) at a ratio of 1:1, in the presence or absence of rDer fII (19, 20), TT or purified Cry j1 (21) (10 μg/ml each), and with or without exogenous IL-13 (250 U) or IL-4 (200 U) for 12 d. As control, B cells only were cultured in the presence of rDer fII. Secreted IgE in culture supernatants was estimated by sandwich ELISA, as described in Methods. Results are expressed as mean ± SD. (b) The neutralizing effect of anti-IL-4/IL-13 and anti-CD40L mAbs (10 μg/ml each) on IgE synthesis was assessed by culturing NMC from PAR patients with B cells, in the presence of relevant mAbs.



**Figure 3.** FcεRI and tryptase expression in NMC. FcεRI and tryptase expression in NMC from PAR and CIR patients was analyzed by FACS after staining with the anti-kit mAb and anti-FcεRIα chain (CRA1) (22, 23)/anti-tryptase mAbs. Correct gating for mast cells was performed as described in Fig 1. (a) FcεRI and tryptase expression in NMC of a PAR and CIR patient. Histograms shown are from a single experiment representative of 15 with similar results. (----) Isotype control; (—) FcεRI<sup>+</sup>/tryptase<sup>+</sup> cells. (b) Comparison of FcεRI expression in NMC of PAR and CIR patients. \*\* $P < 0.01$ .

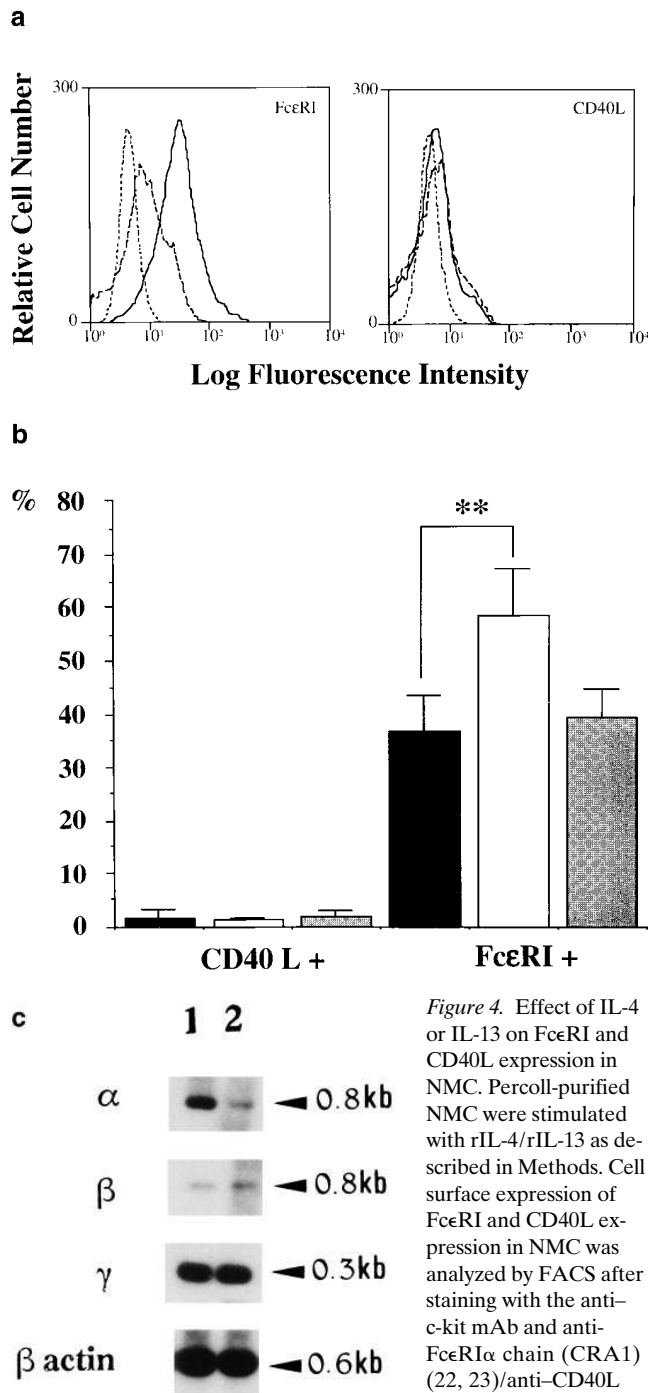


Fig. 1. (a) Effect of IL-4 (10 ng/ml) on FcεRI and CD40L expression in a CIR patient. Histograms shown are representative of ten experiments with similar results. (.....) Isotype control; (----) Unstimulated; (—) Stimulated with IL-4. IL-4 upregulated FcεRIα chain expression in NMC. (b) Effect of IL-4 or IL-13 (10 ng/ml) on FcεRI and CD40L expression in NMC of 10 patients with CIR was compared. Results are expressed as mean ± SD. ■: Freshly isolated; □: Stimulated with IL-4; ▨: Stimulated with IL-13; \*\**P* < 0.01. (c) Effect of IL-4 on FcεRI α, β, γ chain and β-actin gene expression in NMC (purity > 85%) was analyzed by RT-PCR using specific sense and antisense primers, as described in Methods. Southern blotting using digoxigenin-labeled probes specific for FcεRI α, β, and γ chains and β-actin was performed to confirm the PCR products. Lane 1: IL-4-stimulated NMC. Lane 2: unstimulated NMC. Although IL-4 upregulated FcεRIα chain gene expression in NMC, the FcεRIβ and γ chain, and β-actin gene expression, were unaltered.

expression (Fig. 4 C), indicating that IL-4 upregulated FcεRI expression in NMC by enhancing the FcεRIα chain expression at the mRNA level.

## Discussion

Recent studies demonstrated that, when human cell lines, KU812 (basophilic leukemia cell), and HMC-1 (immature mast cell) were stimulated with PMA and calcium ionophore they were induced to express the CD40L and provide help to B cells for IgE synthesis *in vitro*, in the presence of exogenous IL-4 (29). In contrast, cord blood-derived human cultured mast cells did not express IL-4 or the CD40L and did not support IgE synthesis (30). However, there is no report yet whether mast cells in patients with allergic diseases can induce IgE synthesis in response to stimulation with specific antigen.

We demonstrated for the first time that NMC from PAR patients expressed the CD40L, IL-4, and IL-13, and induced IgE synthesis in B cells in response to stimulation with specific antigen (Der fII), even without the addition of exogenous IL-4 or IL-13. 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 gut associated lymphoid tissue (GALT), nose associated lymphoid tissue (NALT), or bronchus associated lymphoid tissue (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. The relative lack of IL-4, IL-13, and CD40L, in NMC of CIR patients may explain their inability to support IgE synthesis.

The anti-IL-4 mAb partially inhibited NMC-induced IgE synthesis in B cells, while the anti-IL-13 mAb completely inhibited the synthesis of IgE. This may be explained by a 10-fold greater level of IL-13 secretion by Der fII-stimulated NMC as compared to that of IL-4, suggesting that IL-13 may play a more important role in the maintenance of IgE synthesis in PAR. Kuhn et al. reported that B cells in IL-4 deficient mice could not be induced to synthesize IgE (31), and Zurawski et al. reported that mouse B cells lack the IL-13 receptor (32). In light of the above data, and previous observations of a strong correlation between IL-13 expression in the nasal mucosa of PAR patients and serum-specific IgE (16), it may be considered that IL-13 plays a more crucial role in the maintenance of IgE synthesis in chronic allergic diseases.

FcεRI plays a central role in allergic inflammation by binding specific IgE and multivalent allergens, resulting in the activation of mast cells. It is also known that the extracellular portion of the FcεRIα chain contains the entire IgE-binding site (33, 34), and studies in FcεRIα chain-deficient mice demonstrated the inability of these mice to exert allergen-induced anaphylaxis even with normal number of mast cells (35). Therefore, the increased FcεRIα chain expression in NMC of PAR patients may contribute to enhancing the allergic reaction by binding greater number of allergen-IgE complexes. This may be further supported by the increased occupancy of IgE molecules on NMC of PAR patients (not shown).

More importantly, the level of FcεRIα expression in NMC of PAR patients strongly correlated with the levels of serum total and specific IgE antibodies. Malvaeux et al. previously reported a strong positive correlation between the density of IgE receptors on human basophils and serum IgE (36, 37), and

Pastorello et al. demonstrated a strong positive correlation between serum specific IgE and clinical symptoms in patients with allergic rhinoconjunctivitis (38). Therefore, the enhanced FcεRI expression in NMC of PAR patients may have important clinical implications. Furthermore, our recent studies actually revealed that mite antigen-stimulated NMC from PAR patients released significantly larger amounts of histamine than passively sensitized NMC from CIR patients (not shown), even though there was no significant difference in the total number of NMC between both groups.

We hypothesized that the differential expression of FcεRI in NMC of PAR and CIR patients may be because of the differential expression of Th2 type cytokines, like IL-4 or IL-13, in situ. We demonstrated that IL-4 upregulated FcεRI expression in NMC. This is of importance since several studies have shown increased numbers of IL-4+ mast cells/T cells in nasal biopsies of PAR patients (15, 39), suggesting that increased expression of FcεRI in NMC of PAR patients may be attributed to increased autocrine/paracrine production of IL-4, in situ.

In conclusion, we report that nasal mast cells in PAR patients expressed increased levels of the FcεRI, and induced IgE synthesis in B cells. These findings endow novel and critical roles for mast cells in perpetuating chronic allergic inflammation, by increasing allergen specific activation of mast cells, and amplifying as well as maintaining the elevated levels of specific IgE, at peripheral sites. Furthermore, the strong positive correlation between FcεRI expression in nasal mast cells of PAR patients and serum specific IgE, and the IL-4-induced upregulation of FcεRI expression, may have important clinical and therapeutic implications.

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