

Human dendritic cell 1 and dendritic cell 2 subsets express FcεRI: Correlation with serum IgE and allergic asthma

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Background: Type 1 dendritic cells (DC1) express the high-affinity IgE receptor (FcεRI); however, the regulation of FcεRI expression by DCs is not well understood. Type 2 DC (DC2) expression of FcεRI has not been demonstrated.

Objective: We hypothesized that DC2 cells also express FcεRI and that expression of FcεRI by the DC1 and DC2 subsets correlates with serum IgE and allergic asthma disease status.

Methods: To test these hypotheses, we quantitated FcεRI α chain expression by the peripheral blood precursor DC1 (pDC1) and pDC2 subsets by using flow cytometry.

Results: FcεRI was expressed by the pDC1 and pDC2 subsets, as well as tissue DCs from tonsils. Relative FcεRI expression by basophil, pDC1, and pDC2 subsets was 12:6.5:1, respectively. In both pDC subsets, FcεRI expression was significantly greater in allergic asthmatic subjects than in nonatopic control subjects. pDC1 and pDC2 expression of FcεRI was highly correlated to serum IgE concentration. The pDC1, pDC2, and basophil subsets demonstrated a similar magnitude of increase in FcεRI expression relative to changes in serum IgE.

Conclusions: FcεRI expression is characteristic of both the DC1 and DC2 subsets. Furthermore, FcεRI expression by these cells is highly correlated to serum IgE and to basophil FcεRI expression and is greater in subjects with allergic asthma. These data support the concept that novel therapeutic approaches directly targeted at FcεRI expression would affect both the sensitization and the effector phases of the allergen-specific immune response. (*J Allergy Clin Immunol* 2003;112:1132-8.)

Key words: Dendritic cell, IgE, IgE receptor, asthma, allergy, flow cytometry

The high-affinity IgE receptor (FcεRI) and allergen-specific IgE play an essential role in immediate-type hypersensitivity by inducing mast cell and basophil activation.¹ In addition to FcεRI expression by mast cells and basophils, in human subjects antigen-presenting cells (APCs), such as dendritic cells (DCs) and monocytes, express FcεRI.² Among DCs, only DC1 cells or DC1-like populations have been reported to express FcεRI, including the peripheral blood precursor DC1 (pDC1) subset,³ skin-derived Langerhans cells,⁴ inflammatory dendritic epidermal cells,⁵ and in vitro differentiated DC1 cells.⁶ In

Abbreviations used

APC: Antigen-presenting cell
BDCA: Blood dendritic cell antigen
Cy5: Cyanin-5
DC: Dendritic cell
lin-1: Lineage cocktail 1
MEPE: Molecules of equivalent phycoerythrin
pDC: Precursor dendritic cell
PE: Phycoerythrin

contrast, the one previous study to specifically examine the DC2 subset concluded that pDC2 cells from healthy control subjects did not express FcεRI.³ FcεRI expression by the DC1 subset appears to increase the efficiency of allergen presentation up to 1000-fold through an antigen-focusing mechanism in which FcεRI-bound IgE on such APCs captures allergen and thus enhances MHC presentation.⁷ FcεRI expression by the DC2 subset would similarly be expected to augment the differentiation and activation of allergen-specific T_H2 cells, thereby increasing allergic inflammation.⁸

The upregulation of FcεRI expression by DC1-like DC populations in human allergic disease would thus promote allergic inflammation and is an area of active study.^{2,9} In atopic dermatitis Langerhans cells isolated from lesional skin expressed increased surface levels of FcεRI,^{4,5} and this FcεRI expression correlated with serum IgE levels.⁵ In contrast, a more recent study found no difference between allergic asthmatic subjects and nonatopic control subjects in FcεRI expression by pDC subsets,¹⁰ suggesting that atopy and increased serum IgE levels might not affect DC expression of FcεRI before their trafficking into tissue.

These previous observations and the unequivocal documentation that increasing levels of IgE result in greater levels of FcεRI expression in mast cells and basophils led us to carefully reexamine whether the serum levels of IgE would similarly influence FcεRI expression on the pDC1 subset and indeed whether the pDC2 population might also express FcεRI and be similarly regulated. To address these questions, we measured FcεRI α chain (FcεRIα) expression by means of flow cytometry in the DC1 and DC2 subsets in peripheral blood samples taken from allergic asthmatic subjects and nonatopic control subjects. In this work we show clear evidence of FcεRI expression by both DC1 and DC2 subsets. We then explored whether FcεRI expression by both pDC1 and pDC2 subsets is correlated to serum IgE and asthma disease status and discuss the implications of these findings.

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METHODS

Antibodies

Anti-IL-3R/CD123 phycoerythrin (PE), PE/cyanin-5 (PE/Cy5), biotin; mouse IgG1 unlabeled, biotin, PE; mouse IgG2b unlabeled, PE, PE/Cy5; mouse IgG2a PE; streptavidin-PE; HLA-DR PE, PE/Cy5; CD3 FITC; and CD11c PE/Cy5 were obtained from BD-PharMingen Corporation (San Diego, Calif). Lineage cocktail 1 (lin-1: CD3, CD14, CD16, CD19, CD20, and CD56) FITC, CD4 allophycocyanin (APC; Becton-Dickinson Biosciences, San Jose, Calif); anti-CD1c (clone: blood dendritic cell antigen [BDCA] 1) biotin, PE; anti-BDCA-2 PE, APC; anti-BDCA-3 APC (Miltenyi Biotec, Auburn, Calif); G α M IgG1 PE, Alexa 647; streptavidin-APC (Molecular Probes, Eugene, Ore); CD20 FITC (Caltag Labs, Burlingame, Calif); anti-Fc ϵ RI α (clone AER-37) unlabeled, PE, biotin (eBiosciences, San Diego, Calif); human myeloma IgE (BioDesign, Kennebunk, Me); CD4 PE (Sigma-Aldrich, St Louis, Mo); CD14 FITC (Immunotech, Marseille, France); CD16 FITC (BioSource, Camarillo, Calif); and CD19 FITC (Serotec, Raleigh, NC) were obtained from the manufacturers. Anti-tryptase (clone G3) biotin and the basophil granule-specific mAb 2D7¹¹ were a gift from Dr Lawrence Schwartz (Virginia Commonwealth University). The study was begun by using the 22E7 anti-Fc ϵ RI α mAb (Dr Wayne Levin, Roche), but we were unable to obtain sufficient mAb to complete the study. Thus for later experiments we used the AER-37 anti-Fc ϵ RI clone. In pilot experiments both 22E7 and AER-37 efficiently competed the binding of the other, and neither mAb's binding was affected by means of preincubation with IgE (data not shown). This suggests that the 2 clones recognize identical or closely related epitopes on Fc ϵ RI α .

Reagents

Normal mouse serum (Caltag), saponin (Fluka, Ronkonkoma, NY), paraformaldehyde, dimethyl sulfoxide, collagenase II (Sigma); deoxyribonuclease I (Calbiochem, La Jolla, Calif); and Sphero Rainbow Calibration Particles (BD-PharMingen) were obtained commercially.

Study subjects and cells

The National Institute of Allergy and Infectious Diseases Institutional Review Board approved the clinical protocol for this study. All subjects signed an informed consent document. Healthy control subjects had no history of allergic disease or asthma, and no more than one immediate-type epidermal skin test response with induration of 2 mm or less (one healthy control subject had a single skin test response with an induration of 5 mm). Allergic asthmatic subjects had a minimum 1-year history of episodic bronchospasm relieved by β -agonist medications and 3 or more positive skin test responses (≥ 3 mm) out of a panel of 10 aeroallergens and were not experiencing an exacerbation at the time of the study. PBMCs were isolated from EDTA-anticoagulated blood by means of density gradient separation with Histopaque-1083 (Sigma), fixed in 4% paraformaldehyde for 5 minutes at 37°C, and cryopreserved in 10% dimethyl sulfoxide/PBS at -80°C, according to previously published methods.¹² Serum IgE determinations were performed by the National Institutes of Health Clinical Center Department of Laboratory Medicine by using a chemiluminescence immunoassay.

Human tonsils were obtained from surplus surgical samples and were exempted from institutional review board review by the National Institutes of Health Office of Human Subjects Protection. The tissue was first cut into 1- to 2-mm² pieces and then treated with collagenase (50 U/mL) and deoxyribonuclease I (2500 Dornase Units/mL) in a shaking water bath for 30 minutes at 37°C. The samples were then filtered through a 70- μ m cell strainer, fixed, and cryopreserved as above.

Antibody staining

Multiple combinations of antibodies were used in the experiments, which used a nonpermeabilizing adaptation of described procedures.^{12,13} The following protocol is an example of extracellular staining for pDC2 Fc ϵ RI α . Cryopreserved fixed cells were thawed, washed once in PBS with 0.1% BSA (PBS-BSA), and then blocked in PBS-BSA-5% nonfat dry milk (PBS-BSA-milk) for 1 hour on ice. Cells were incubated with 22E7 mAb or mouse IgG1 isotype control in PBS-BSA-milk for 30 minutes at 4°C, washed twice in PBS-BSA, and incubated with G α M IgG1 PE in PBS-BSA-milk for 30 minutes at 4°C and again washed. Cells were then incubated for 1 hour in 2% normal mouse serum in PBS-BSA-milk to block further G α M binding. Cells were then incubated with lin-1 FITC, CD123 PE/Cy5, and BDCA-2 APCs for 30 minutes. The cells were then washed twice in PBS-BSA and analyzed by means of flow cytometry. In experiments performed with the AER-37 PE-labeled anti-Fc ϵ RI mAb, all mAb staining was performed simultaneously.

The pDC1 subset was identified by first gating on CD1c⁺, lin-1⁻ cells after gating on these cells and back gating on cells of the corresponding scatter, after which a distinct pDC1 population was noted (polygon in Fig 1, A), which was then further gated on CD11c (Fig 1, B). The pDC2 and basophil populations were identified by first gating on the population of CD123⁺, lin-1⁻ cells (polygon in Fig 1, C) and back gating on cells of the corresponding scatter, which yielded 2 distinct populations of cells differentially expressing BDCA-2 (Fig 1, D). The pDC2 and basophil populations were identified as the BDCA-2 high and low populations, respectively. Antibody competition experiments were performed as described¹³ by using Zenon-1 APCs (Molecular Probes, Eugene, Ore) labeled with 22E7. Intracellular staining for tryptase and 2D7 was also performed, as previously described.^{12,13} Lactic acid stripping (Fig 2, H) was performed, as previously described.¹⁴ Briefly, PBMCs were treated with 0.01 molar lactic acid buffer, pH 3.9, for 5 minutes on ice, washed twice, and then incubated with either IgG or IgE (10 μ g/mL). Cells were then stained with IgE biotin (0.5 μ g/mL), washed twice, and stained with streptavidin-PE and the DC2 mAbs noted above.

Flow cytometry

Data were acquired with a 2-laser, 4-parameter FACSCalibur flow cytometer (Becton-Dickinson Biosciences) and analyzed on Cellquest (Becton-Dickinson Biosciences) or FlowJo (Tree Star, San Carlos, Calif) software. Typically, 600,000 to 1,000,000 total events were acquired to obtain adequate numbers of DCs (1000-3000). Fc ϵ RI α expression was quantitated as molecules of equivalent PE (MEPE) by using Sphero Rainbow Calibration particles, as per the manufacturer's instructions. Statistical markers were placed on the basis of the isotype-matched controls.

Statistical analysis

The Mann-Whitney *U* test was used to compare differences of Fc ϵ RI α expression between healthy donors and allergic asthmatic donors. A *P* value of less than .05 was considered significant. The Spearman rank correlation test was used to evaluate correlative data. Statistical calculations and linear regression analysis were performed with Prism software (GraphPad Software, San Diego, Calif). Relative Fc ϵ RI expression between cell lineages ("Results" text supporting Fig 3, A-C) was first determined in each subject by determining the ratio of Fc ϵ RI MEPE for 2 subsets and then calculating the median value of this ratio for the entire study population.

RESULTS

Fc ϵ RI α expression by DC subsets

We first sought to unambiguously identify pDC subsets by using the recently characterized BDCA mAb

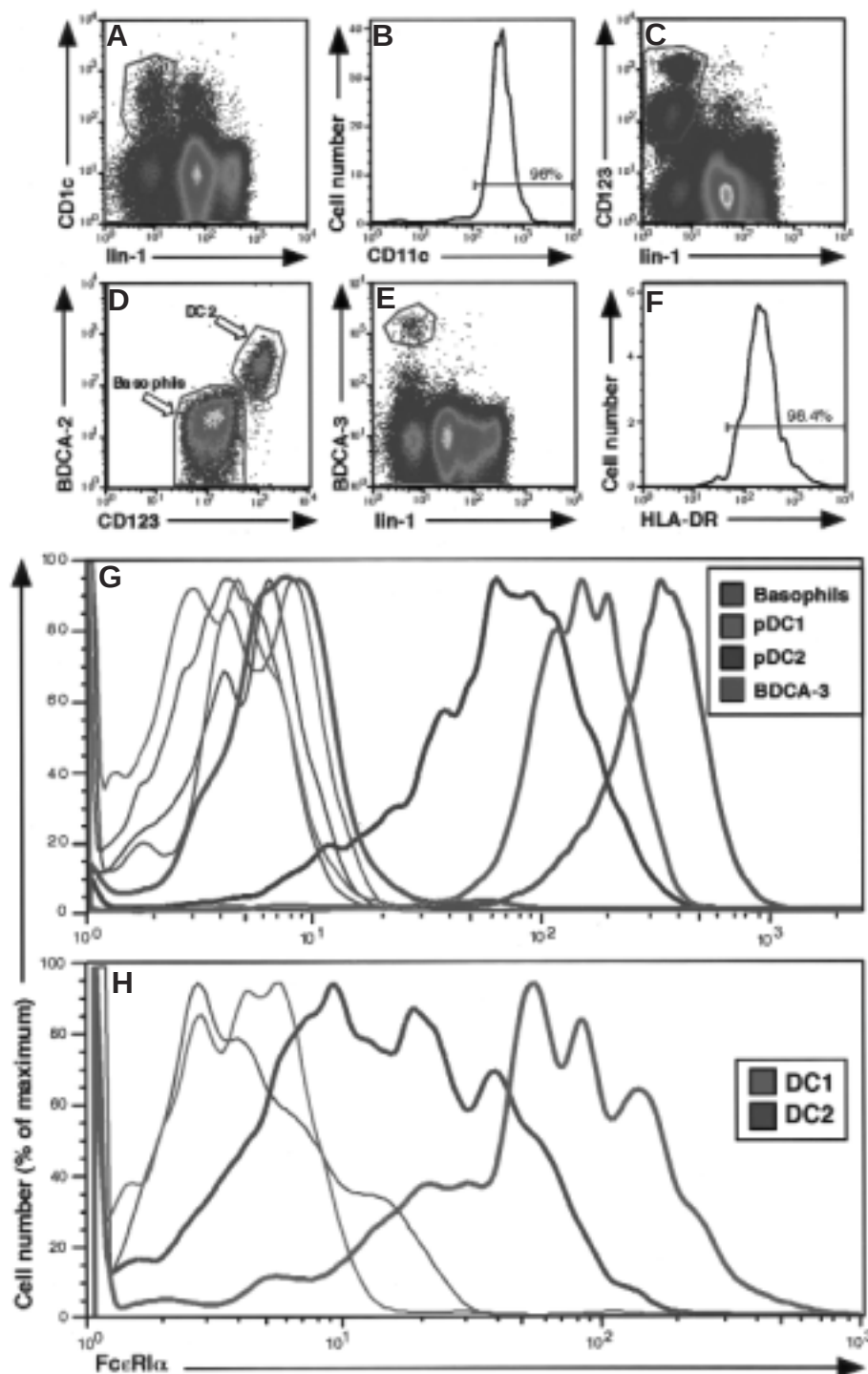


FIG 1. FcεRIα expression by pDC subsets. The pDC1 subset was identified as a CD1c⁺, lin-1⁻ population (A) and further gated on CD11c (B). After gating on CD123⁺, lin-1⁻ cells (C), the pDC2 and basophil populations were identified as 2 distinct populations of cells differentially expressing BDCA-2 (D). pDCs expressing the BDCA-3 marker were identified as being BDCA-3⁺, lin-1⁻ (polygon in E), and their HLA-DR expression was determined (F). PBMCs (G) or tonsillar mononuclear cells (H) were stained for pDC subsets and either FcεRIα (bold lines) or isotype control (thin lines) after gating on each subset. Results are representative of 4 subjects and 3 tonsil samples.

clones.^{3,15} pDC1 cells were identified by their characteristic scatter and CD1c⁺, CD11c⁺, lin-1⁻ phenotype (Fig 1, A and B). The identity of this pDC1 population was

confirmed by means of its uniform positive staining for HLA-DR (data not shown) and FcεRI (Fig 1, G). pDC2 and basophil subsets were identified by their characteris-

tic CD123⁺, lin-1⁻ phenotype (Fig 1, C) and were differentiated from each other by their expression of BDCA-2 and CD123 (Fig 1, D). The identity of the pDC2 subset was confirmed on the basis of uniform positive staining for HLA-DR and CD4 (data not shown). The identity of the basophil subset was confirmed on the basis of positive staining for 2D7 and negative staining for HLA-DR (data not shown). A third minor pDC subset was identified on the basis of the BDCA-3⁺, lin-1⁻ phenotype (Fig 1, E), which was confirmed on the basis of positive staining for HLA-DR (Fig 1, F).

As reported, basophils and pDC1 cells yielded uniform positive FcεRIα staining (Fig 1, G). The pDC2 subset, which had been reported to not express FcεRI,³ exhibited positive FcεRIα staining in all subjects examined. The median pDC2 FcεRI fluorescence was 20 times brighter than that of the isotype control (median value; range, 3.1-64; n = 40 subjects, 19 allergic asthmatic subjects and 21 nonatopic control subjects). In contrast, the BDCA-3⁺ pDC subset did not express surface FcεRI. These data demonstrate that both the pDC1 and pDC2 subsets express FcεRI.

Tissue DC expression of FcεRI

After antigen exposure, pDCs traffic into tissue, whereupon they capture antigen and ultimately traffic to secondary lymphoid tissue. To determine whether tissue DCs expressed FcεRI in a similar manner to what we found in peripheral blood pDCs, we examined tonsillar DC subpopulations for their expression of FcεRI. Both tonsillar DC1 and DC2 cells expressed FcεRI, with the DC1 subset consistently demonstrating greater expression (Fig 1, H). These data demonstrate that secondary lymphoid tissue DCs and peripheral blood pDCs express FcεRI in a similar manner.

Specificity of staining

pDC2 cells and basophils share a number of phenotypic features, including expression of FcεRI and CD123 and the lack of lineage marker expression, which could confound attempts to specifically quantitate FcεRI on blood DC populations. Additionally, mast cells express FcεRI, and thus it is possible that peripheral blood mast cell precursors could artifactually contribute to FcεRI staining of pDCs. We therefore confirmed that the pDC populations expressing CD1c and BDCA-2 did not costain for either the basophil marker 2D7 or for mast cell tryptase (Fig 2, A-D). We further validated the specificity of FcεRIα staining in these DC subsets in a competition experiment in which specific staining was blocked by means of preincubation with unlabeled mAb (Fig 2, E-G). These results demonstrate that FcεRI staining of pDCs is specific.

Our approach, using an mAb against FcεRIα only demonstrates expression but not that the receptor is capable of binding IgE. Given that FcεRI expression by the DC2 subset represents a new finding, we first determined that pDC2 cells stain with anti-IgE (data not shown), suggesting that the DC2 subset had bound IgE in vivo. To

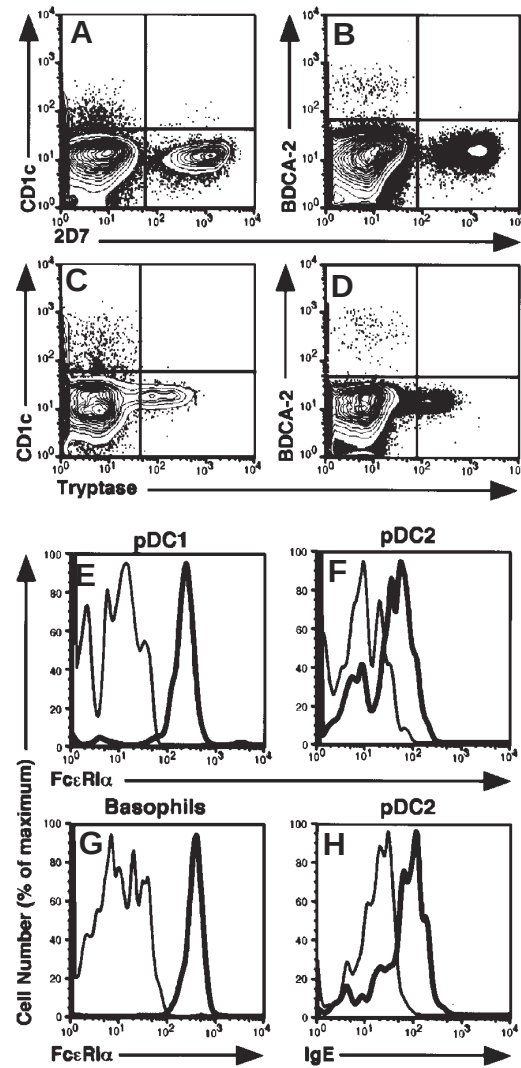


FIG 2. Specificity of staining. After gating on lin-1⁻ cells, dot plots showing CD1c or BDCA-2 versus either intracellular 2D7 (A and B) or tryptase (C and D) were generated. E-G, In the gated subsets noted, binding of labeled anti-FcεRI mAb 22E7 was blocked by using either an excess of 22E7 mAb (thin histograms) or an excess of IgG1 isotype control (bold histograms); results are representative of data from 4 subjects. H, PBMCs were stripped of IgE by using a lactic acid buffer, blocked with either IgG (bold histogram) or IgE (thin histogram), and then stained with labeled IgE and DC2 markers; results are representative of data from 5 subjects.

verify that FcεRI on the pDC2 subset is capable of binding IgE, we performed lactic acid stripping and then reloaded the cells with labeled IgE (Fig 2, H).

FcεRI expression in allergic asthma

We next examined FcεRI expression by pDC subsets in allergic asthmatic subjects and nonatopic control subjects, the clinical characteristics of which are noted in Table I. We found that both the pDC1 and pDC2 subsets from allergic asthmatic subjects expressed FcεRI at levels significantly greater than those of control nonatopic

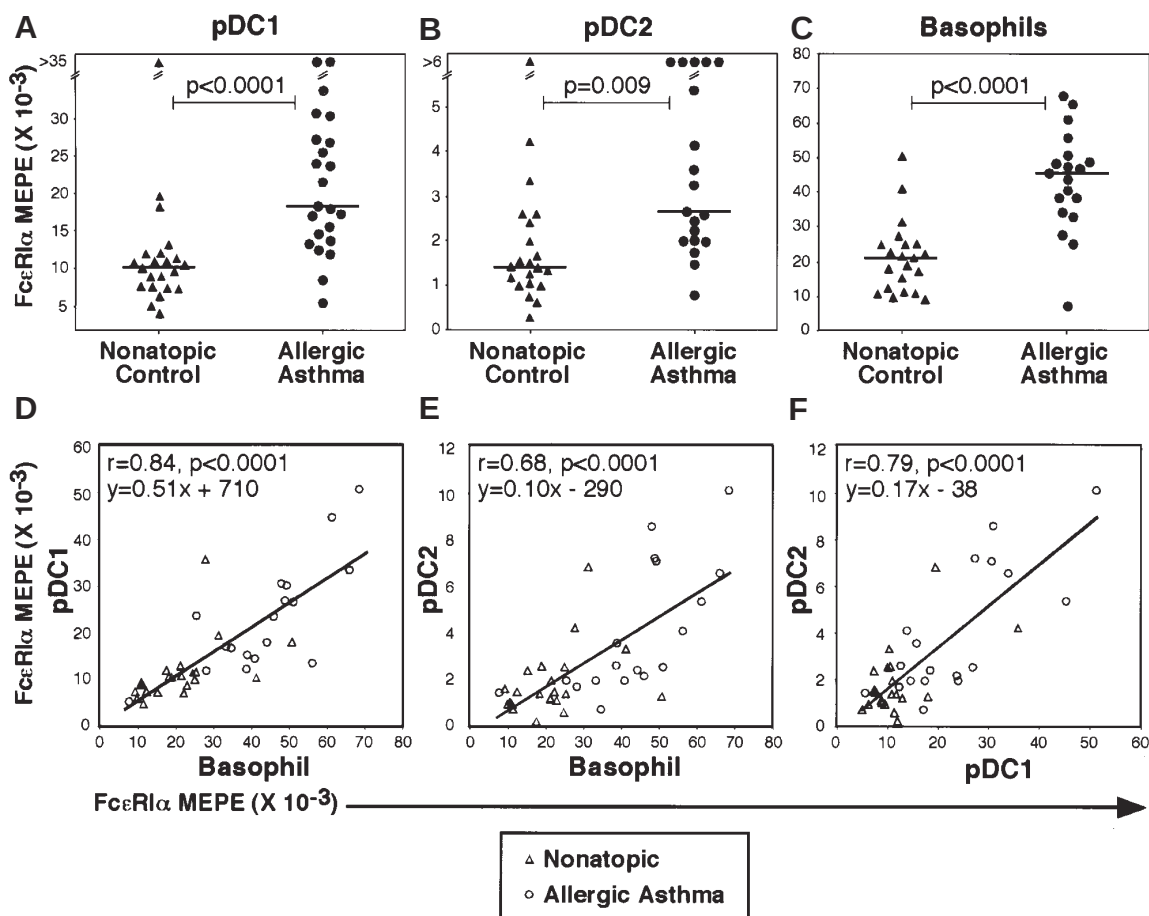


FIG 3. Differential DC FcεRIα expression in allergic asthmatic subjects versus nonatopic control subjects. **A-C**, PBMCs were obtained from 21 healthy nonatopic control subjects (triangles) and 23 allergic asthmatic subjects (circles), and FcεRI expression was determined by gating on the pDC1, pDC2, and basophil subsets. Horizontal lines indicate median values. Each symbol represents a unique subject. **D-F**, In the same subjects correlation of FcεRI expression between the DC and basophil subsets was determined by using the Spearman rank correlation test. The line fitting these results was determined by using linear regression analysis.

TABLE I. Clinical characteristics of the study population

Subjects	n	Serum IgE	No. of positive skin test responses	FEV ₁ (% predicted)	Age (y)	Sex (F/M)
Allergic asthmatic subject	23	210	6	69	39	10/13
Nonatopic control subject	21	23	0	95	35	13/8

Values shown in columns 3 to 6 represent median values. Skin tests with wheals of 3 mm or larger than those produced by the negative control were considered positive, as noted, out of a panel of 10 aeroallergens tested.

subjects (Fig 3, A and B). As expected from previous studies, basophils demonstrated a similar pattern of expression (Fig 3, C). FcεRI expression was 1.8, 1.9, and 2.2 times greater in the allergic asthmatic subjects versus nonatopic control subjects for the pDC1, pDC2, and basophil populations, respectively. The ratio of FcεRI expression was 12:6.5:1 for the basophil, pDC1, and pDC2 populations, respectively (median values calculated from intrasubject MEPE ratios, n = 23 allergic asthmatic subjects and 21 nonatopic control subjects).

Additionally, FcεRI expression was highly correlated between the pDC and basophil subsets (Fig 3, D-F). The slopes of the fitted lines indicate a ratio of FcεRI expres-

sion of 10:5:1 for the basophil, pDC1, and pDC2 populations, respectively. These results demonstrate that FcεRI expression by both the pDC1 and pDC2 subsets is increased in subjects with allergic asthma.

Relationship between FcεRI expression and serum IgE levels

To determine whether peripheral blood pDC expression of FcεRI correlates to serum IgE concentration, we simultaneously examined pDC FcεRI expression and serum IgE levels in individuals with a wide range of serum IgE concentrations. pDC1, pDC2, and basophil FcεRI expression were all highly correlated with serum

IgE (Fig 4). Analysis of the allergic asthmatic subjects alone yielded correlations of similar magnitude. The slopes of the fitted lines indicate that a 10-fold increase in serum IgE concentration is associated with increases in FcεRI of 5200, 2500, and 3700 MEPE units for the pDC1, pDC2, and basophil populations, respectively. These results demonstrate that FcεRI expression by both pDC subsets is highly correlated to serum IgE concentration.

DISCUSSION

In this study we demonstrate that FcεRIα is expressed by both the DC1 and DC2 subsets. We furthermore asked whether FcεRI expression by precursor DCs correlates with serum IgE or the presence of allergic asthma. We found highly significant correlations between DC FcεRI expression with both serum IgE and allergic asthma disease status. These results are consistent with the conclusion that IgE is a major factor regulating FcεRI expression by human DCs *in vivo*.

The readily detectable expression of FcεRIα by both peripheral blood pDC2 and tissue DC2 subsets is a new observation (Figs 1 and 3). The one previous study to specifically examine a pDC2 population reported that this subset did not express FcεRI,³ possibly because of the use of the 15.1 anti-FcεRI mAb clone, which stains dimly because of its competition with IgE for receptor binding.¹⁶ In this study we used anti-FcεRI mAb clones that recognize noncompeting epitopes and thus yield brighter staining. The demonstration that pDC2 cells express FcεRI suggests the possibility of a positive feedback loop in which T_H2-driven IgE production results in greater DC2 expression of FcεRI and, consequently, more efficient T_H2 cell priming and activation.⁸

Regulation of mast cell and basophil FcεRIα expression by IgE is well documented.¹⁷⁻²² Here we similarly present evidence that FcεRIα expression by both the pDC1 and pDC2 subsets is highly correlated to serum IgE. These data are consistent with the conclusion that DCs upregulate FcεRI even before they traffic into tissue and that IgE upregulation of pDC FcεRI is a systemic phenomenon. These results supplement the previous observation that in Langerhans cells isolated from the lesional skin of patients with atopic dermatitis, FcεRI expression correlates with serum IgE.^{4,5} A strength of our results using pDCs is that they provide evidence of FcεRI upregulation at an early stage of DC development before they have trafficked to tissue. Furthermore, because pDCs are not directly exposed to the local inflammatory milieu, they might more directly reflect systemic influences on FcεRI expression.

We found a highly significant association of DC FcεRI expression with allergic asthma for both the pDC1 and pDC2 subsets. A previous study using similar methods to examine FcεRI expression by pDCs concluded that asthma was not associated with increased surface expression of FcεRI by pDCs.¹⁰ This divergence of findings is most likely a result of technical differences in study design, particularly the previous report's use of the dimmer 15.1 anti-FcεRI mAb.¹⁶ However, our results provide a likely

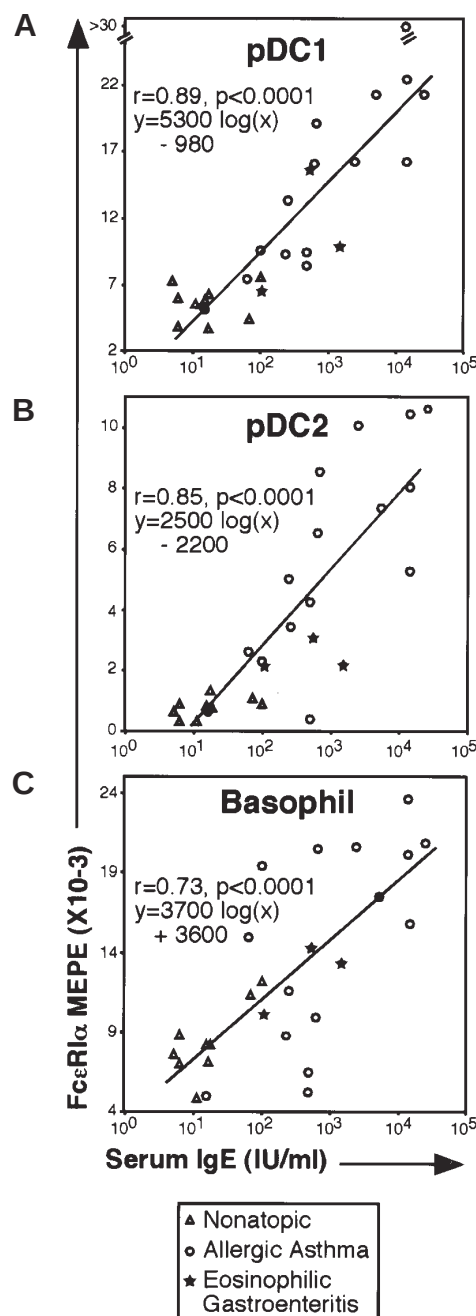


FIG 4. Correlation of serum IgE with DC FcεRIα expression. PBMCs were obtained from 15 allergic asthmatic subjects, 3 subjects with eosinophilic gastroenteritis, and 9 healthy nonatopic control subjects. For each subject, FcεRI expression for the pDC1 (A), pDC2 (B), and basophil (C) subsets was plotted against the serum IgE concentration. Correlation was determined by using the Spearman rank correlation test. The line fitting these results was determined by using linear regression analysis.

explanation for the major positive finding from that previous study, which showed that pDCs from allergic asthmatic subjects have an increased IgE binding capacity. This same group also examined bronchial biopsy specimens and found an increased number of FcεRI⁺ cells in allergic asthmatic subjects; however, the immunohisto-

chemical techniques used did not allow quantitation of FcεRI expression per cell.^{23,24} A major strength of the flow cytometry instrumentation used in this study is that it provides a quantitative measure of expression. Such increased levels of FcεRI expression by DCs would be expected to result in enhanced activation of allergen-specific T_H2 cells, thus promoting allergic inflammation.

We noted consistent differences in the absolute magnitude of FcεRI expression among the pDC1, pDC2, and basophil subsets and were particularly struck by the higher than expected FcεRI expression by the DC1 subset (Figs 1 and 3). Our results suggest that pDC1 cells express approximately half the amount of FcεRI per cell as that of basophils. Although still lower than that of basophils, this level of FcεRI expression is 5- to 50-fold greater than is generally appreciated for APCs.¹ This high level of expression of FcεRI by DCs highlights that such expression might influence DC function.

In a given subject FcεRI expression in one pDC subset was reflected in similar relative FcεRI expression in the basophil or other pDC subset. For example, subjects whose pDC1 population expressed high FcεRI also had pDC2 and basophil populations with high FcεRI expression relative to that of other subjects. Evidence for this parallel response is seen in Fig 3, D-F, in which FcεRI expression was highly correlated among the 3 subsets. Additionally, the slopes describing FcεRI expression as a function of serum IgE were similar among the pDC1, pDC2, and basophil subsets (Fig 4). Taken together, these results suggest similar regulation of FcεRI expression in both the pDC and basophil subsets, despite their expression of different forms of FcεRI.^{22,25,26} As such, the genetic basis and pathways controlling FcεRI expression might be a productive area for future research that could directly affect the care of those with allergic diseases.

In summary, there are several important implications of the research observations reported in this article. First, our findings show that FcεRIα expression is a typical characteristic of both the DC1 and DC2 subsets. Second, the fact that we found this expression at the pDC level underscores the potential for IgE to increase FcεRI expression on a wide range of DCs and to do so at the earliest stages of development before trafficking into tissue and differentiation into mature DCs. Lastly, the direct therapeutic down-regulation of FcεRI represents a unique molecular target whereby both the sensitization and effector phases of the allergen-specific immune response could be inhibited.

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