

Expression of the high-affinity IgE receptor on peripheral blood dendritic cells: Differential binding of IgE in atopic asthma

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Background: Dendritic cells can express the high-affinity IgE receptor (FcεRI), which, in the presence of specific IgE, facilitates the uptake of allergen, leading to increased activation of allergen-specific T cells. FcεRI expression by dendritic cells is higher in the airways of atopic asthmatic subjects than in those of healthy, nonatopic control subjects.

Objective: The aims of this study were to determine whether a similar difference in FcεRI expression occurs between dendritic cells in the peripheral blood of atopic asthmatic subjects and healthy individuals and also whether an altered ability of FcεRI⁺ peripheral blood dendritic cells to bind IgE accompanies the atopic asthmatic state.

Methods: Flow cytometry was used to analyze the surface expression of FcεRI and exogenously bound IgE on dendritic cells identified as lineage negative (CD3, CD14, CD16, CD19, and CD56) and HLA-DR bright.

Results: The total expression of FcεRI on the surface of dendritic cells from healthy and asthmatic subjects was not significantly different. However, *in vivo*, dendritic cells from atopic asthmatic subjects had higher levels of receptor occupancy by IgE and bound exogenous IgE *in vitro* more efficiently than dendritic cells from healthy subjects.

Conclusion: The similar levels of expression of FcεRI on peripheral blood dendritic cells from healthy and asthmatic subjects suggest that the local environment in the airway is responsible for the upregulation of surface FcεRI on airway dendritic cells in asthma. The results also suggest that the functional ability of FcεRI to bind IgE is differentially controlled in the atopic state. (*J Allergy Clin Immunol* 2001;107:1009-18.)

Key words: IgE, FcεRI, dendritic cell, asthma, atopy, peripheral blood, flow cytometry

Abbreviations used

APC:	Antigen-presenting cell
FcεRI:	High-affinity IgE receptor
MFI:	Mean fluorescence intensity
PE:	Phycoerythrin
PerCP:	Peridinin chlorophyll protein
SPT:	Skin prick test

IgE and its high-affinity receptor (FcεRI) play a pivotal role in the pathophysiology of asthma and other allergic diseases. Within minutes of exposure to allergen, FcεRI⁺ mast cells bearing IgE directed against the relevant allergen become activated and release a variety of mediators, chemotactic factors, and cytokines.¹⁻³ However, the expression of FcεRI on other cells present at allergic reaction sites, such as eosinophils,⁴ suggests that the function of FcεRI goes beyond its role in type I hypersensitivity. Recently, it is the expression of FcεRI on antigen-presenting cells, notably monocytes and dendritic cells, that has received particular attention.⁵

When expressed on antigen-presenting cells, FcεRI is directly involved in antigen capture for subsequent presentation to T cells.^{6,7} Allergen-specific IgE bound to the receptor through its Fc region can capture multimeric allergens, leading to receptor cross-linking and internalization. The internalized allergen is then proteolytically cleaved in cathepsin S-enriched intracellular compartments,^{8,9} and the resulting allergen peptides are loaded onto MHC class II molecules for presentation to T cells. FcεRI-mediated allergen uptake is up to 1000 times more efficient than allergen handled by any other route and results in greatly enhanced activation of allergen-specific T helper cells.⁶ Thus in allergic diseases, in which levels of allergen-specific IgE are elevated, this mechanism may be critically important in enhancing and perpetuating allergic inflammation and could thus contribute to the chronicity of the asthmatic inflammatory response.

The contribution of FcεRI-mediated allergen presentation by dendritic cells to the pathogenesis of allergic diseases may be further augmented by the fact that FcεRI expression is increased on dendritic cells present at the sites of allergic disease.⁵ In this context we have reported a significant increase in the number of FcεRI-bearing dendritic cells in the airways of atopic asthmatic subjects when compared with the airways of healthy subjects.^{10,11} It is therefore of critical importance to understand how FcεRI

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expression by dendritic cells is regulated in atopic asthma and to probe the functional competence of this receptor.

To gain an insight into the *in vivo* regulation of FcεRI expression by dendritic cells, we have investigated whether peripheral blood dendritic cells, the stage in the dendritic cell life cycle that immediately precedes recruitment into the lung, also show increased expression of FcεRI in atopic asthmatic subjects compared to healthy, nonatopic subjects. Furthermore, the possibility that the function of FcεRI on dendritic cells may be differentially regulated in atopic asthmatic subjects and healthy subjects has been explored by examining the *ex vivo* occupancy of FcεRI by IgE and its ability to bind exogenous IgE *in vitro*.

To avoid artifactual changes in FcεRI expression induced during cell purification, we have used 3-color flow cytometry to determine FcεRI surface expression on dendritic cells present at low frequency within PBMC preparations. This relatively simple and rapid method for the phenotypic analysis of dendritic cells enables the cells to be studied with minimal alteration to their phenotype.

METHODS

Materials

For skin prick tests (SPTs), aeroallergens and the saline (negative) control were obtained from Hollister Stier (Spokane, Wash) and the histamine acid phosphatase (positive) control was from ALK A/S (Hørsholm, Denmark). The dry wedge spirometer was from Vitalograph Ltd (Buckingham, United Kingdom). Cell purification reagents and chemical supplies were from Life Technologies (Paisley, United Kingdom) or Sigma-Aldrich Chemical Company (Dorset, United Kingdom). Sulfo-NHS-LC-biotin was from Pierce and Warriner (Cheshire, United Kingdom). FITC was from Sigma-Aldrich Chemical Company, and protein G and diethylaminoethyl cellulose was from Pharmacia Biotech (Hertfordshire, United Kingdom).

Antibodies

The murine mAb 15.1 against the α subunit of FcεRI (FcεRI α; kindly provided by Professor Jean-Pierre Kinet, Boston, Mass) and anti-CD23 (MHM6; American Type Culture Collection) were conjugated to biotin by using sulfo-NHS-LC-biotin according to the manufacturer's recommendations. Streptavidin quantum red was purchased from Sigma-Aldrich Chemical Company. HLA-DR-FITC, HLA-DR-peridinin chlorophyll protein (PerCP; both clone L243), and phycoerythrin (PE)-conjugated antibodies against CD3 (clone SK7), CD14 (clone MφP9), CD20 (clone L27), CD16 (clone B73.1), and CD56 (clone MY31) were obtained from Becton Dickinson (Oxford, United Kingdom). Biotinylated IgG1 and IgG2a isotype control antibodies were from Dako A/S (Glostrup, Denmark), and IgG2a FITC and IgG2a PerCP were from Becton Dickinson. IgE obtained from a patient with myeloma in the area served by the Southampton University Hospitals Trust was a kind gift from Professor Martin Glennie (Tenovus Laboratories, Southampton, United Kingdom). Myeloma IgE was purified by means of isolation with a protein G column and was either left unconjugated or was FITC conjugated, followed by ion exchange chromatography on diethylaminoethyl cellulose.

Study population

Ten healthy, nonatopic volunteers; 10 atopic subjects with mild asthma receiving treatment with inhaled β₂-agonists as required; 5

atopic nonasthmatic subjects; 5 nonatopic asthmatic subjects with mild symptomatic disease; and 5 atopic subjects with moderate asthma treated with inhaled corticosteroids took part in the study. Subjects were characterized with respect to atopic status (SPT reactivity and plasma total IgE levels), FEV₁, and a clinical history of asthmatic episodes. SPTs were carried out with the following aeroallergens: *Dermatophagoides pteronyssinus*, mixed grasses, cat fur, and dog hair. A skin wheal response was scored as positive if larger than 3 mm in diameter in the presence of negative and positive control reactions. Total IgE was measured by using a sandwich ELISA of plasma samples. Nonatopic subjects were defined as having a negative SPT response to all the aeroallergens and circulating levels of total IgE of less than 100 IU/mL.¹² Subjects with a positive SPT response and clinical history of atopy, irrespective of the level of total IgE, were regarded to be atopic. Lung function was determined by measurement of FEV₁ with a dry wedge spirometer, with the highest of 3 values recorded. The FEV₁ was expressed as a percentage of predicted value adjusted for sex, height, and age.¹³ All subjects gave informed consent, and the study was approved by the Southampton Combined University and Hospital Ethics Committee (246/97).

Preparation of PBMCs

PBMCs were separated from 70 mL of EDTA-treated blood by means of standard centrifugation over Lymphoprep (20 minutes at 20°C and 1000g). Plasma was collected for subsequent analysis of total IgE levels. Once washed, PBMCs were divided into 2 fractions, and all further processing was performed at 4°C. The first fraction was incubated for 5 minutes with 0.01 mol/L lactic acid-buffered saline solution (0.13 mol/L NaCl, 0.005 mol/L KCl, and 0.01 mol/L lactic acid [pH 3.9])¹⁴ to remove *in vivo*-bound IgE. In preliminary time-course studies, this time point was determined as being most efficient at removal of IgE with maximum retention of cell surface FcεRI (see Results section). The second fraction was incubated in PBS (Dulbecco's PBS without Ca²⁺ and Mg²⁺) as a control, allowing analysis of the level of *ex vivo* occupancy of the IgE receptors. Cells were then immediately washed twice with cold PBS with 0.1% sodium azide (NaN₃; wt/vol) and 1% BSA (wt/vol).

Immunostaining for flow cytometry

For each condition, 1 × 10⁶ cells were incubated for 30 minutes in PBS with 0.1% NaN₃ and 1% BSA containing 50 μg/mL human Fcγ fragments (blocking buffer) to prevent nonspecific binding by means of Fcγ receptors. Cells were then incubated for 30 minutes with 50 μL of biotinylated mAb 15.1, anti-CD23, or isotype controls, all diluted to their optimal concentration in blocking buffer. The cells were washed 3 times in wash buffer. Cells were then labeled with streptavidin quantum red in conjunction with FITC-conjugated anti-HLA-DR and a cocktail of PE-conjugated antibodies against CD3, CD14, CD20, CD16, and CD56 and incubated for 30 minutes. In parallel, cells were also labeled with appropriate fluorochrome-conjugated isotype control antibodies, anti-HLA-DR FITC, and the cocktail of PE-conjugated antibodies. PBMCs were washed 3 times and placed on ice in the dark pending analysis.

Assessment of the ability of unoccupied FcεRI on dendritic cells to bind IgE *in vitro*

Flow cytometry was used to assess the *in vitro* binding of IgE by unstripped and lactic acid-stripped FcεRI. Cells were stained simultaneously with IgE conjugated to FITC, an HLA-DR PerCP conjugate, and the cocktail of PE-conjugated antibodies against lineage markers. As a control, cells were preincubated on ice for 30 minutes with 10 μg/mL unconjugated myeloma IgE and then stained with IgE-FITC as above. To determine the contribution of FcεRI to IgE binding, stripped cells were preincubated with 5 μg/mL mAb 15.1

TABLE I. Clinical and physiologic characteristics of the patients studied

Subject category (sex, M:F)	Age (y)	FEV ₁ (% predicted)	IgE (IU/mL)
Healthy subjects (4:6)*			
Range	18-46	93-130	5-42
Mean	32.40 [†]	107.60 [‡]	13.20
SE	2.6	3.7	3.7
Atopic nonasthmatic subjects (1:4)*			
Range	27-36	88-104	14-333
Mean	30.20 [†]	97.80	103.40
SE	1.6	3.0	58.6
Nonatopic asthmatic subjects (1:4)*			
Range	25-45	78-94	5-25
Mean	31.00 [†]	84.80	10.00
SE	3.6	2.6	5.0
Atopic subjects with mild asthma (6:4)*			
Range	19-45	73-95	28-533
Mean	30.30 [†]	84.30 ^{‡§}	174.00
SE	2.6	2.4	58.4
Atopic subjects with moderate asthma (2:3)*			
Range	33-46	65-85	25-377
Mean	36.60 [†]	71.00 [§]	174.00
SE	2.4	3.6	58.4

*No significant difference among the subject groups with respect to sex ($P = .541$).

[†]No significant difference among the subject groups with respect to age ($P = .589$).

[‡]Significantly lower lung function in atopic subjects with mild asthma than in healthy subjects ($P < .01$).

[§]Significantly lower lung function in atopic subjects with moderate asthma than in subjects with mild asthma ($P < .01$).

^{||}Significantly higher total IgE levels in atopic asthmatic subjects than in healthy subjects ($P < .01$).

before labeling with FITC-conjugated myeloma IgE. PBMCs were washed 3 times and placed on ice in the dark pending analysis.

Flow cytometric analysis

Events were acquired by using a FACScan flow cytometer (Becton Dickinson, Oxford, United Kingdom) and analyzed by with WinMDI 2.8 software (Scripps Research Institute, La Jolla, Calif). Cells were passed through the flow cytometer at a flow rate of 1000 events per second. Dendritic cells were identified as showing no labeling for CD3, CD14, CD16, CD20, and CD56 and strong labeling for HLA-DR. The following parameters were used to define the dendritic cell gate. Cells negatively stained with PE were defined as lying below 10¹ on the FL2 axis, corresponding to the 99th percentile of PE-isotype control-stained dendritic cells. Having excluded all PE⁺ cells, the FITC gate was set (on PE⁻ cells) to include 99% of the HLA-DR bright cells. The exact position of this gate was donor specific but always lay in the second log decade on the FL1 axis.

In excess of 1×10^6 events were required to selectively acquire 5000 dendritic cells, a process taking approximately 20 minutes per sample. By using 3-color analysis, the presence of surface FcεRI α, CD23, and in vitro-bound myeloma IgE on these dendritic cells was determined. All positive staining was compared with that of appropriate isotype controls. The percentage of positive cells was defined as the proportion expressing higher levels of fluorescence than the isotype control. Mean fluorescence intensity (MFI) was used to compare the relative density of the receptor on the cell surface. MFI was always measured on the entire ungated cell population.

Statistical analyses

FEV₁ and total plasma IgE levels were expressed as means $\pm$ SEM. Comparisons between all 5 subject groups were made by using multifactorial analysis on the basis of ANOVA. A comparison between atopic asthmatic and healthy subjects, subjects with atopy

or asthma alone, or atopic asthmatic subjects with and without inhaled steroid treatment were then made by using a 2-tailed independent Student *t* test. A *P* value of less than .05 was considered significant. Statistical analysis was performed with SPSS version 8 software. The Spearman rank correlation coefficient was calculated to determine the relationship between plasma IgE and total FcεRI expression on dendritic cells and was considered significant at a *P* value of less than .05.

RESULTS

Clinical and physiologic characteristics of the subjects

The clinical and physiologic details of the subjects studied are summarized in Table I.

Identification of peripheral blood dendritic cells and their expression of surface FcεRI

By using 2-color flow cytometry, dendritic cells were identified as cells staining brightly for HLA-DR but negative for a cocktail of antibodies against markers of T-cell, B-cell, monocyte, and NK cell lineages (Fig 1, A, region 1). The proportion of dendritic cells in PBMCs was found to be in the range 0.15% to 1.1% (mean, $0.40\% \pm 0.19\%$; $n = 35$). To identify surface FcεRI on dendritic cells, 5000 dendritic cell events were collected in region 1 (Fig 1, B), and those showing immunoreactivity to mAb 15.1 were detected in a third color (Fig 1, C). In the case of the atopic asthmatic subject shown in Fig 1, 59% of peripheral blood dendritic cells expressed moderate levels of surface FcεRI α (MFI of 15).

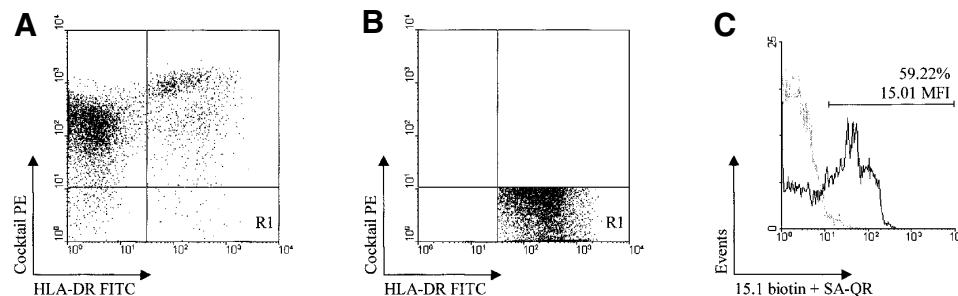


FIG 1. Identification of dendritic cells and their surface receptors by means of 2- and 3-color flow cytometry. **A**, Dendritic cells, identified as events lying within region 1 (*R1*), were strongly stained with an FITC-conjugated antibody against HLA-DR but showed no immunoreactivity with a cocktail of PE-conjugated antibodies against the lineage markers CD3, CD14, CD16, CD20, and CD56. In this representative experiment dendritic cells comprised 0.74% of PBMCs. **B**, Five thousand dendritic cell events within *R1* were collected and analyzed in a third color for FcεRI surface staining. **C**, The percentage of dendritic cells stained with biotinylated mAb 15.1 against FcεRI α was 59.22% (visualized with streptavidin quantum red). The MFI was 15.01. The biotinylated mouse IgG1 isotype control antibody detected with streptavidin quantum red is overlaid in grey.

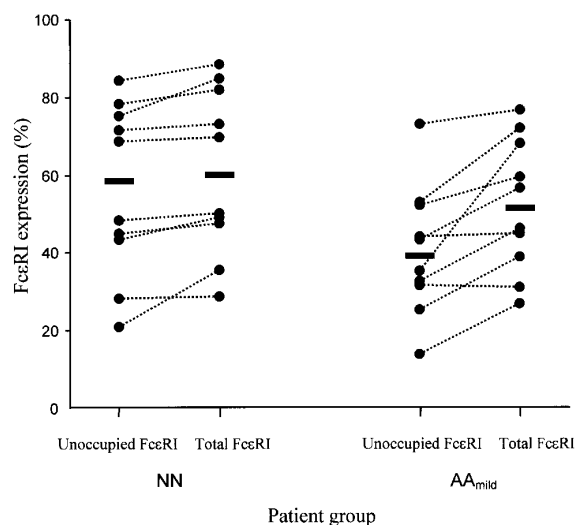


FIG 2. Summary of the effects of acid stripping on immunoreactivity with mAb 15.1. PBMCs from healthy subjects (*NN*, *n* = 10) and atopic asthmatic subjects with mild disease (*AA_{mild}*, *n* = 10) were labeled with mAb 15.1 either before (*Unoccupied FcεRI*) or after (*Total FcεRI*) removal of IgE by means of a 5-minute exposure to lactic acid-buffered saline solution. The ability to detect FcεRI α on peripheral blood dendritic cells was determined by means of flow cytometry. In all cases acid stripping either enhanced receptor expression or left it unchanged; receptor levels were never decreased by using acid treatment.

Effect of dissociation of in vivo bound surface IgE from dendritic cells

The mAb 15.1 only recognizes FcεRI α that is not occupied by IgE. To estimate total receptor expression by using this antibody, it has therefore been necessary to acid strip IgE from the dendritic cell surface before immunostaining with 15.1. In all the subjects tested, after 5 minutes of acid stripping at 4°C, levels of staining with 15.1 either remained unchanged or were increased (Fig 2). Irrespective of ex vivo occupancy of FcεRI by IgE, staining of dendritic cells with mAb 15.1 (both percentage and MFI) was never decreased by lactic acid strip-

ping (Fig 2). Therefore cells exhibiting mAb 15.1 immunoreactivity before acid stripping were considered to express unoccupied FcεRI, whereas the level of staining observed with mAb 15.1 after dissociation of IgE has been taken to represent total FcεRI expression.

The effect of acid stripping on the ability to detect FcεRI on peripheral blood dendritic cells from an atopic asthmatic subject is shown in Fig 3. Dendritic cells expressing unoccupied FcεRI increased from 60% to 68% after acid treatment, suggesting that IgE had been effectively removed from 8% of dendritic cells. The density of detectable FcεRI also increased, as indicated by a change in MFI from 13 to 27. After removal of in vivo-bound IgE, 24% of dendritic cells were able to bind IgE added in vitro compared with 3% before treatment. Acid stripping did not induce nonspecific antibody binding because neither the percentage nor MFI of cells stained with the isotype control changed (0.9% and MFI of 2.4 compared with 0.4% and MFI of 2.2 after acid stripping).

Levels of expression of the low-affinity IgE receptor (CD23) were negligible on the peripheral blood dendritic cells and remained unchanged after lactic acid stripping (Fig 4, *A*). Incubation of cells with mAb 15.1 before exposure to exogenous IgE inhibited IgE binding (Fig 4, *B*).

FcεRI expression on peripheral blood dendritic cells from healthy and asthmatic subjects

Total FcεRI expression on dendritic cells from 10 healthy and 10 atopic subjects with mild asthma was compared after treatment of the cells with acid-buffered saline solution (Fig 5). There was no significant difference in the percentage of dendritic cells positive for FcεRI ($60.73\% \pm 6.7\%$ for healthy nonatopic subjects compared with $51.79\% \pm 5.5\%$ for atopic subjects with mild asthma, *P* = .318). There was also no significant difference in the density of receptor on the dendritic cell surface (26.74 ± 5.9 for healthy nonatopic subjects compared with 21.82 ± 4.8 for atopic subjects with mild

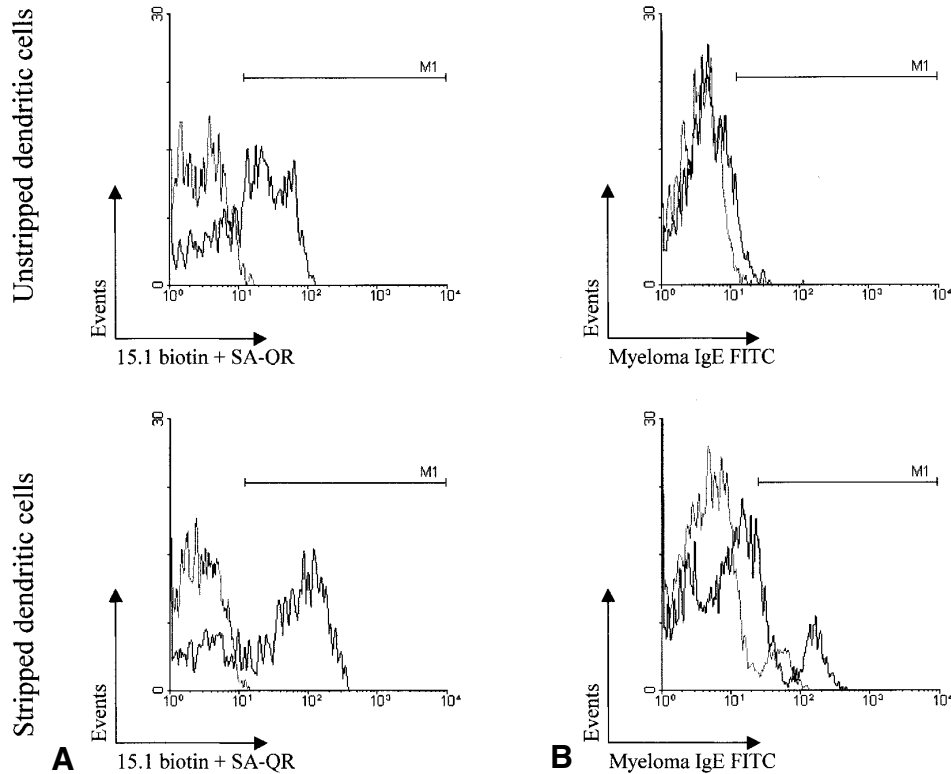


FIG 3. Effect of dissociation of IgE from dendritic cells. PBMCs from an atopic asthmatic subject were left untreated or treated with 0.01 mol/L lactic acid-buffered saline solution for 5 minutes before immunostaining. In this representative experiment there was no change in the percentage or MFI of cells stained with the isotype control (*grey overlay*). After acid treatment, the percentage of dendritic cells expressing FcεRI detected by staining with mAb 15.1 increased from 60.50% to 67.60%, and the MFI increased from 12.64 to 26.71 (**A**). The percentage of dendritic cells able to bind exogenous IgE added in vitro increased from 3.50% to 23.60%, and the MFI increased from 4.57 to 9.51 (**B**).

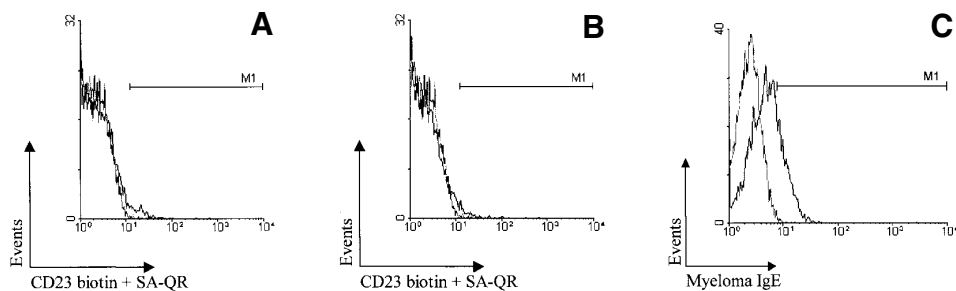


FIG 4. The contribution of CD23 and FcεRI to the binding of exogenous IgE by dendritic cells. 3-color flow cytometry showed the expression of CD23 by dendritic cells to be negligible either before (**A**) or after (**B**) lactic acid stripping (*grey overlay*, isotype control). **C**, After lactic acid treatment (*black curve on histogram*), 25.76% of dendritic cells were able to bind myeloma IgE (MFI of 4.9). Preincubating these acid/saline solution-treated PBMCs with unconjugated mAb 15.1 (*grey overlay*) reduced the proportion of dendritic cells able to bind exogenous IgE to 0.7% (MFI of 2.33).

asthma, $P = .527$). Although fewer dendritic cells from atopic asthmatic subjects had unoccupied FcεRI before lactic acid treatment, and the density of free receptor was lower than that found on dendritic cells from healthy subjects, this failed to reach statistical significance ($P = .085$ for percentage expression and $P = .062$ for MFI, data not shown).

Comparing peripheral blood dendritic cells from asthmatic subjects with mild versus moderate disease, there was a significant increase with disease severity in both the number of dendritic cells positive for total FcεRI ($P = .036$) and in the density of the receptor after acid stripping ($P = .013$; Fig 5, *B*). The density of unoccupied FcεRI was significantly increased on dendritic cells from subjects

with moderate asthma ($P = .013$), although the increase in the percentage of cells expressing unoccupied receptor failed to reach significance ($P = .078$, data not shown).

Total FcεRI expression was compared with the levels of unoccupied receptor before the removal of IgE to obtain an indirect measure of the occupancy of FcεRI by endogenous IgE *in vivo*. The ratio of total FcεRI minus unoccupied receptor divided by the total levels of FcεRI for both percentage and MFI values was calculated. When compared with healthy subjects, peripheral blood dendritic cells from atopic asthmatic subjects showed increased *in vivo*-bound IgE, which was significant for the percentage of positive cells ($P < .001$) and for the MFI ratio ($P = .005$; Fig 5, C). Dendritic cells from subjects with moderate asthma showed a further increase in bound endogenous IgE when compared with those from subjects with milder disease ($P = .002$, data not shown).

Pooling the data from all 5 subject groups, correlations were sought between plasma total IgE levels and the levels of either unoccupied FcεRI or total FcεRI expressed on dendritic cells. There was a weak negative correlation between plasma total IgE levels and the level of unoccupied FcεRI expressed on dendritic cells ($r = -0.339$, $P = .046$), suggesting that as the level of plasma total IgE rises, the amount of detectable free FcεRI decreases. No correlation was found between plasma total IgE levels and the expression of total FcεRI revealed by means of acid stripping.

Ability of unoccupied FcεRI expressed on dendritic cells to bind IgE *in vitro*

Peripheral blood dendritic cells from healthy subjects were unable to bind IgE either before or after lactic acid stripping (Fig 6, A). In contrast, although cells from atopic subjects with mild asthma were also unable to directly bind IgE *ex vivo*, after removal of *in vivo*-bound IgE by means of acid stripping, there was a significant increase in the binding of exogenous IgE *in vitro* ($P = .009$; Fig 6, A). After acid stripping, $13.6\% \pm 4.1\%$ of dendritic cells from healthy subjects were able to bind myeloma IgE compared with $33.0\% \pm 4.3\%$ of dendritic cells from atopic asthmatic subjects (Fig 6, B), a difference statistically significant at the 99% confidence level. Although dendritic cells from atopic asthmatic subjects with moderate disease showed a further increase in the ability to bind IgE, this did not achieve statistical significance ($P = .102$).

To determine whether the higher binding of IgE *in vitro* by atopic asthmatic dendritic cells was preferentially associated with atopy or asthma, acid-stripped peripheral blood dendritic cells from subjects with atopy or asthma alone were compared (Fig 6, B). An increased ability to bind exogenous IgE was found in both the atopic and asthmatic groups, with the increase being more pronounced in the atopic group, although the difference failed to reach significance ($P = .074$). There was a significant positive correlation between the level of circulating total IgE and the presence of IgE bound *in vitro* to the surface of dendritic cells that had previously been treated with lactic acid ($r = 0.366$, $P = .030$).

DISCUSSION

To explore how FcεRI expression by dendritic cells may be regulated *in vivo* in allergic asthma, we have investigated the expression of FcεRI by peripheral blood dendritic cells from healthy and atopic asthmatic subjects. It was found that after lactic acid stripping of any receptor-bound IgE, peripheral blood dendritic cells from healthy and atopic asthmatic subjects express FcεRI at similar frequencies and surface densities. In the absence of acid treatment, the occupancy of the receptor by endogenous IgE was found to be higher in atopic asthmatic subjects. Furthermore, after acid stripping, only FcεRI⁺ dendritic cells from atopic asthmatic subjects could bind exogenous IgE *in vitro*. Thus dendritic cells in the peripheral blood of healthy and atopic asthmatic subjects differ with respect to their capacity to bind IgE by means of FcεRI rather than their levels of FcεRI expression.

To avoid purification of dendritic cells that comprise less than 1% of peripheral blood leukocytes,¹⁵ flow cytometry has been used to identify dendritic cells and monitor their receptor expression. Dendritic cells were identified as HLA-DR bright cells that did not express the lineage markers CD3, CD14, CD16, CD20, and CD56. The expression of FcεRI by cells fulfilling these criteria was investigated by using mAb 15.1, which is specific for FcεRI α. Because this antibody can only recognize FcεRI α in the absence of bound IgE (unoccupied FcεRI), to determine the total levels of FcεRI expression, it was necessary to strip any endogenously bound IgE from the receptor by means of incubation in lactic acid-buffered saline solution for 5 minutes before antibody labeling. In all donors this approach consistently led to either enhanced or unchanged detection of FcεRI α by mAb 15.1. Acid stripping did not cause a decrease in detectable FcεRI α, as would be predicted if the receptor itself was being removed by the treatment. This supports the applicability of staining acid-stripped cells with mAb 15.1 to estimate total FcεRI α expression. Further support comes from our current studies, in which levels of FcεRI α detected with mAb 15.1 after acid stripping reach those seen with mAb 22E7, which detects receptor irrespective of its occupancy by IgE (data not shown).

A striking finding of the current study is that after acid stripping, there was no significant difference in FcεRI expression on peripheral blood dendritic cells from healthy subjects or atopic subjects with mild asthma. Substantial numbers of dendritic cells from the blood of both subject groups expressed cell surface FcεRI ($60.73\% \pm 6.7\%$ for healthy nonatopic subjects compared with $51.79\% \pm 5.5\%$ for atopic subjects with mild asthma) at densities within the second log decade of fluorescence intensity (26.74 ± 5.9 for healthy nonatopic subjects compared with 21.82 ± 4.8 for atopic subjects with mild asthma). This is in contrast to the airways, in which atopic asthmatic subjects have an increased number of FcεRI⁺ dendritic cells compared with the situation in healthy subjects.¹¹ This suggests that in asthmatic subjects either FcεRI⁺ dendritic cells

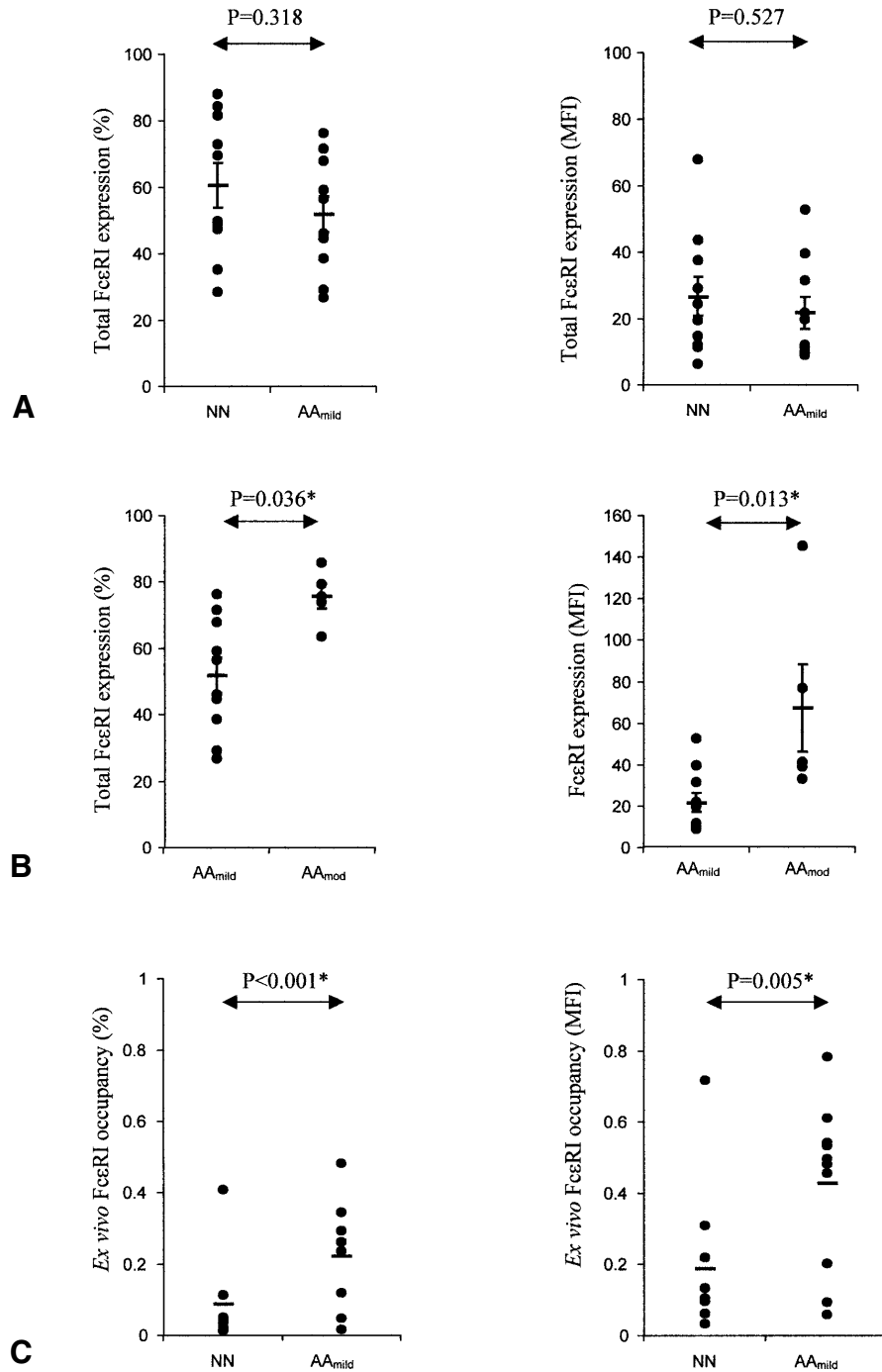


FIG 5. FcεRI expression and IgE binding by dendritic cells in healthy and atopic asthmatic subjects. **A**, Total FcεRI expression on dendritic cells from 10 healthy (NN) and 10 atopic asthmatic subjects with mild disease (AA_{mild}) was measured, as shown in Fig 2, A. There was no significant difference in cell surface FcεRI expression by dendritic cells from the 2 subject groups with respect to either the percentage of dendritic cells positive for FcεRI ($P = .318$) or the density of receptor on the cell surface (MFI, $P = .527$). **B**, Comparing FcεRI expression on dendritic cells from 10 asthmatic subjects with mild disease (AA_{mild}) versus 5 with moderate disease (AA_{mod}), there was a significant increase in total FcεRI expression with disease severity, in terms of both the percentage of dendritic cells expressing the receptor ($P = .036$) and its density of expression ($P = .013$). $^*P < .05$. **C**, As a measure of the occupancy of FcεRI by endogenous IgE, the ratio of total FcεRI expression minus unoccupied receptor divided by the total levels of FcεRI for both percentage and MFI was calculated for 10 nonatopic nonasthmatic subjects (NN) and 10 atopic asthmatic subjects (AA_{mild}). There was a highly significant increase ($P < .01$) in in vivo-bound IgE on dendritic cells from atopic asthmatic subjects with mild disease compared with that in healthy subjects, which was true whether the ratio had been calculated with percentage values ($P < .001$) or MFI values ($P = .005$).

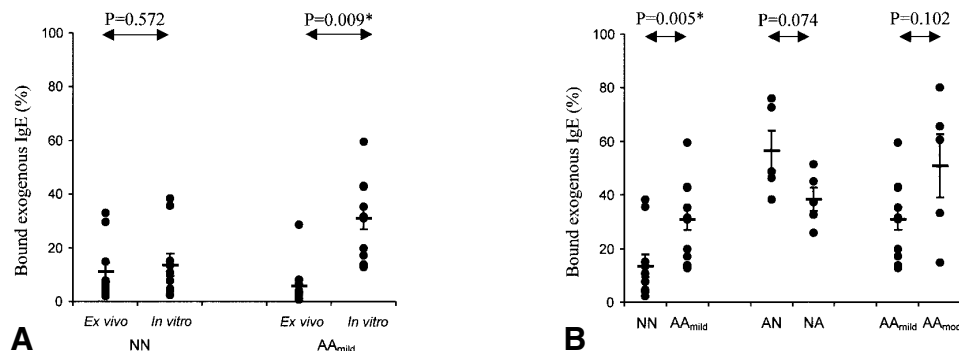


FIG 6. Binding of IgE to FcεRI on dendritic cells before and after treatment with lactic acid. **A**, Dendritic cells from healthy subjects (NN) showed no difference in the ability to bind IgE before (ex vivo) and after (in vitro) treatment with lactic acid to remove surface bound IgE ($P = .572$). In contrast, cells from atopic asthmatic subjects with mild disease (AA_{mild}) were shown to bind significantly more IgE in vitro after removal of in vivo-bound IgE ($P = .009$). * $P < .01$. **B**, After lactic acid treatment, the ability of dendritic cells from all the subject groups to bind exogenous IgE was compared. Dendritic cells from atopic asthmatic subjects with mild disease (AA_{mild}) bound significantly more IgE in vitro than those from healthy subjects (NN; $P = .005$), and subjects with more severe disease (AA_{mod}) showed a trend for a further increase in the proportion of dendritic cells able to bind IgE in vitro, although this trend failed to reach significance ($P = .102$). Dendritic cells from subjects with either atopy (AN) or asthma (NA) did not differ significantly in their ability to bind exogenous IgE ($P = .074$). * $P < .01$.

are preferentially recruited from the peripheral blood into the lung or mediators within the environment of the atopic asthmatic lung act locally to upregulate expression of the receptor on dendritic cells.

FcεRI expression on mast cells has been linked to levels of serum total IgE¹⁶; therefore, a correlation was sought between levels of circulating total IgE and the expression of FcεRI on peripheral blood dendritic cells. Although the atopic asthmatic subjects used in this study had significantly higher levels of plasma total IgE than healthy subjects, an increase associated significantly with atopy rather than with asthma, no correlation was found between plasma IgE and the expression of total or unoccupied FcεRI on dendritic cells. However, by using an indirect measure of FcεRI occupancy in vivo, the amount of occupied FcεRI on dendritic cells did correlate with total IgE levels, as shown previously for basophils and monocytes.¹⁷

That total FcεRI expression by peripheral blood dendritic cells does not differ significantly between healthy and atopic subjects is in keeping with reports of FcεRI expression by monocytes presented by Reischl et al.¹⁸ However, it contrasts with data from Maurer et al,¹⁹ who found a positive correlation between the amount of serum total IgE and FcεRI expression by monocytes. The difference in results among these 3 studies is probably attributable to the methodologies used. The present study applied a rapid method that avoided the need for cell purification and enabled the cells to be kept cool throughout. Similarly, Reischl et al¹⁸ worked preferentially with unfractionated PBMCs. In contrast, Maurer et al¹⁹ used lengthy cell-separation procedures with extended periods at ambient temperature followed by acid stripping. Because IgE bound to FcεRI stabilizes surface expression of the receptor,²⁰ leaving acid stripping until

the end of a lengthy protocol could allow the unoccupied receptor on monocytes from healthy subjects to be down-regulated, whereas the occupied FcεRI on monocytes from atopic subjects might be retained.

The present study has extended its observations to peripheral blood dendritic cells from asthmatic subjects with moderate disease. FcεRI expression on dendritic cells from these subjects was higher than that found on cells from subjects with mild asthma. The levels of unoccupied receptor were also higher in the group with more severe asthma, although it cannot be ruled out that the level of in vivo receptor occupancy by IgE between the 2 asthmatic groups was equivalent. Because no difference in the contribution of atopy or asthma alone to FcεRI expression on dendritic cells was found, either phenotypic component could be contributing to the increased total FcεRI expression seen with increasing disease severity. Alternatively, the increased receptor expression may be due to the inhaled corticosteroid treatment the subjects with more severe disease were receiving. Corticosteroids may cause an increase in FcεRI expression on dendritic cells by changing the differentiated state of the cell,²¹⁻²³ with FcεRI being maximally expressed on immature dendritic cells.

A major finding of the present work is that despite expressing FcεRI, dendritic cells from healthy individuals fail to bind exogenous IgE in vitro, either before or after acid stripping. They also carry negligible levels of in vivo-bound IgE (determined indirectly by the inability to detect increased FcεRI immunoreactivity after acid stripping). When these experiments were performed on atopic asthmatic subjects, variable levels of occupied FcεRI were detected on the dendritic cell surface, but there was also free FcεRI, to which no additional IgE could be bound in vitro. However, if in vivo-bound IgE

was removed from atopic asthmatic dendritic cells before in vitro addition of IgE, it was possible for the ligand to bind to the cell surface. Preincubating peripheral blood dendritic cells with mAb 15.1 blocked the binding of exogenous IgE, showing that IgE binding to dendritic cells was mediated by FcεRI. Furthermore, dendritic cells from all subject groups expressed negligible levels of CD23.

These findings are in contrast to those of another study of peripheral blood dendritic cells⁷ in which exogenously added IgE was found to bind to acid-stripped cells from both healthy individuals and subjects with atopic dermatitis, although as reported here, the FcεRI-dependent IgE-binding capacity of these cells was again significantly higher in subjects with atopic disease. Why in the present study acid-stripped dendritic cells from healthy individuals could not bind IgE in vitro is unclear, although differences in the studies may arise from the use of IgE preparations, differing, for example, in their glycosylation.

In the current study a significant correlation was found between plasma IgE levels and the ability of stripped dendritic cells to rebind IgE in vitro. This relationship, and the fact that only when dendritic cells had bound IgE in vivo could they subsequently bind IgE in vitro after acid stripping, suggests that if IgE is available when FcεRI first appears at the cell surface, it will bind to and stabilize the receptor. In the absence of exogenous IgE, the receptor may rapidly lose the ability to bind IgE, and the subsequent availability of exogenous IgE cannot restore IgE-binding ability.

The mechanisms by which FcεRI may lose the capacity to bind IgE are unknown. Secondary molecules may be preventing the binding of whole IgE molecules. It has been suggested that the IgE-binding lectin galectin-3 prevents IgE binding to FcεRI on human Langerhans' cells.²⁴ However, it has since been reported that galectin-3 is rarely expressed on the cell surface,^{18,25} and when it is, the carbohydrate-binding site involved in IgE binding is required to anchor galectin-3 to the membrane, rendering it unavailable for IgE binding (personal communication from Professor Fu-Tong Liu, La Jolla, Calif). Therefore the presence of galectin-3 on the surface of dendritic cells was not tested in this study.

In conclusion, the finding that total FcεRI expression by peripheral blood dendritic cells neither differs significantly between healthy and atopic subjects nor correlates with levels of circulating IgE suggests that there is an IgE-independent regulatory mechanism that controls overall FcεRI expression by dendritic cells in the blood. Once a dendritic cell enters the lung, local microenvironmental factors may be responsible for the greater expression of FcεRI reported for dendritic cells in the asthmatic airway.^{10,11} Similar local systems may operate at other sites affected by allergic disease (eg, the skin), determining the high expression of FcεRI by Langerhans' cells in atopic dermatitis.²⁶ The second major finding of this study, namely that the functional competence of the receptor differs between atopic asthmatic subjects and healthy subjects, with FcεRI⁺ peripheral blood dendritic

cells from healthy subjects being unable to bind IgE in vitro, implies that the ability of dendritic cells to carry out FcεRI-mediated allergen presentation is governed not only at the level of receptor surface expression but also by unknown mechanisms that control the ability of the receptor to bind IgE. These multiple points of control need to be considered in any study aimed at targeting FcεRI on dendritic cells for therapeutic purposes.

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