

Altered expression and action of the low-affinity IgE receptor FcεRII (CD23) in asthmatic airway smooth muscle

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Background: Changes in cell surface expression of certain immunoglobulin Fc receptors have been demonstrated in leukocytes isolated from the lungs of atopic asthmatic individuals. This, together with emerging evidence that Fc receptors can also be expressed and activated in non-bone marrow-derived cell types, including airway smooth muscle (ASM), raises the hypothesis that the atopic asthmatic ASM phenotype is associated with an altered endogenous expression and action of specific Fc receptors present in the ASM itself. **Objective:** The current study addressed the above hypothesis by examining (1) whether the expression of certain key Fc receptor subtypes for IgE and IgG is altered in ASM tissue isolated from human atopic asthmatic individuals and (2) whether this altered Fc receptor expression is comparably induced in naive human ASM tissue and cultured cells after their passive sensitization with human atopic asthmatic serum or IgE immune complexes.

Methods: Messenger RNA and cell surface protein expression of the individual IgG receptor subtypes FcγRI, FcγRII, and FcγRIII, as well as the IgE receptor subtypes FcεRI and FcεRII, were examined in human ASM tissue isolated from atopic asthmatic and control (nonatopic/nonasthmatic) individuals. In addition, we examined the effects of passive sensitization of ASM tissue and cultured ASM cells with control serum, atopic asthmatic serum, or exogenously administered IgE immune complexes on Fc receptor expression and action (ie, induction of proinflammatory cytokine release).

Results: The observations demonstrate that (1) human ASM tissue expresses messenger RNA and surface protein for FcεRII, as well as for all the Fcγ receptor subtypes, (2) in contrast to unaltered Fcγ subtype expression, however, relative to control human ASM, FcεRII is significantly up-regulated in inherently asthmatic ASM tissue, (3) up-regulated expression of FcεRII represents, at least in part, an inducible phenomenon that is largely attributed to IgE immune complex-coupled

activation of the receptor, and (4) the latter action is associated with FcεRII-induced autologous elaboration of the proinflammatory cytokine, IL-1β, by the atopic sensitized ASM.

Conclusion: These observations provide new evidence that human ASM tissue expresses FcεRII in addition to all 3 subtypes of Fcγ receptors and that the expression of FcεRII is selectively increased in atopic asthmatic ASM, a phenomenon associated with IgE immune complex/FcεRII-mediated elaboration of IL-1β by the ASM itself. (*J Allergy Clin Immunol* 1999;104:575-84.)

Key words: Fc receptor expression, immunoglobulins, IL-1β, human airway smooth muscle, asthma

Exaggerated agonist-induced bronchoconstriction, attenuated β-adrenoceptor-mediated airway relaxation, and airway inflammation are all characteristic features of bronchial asthma.¹⁻³ Although the mechanism underlying these inflammation-associated changes in airway responsiveness remains largely unknown, numerous reports support a role for Fc receptor activation in both initiating and propagating the pulmonary inflammatory response in asthma.⁴⁻⁷ Thus mast cell stimulation has been implicated in mediating the immediate bronchoconstrictor response after antigen inhalation in individuals with atopic asthma, wherein IgE-coupled activation of its high-affinity receptor (ie, FcεRI) on mast cells leads to cellular degranulation and the release of various proinflammatory mediators and cytokines. The recent identification of Fc receptors on other cell types in the lung (eg, mononuclear cells, eosinophils, dendritic cells, and epithelial cells) suggests that their activation may also serve to propagate the proinflammatory airway response, most likely by the orchestrated extended release of specific cytokines.⁷⁻¹⁴ Moreover, apart from leukocytes per se, the identification of Fc receptors in resident tissue cells, including airway smooth muscle (ASM) cells,¹⁵ suggests an expanded role for Ig receptors in regulating local tissue immune responses. In this regard, we recently identified that rabbit ASM tissue and cultured ASM cells express the low-affinity receptor for IgE, FcεRII (CD23) and that activation of the latter receptor is associated with the induction of "proasthmatic" changes in agonist-mediated ASM constrictor and relaxant responsiveness.¹⁵

In light of the above evidence, the current study examined whether expression of specific Fc receptor messenger RNAs (mRNA) and proteins is altered in inherently asthmatic human ASM tissue and whether this modulated Fc receptor expression represents a potentially inducible

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Abbreviations used

ASM:	Airway smooth muscle
cDNA:	Complementary DNA
FcεRII (CD23):	Low-affinity receptor for IgE
HBSM:	Human bronchial smooth muscle
mRNA:	Messenger RNA
NaCl:	Sodium chloride
RT:	Reverse transcriptase
SFM:	Serum-free medium
SMBM:	Smooth muscle basal medium
SSC:	Standard saline citrate

phenomenon in naive ASM passively sensitized with human atopic asthmatic serum or exogenously administered IgE immune complexes. The results demonstrate that (1) human ASM tissue expresses mRNA and surface protein for the low-affinity IgE receptor FcεRII as well as for all 3 Fcγ receptor subtypes that bind to IgG, (2) in contrast to unaltered Fcγ receptor expression, relative to naive (control) human ASM, expression of FcεRII is significantly up-regulated in inherently asthmatic ASM, (3) up-regulated expression of FcεRII in human ASM is, at least in part, an inducible phenomenon, largely attributed to immune-complexed IgE/CD23-coupled interaction, and (4) the latter phenomenon is accompanied by the induced expression and elaboration of the proinflammatory cytokine IL-1β by the ASM itself. Collectively, these findings suggest a critical role for up-regulated FcεRII receptor expression and activation in mediating the proasthmatic phenotype in human ASM.

METHODS**Preparation of human ASM tissue**

Human ASM tissue (both tracheal and bronchial sections) was isolated from a 12-year-old asthmatic boy who died from status asthmaticus (patient 1). Although the patient's cardiopulmonary status responded to resuscitation, he was determined to be dead on the basis of brain perfusion scan criteria. The lung and airway tissue were freshly obtained under sterile conditions during a subsequent organ donation procedure. The ASM tissue was carefully scraped of loose connective tissue and epithelium under a microscope, prepared for RNA and protein extraction, and examined for Fc receptor expression, as described below. ASM tissue was also prepared from a 25-year-old African American woman with asthma who died from an exacerbation complicated by pneumonia (patient 2). Of note, microscopically the airway tissue specimens from both patients had all the characteristic features of asthmatic airway tissue, including the presence of ASM hypertrophy-hyperplasia. Age-matched ASM tissue from 6 individuals who had no history of asthma and who either had a lung resection for a peripheral lung carcinoma or who died from nonpulmonary related causes served as controls. The latter tissue specimens were tumor free and microscopically normal.

In extended experiments, ASM tissue from 3 of the above 6 nonasthmatic subjects was passively sensitized with either human control serum, atopic asthmatic serum, IgE alone (2-15 μg/mL), or IgE immune complexes (2-15 μg IgE, 1-5 μg/mL anti-IgE) for various time points (see below). The atopic asthmatic serum was obtained from allergic patients with moderate to severe asthma and

serum IgE levels >1000 IU/mL and 4 to 5 of 6 plus radioallergen sorbent tests positive (specific IgE concentration of > 17.5 Phadebas radioallergen sorbent test units/mL) to *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, and ragweed and positive skin test results to these antigens. The control serum was obtained from nonatopic nonasthmatic subjects with normal IgE levels (ie, <70 IU/mL) and negative skin test reactivity. The tissues' mRNA and protein were subsequently isolated for analysis of Fc receptor mRNA and protein expression (see below). The participation of human subjects in this study was approved by the Institutional Review Board of the Joseph Stokes, Jr Research Institute at the Children's Hospital of Philadelphia.

Preparation and sensitization of human ASM cells

To examine Fc receptor regulation in ASM cells, we used cultured human bronchial smooth muscle (HBSM) cells obtained from Clonetics (San Diego, Calif). The HBSM cells were derived from 2 healthy male donors aged 16 and 21 years. The cells were fully characterized with specific markers to confirm their selective smooth muscle phenotype and to exclude contamination with other cell types, according to the manufacturer's protocol. The cells were grown in smooth muscle basal medium (SMBM) supplemented with 5% FBS, insulin (5 ng/mL), epithelial growth factor (10 ng/mL, human recombinant), fibroblast growth factor (2 ng/mL, human recombinant), gentamicin (50 ng/mL), and amphotericin B (50 ng/mL). The standard experimental protocol involved growing the cells to confluence in the above medium. The cells were then starved in unsupplemented SMBM (SFM) for 24 hours, at which time the cells were treated with either human control serum from nonatopic-nonasthmatic subjects or serum from subjects with atopic asthma in the absence and presence (40 μg/mL) of an anti-CD23 monoclonal blocking antibody (anti-CD23 mAb). In other experiments cells were treated with SFM alone or SFM in the presence of maximum effective concentrations of IgE or IgE immune complexes. The cells were then examined for Fc receptor mRNA and protein expression, as described below.

Determination of Fcγ and Fcε receptor mRNA expression

Total RNA was isolated from the above tissue and cell preparations with use of the modified guanidinium thiocyanate-phenol-chloroform extraction method to include proteinase K (in 5% SDS) digestion of protein in the initial RNA pellet.¹⁶ The concentration of each RNA sample was then determined spectrophotometrically. This procedure consistently produced yields of 15 to 25 μg of intact RNA per each tissue specimen under study or T-75 flask of HBSM cells. To analyze for mRNA expression of the FcγRI, FcγRII, FcγRIII, FcεRI, and FcεRII receptor subtypes, we used a reverse transcriptase (RT)-PCR protocol and human specific primers for these Fc receptor subtypes. Complementary DNA (cDNA) was synthesized from total RNA isolated from (1) naive versus inherently atopic asthmatic human ASM tissue and (2) human ASM tissue or ASM cells passively sensitized with either IgE alone, IgE immune complexes, human control serum, or atopic asthmatic serum in the absence and presence of anti-CD23 mAb. cDNA from activated peripheral blood mononuclear cells served as positive controls for the individual Fcγ and Fcε receptor expression analyses. The cDNA was primed with oligodeoxythymidine 12-18 and extended with Superscript II reverse transcriptase (Gibco BRL). PCR was used to amplify Fc receptor-specific products from each cDNA reaction with use of the following Fc receptor primer pairs, on the basis of published sequences of the human FcγRI, FcγRII, FcγRIII, and FcεRI and FcεRII genes,^{17,18} and included the following primer sets:

FcγRIa:

5'-Primer 5'-ATGTGGTTCTTGACAACTCTGCTC-3'
3'-Primer 5'-ATGTCTGTCTTCTTGAAGGCTGGA-3'
Product is 1038 bp

FcγRIIa,c:

5'-Primer 5'-GACTCCATTCAGTGGTTCCA-3'
3'-Primer 5'-GTCAGCTGTTTCATAGTCATTG-3'
Product is 644 bp

FcγRIII:

5'-Primer 5'-AAGATCTCCCAAAGGCTGTG-3'
3'-Primer 5'-ATGGACTTCTAGCTGCACCG-3'
Product is 254 bp

FcεRI:

5'-Primer 5'-GGAACGGGAATTACCATCCT-3'
3'-Primer 5'-AAAGACGATCATCGGGAACC-3'
Product is 313 bp

FcεRII:

5'-Primer 5'-CGTCTCTCAAGTTTCCAAG-3'
3'-Primer 5'-GCACTTCCGTTGGAATTTG-3'
Product is 333 bp

RPL7:

5'-Primer 5'-AAGAGGCTCTCATTTTCTGGCTG-3'
3'-Primer 5'-TCCGTTCCCTCCCCATAATGTTCC-3'
Product is 157 bp

The cycling profile used was as follows: denaturation 95°C for 1 minute, annealing 52°C to 62°C for 1.0 to 1.5 minutes, and extension 72°C for 1 to 2 minutes and 34 to 40 cycles for the FcγRI, FcγRII, FcγRIII, FcεRI and FcεRII receptor genes and 26 cycles for the RPL7 gene. The number of cycles was determined to be in the linear range of the PCR products. The PCR reactions for the human Fcγ, Fcε, and RPL7 primers were performed with use of equivalent amounts of cDNA prepared from 2.5 μg of total RNA. Equal aliquots of each PCR reaction were then run on a 1.2% agarose gel and subsequently transferred to a zeta-probe membrane overnight in 0.4N sodium hydroxide. After capillary transfer, the DNA was immobilized by UV cross-linking with use of a Stratalinker UV Crosslinker 2400 at 120,000 μJ/cm² (Stratagene). Prehybridization in a Techne hybridization oven was conducted for 2 to 3 hours at 42°C in 50% formaldehyde, 7% (wt/vol) SDS, 0.25 mol/L sodium chloride (NaCl), 0.12 mol/L sodium phosphate (pH 7.2), and 1 mmol/L EDTA. Hybridization was for 20 hours at 42°C in the same solution. The FcγRI, FcγRII, FcγRIII, FcεRI, FcεRII, and RPL7 DNA levels were assayed by Southern blot analysis with phosphorus 32-labeled probes. These probes were prepared by pooling several RT-PCR reactions for the individual Fc and RPL7 PCR fragments and purifying them from a 1.2% agarose gel with use of the Qiaex II agarose gel extraction kit. The individual Fc receptor cDNA fragments were subsequently sequenced for product confirmation. Washes were as follows: 1 for 15 minutes in 2× standard saline citrate (SSC), 0.1% SDS at room temperature; 1 for 15 minutes in 0.1× SSC, 0.1% SDS at room temperature; and 2 for 15 minutes at 50°C in 0.1× SSC, 0.1% SDS. Southern blots were quantitated by direct measurements of radioactivity in each band with use of a PhosphorImager (Molecular Dynamics).

Fifteen micrograms of cytoplasmic RNA pertaining to each time point was also fractionated in 1% agarose, formaldehyde denaturing gels. After capillary transfer, cross-linking, and prehybridization, the levels of mRNA were analyzed with use of the above ³²P-labeled cDNA fragments for the individual Fc receptor genes prepared by random priming, as previously described in our laboratory.¹⁹ Northern blots were quantitated by direct measurement of radioactivity in each band with use of the PhosphorImager.

Determination of Fcγ and Fcε receptor protein expression

Expression of cell surface protein for the FcγRI, FcγRII, FcγRII, and FcεRII receptors was assayed by Western blot analysis of membrane protein samples isolated from naive (control) and human asthmatic ASM tissue. The ASM was then minced and homogenized in 50 mmol/L TRIS-hydrochloric acid, 150 mmol/L NaCl, 1 mmol/L EDTA (pH 7.4) containing 1 mmol/L phenylmethylsulfonyl fluoride, 5 μg/mL aprotinin, and 5 μg/mL leupeptin. Nuclei and large particulates were removed by centrifugation at 100g for 5 minutes. The supernatant was then centrifuged at 100,000g for 1 hour to pellet the membrane fractions. The membrane pellet was resuspended in the same Tris-EDTA buffer, and the protein concentration was measured with the Lowry assay. Equivalent amounts (30-50 μg) of membrane protein were fractionated in 11% SDS-polyacrylamide gels followed by transfer to nitrocellulose membranes. The membranes were then blotted overnight at room temperature in 25 mmol/L TRIS-hydrochloric acid (pH 7.5), 150 mmol/L NaCl, and 0.05% Tergitol NP-40 containing 5% nonfat milk, as previously described in our laboratory.²⁰ The primary mouse antihuman FcγRI, FcγRII, FcγRIII, and FcεRII antibodies used were diluted 1:250 to 1:500 and incubated for 1 hour at room temperature. All primary and secondary antibody incubations and washes were done in 25 mmol/L TRIS-hydrochloric acid (pH 7.5), 150 mmol/L NaCl, and 0.05% NP-40 containing 0.50% nonfat milk. The individual Fc receptor levels were detected with enhanced chemiluminescence after a 1-hour incubation with a 1:1000 dilution of an antimouse horseradish peroxidase-linked secondary antibody and subsequent exposure to autoradiography film. Expression levels of the individual Fc receptor protein were quantitated with use of laser densitometry (Bio-Rad, Hercules, Calif).

Fc receptor cell surface expression was also examined in human ASM cells by flow cytometric analysis with a Coulter EPICS Elite flow cytometer (Coulter EPICS Division, Hialeah, Fla) equipped with a 5-W argon laser operated at 488 nmol/L and 300 mW output. Fluorescence signals were accumulated as 2-parameter fluorescence histograms with both percent positive cells and mean channel fluorescence being recorded. Cells treated for 24 hours with either 20% human control serum or 20% human atopic asthmatic serum were resuspended in buffer, dispersed by passage through a 23-gauge needle, and then separately stained with primary mouse antihuman antibodies specific for the individual FcγRI, FcγRII, FcγRII, FcεRI, and FcεRII receptor genes (Serotec, Raleigh, NC) in 1:250 to 1:500 dilution. After subsequent repeat washing, FITC-labeled F(ab')₂ goat antimouse IgE and IgG fragments were used as secondary antibodies and incubated for 1 hour in 1:500 to 1:1000 dilutions in PBS containing 0.5% BSA. Activated peripheral blood lymphocytes isolated from blood donors were used as the positive controls for the Fc receptor expression assays. The cells were also stained with mouse antibodies of the identical isotypes as the Fc receptor mAbs to measure background fluorescence (ie, mouse IgG1 negative control). The antibody-stained cells were then evaluated by flow cytometry and analyzed with use of Elite Immuno 4 statistical software (Coulter EPICS Division). Fluorescence intensities were expressed as percent positive cells, including mean channel fluorescence.

Finally, an immunofluorescence detection assay was also used to examine for FcεRII surface receptor expression in ASM cells after a 24-hour treatment with human control versus atopic asthmatic serum, as described above. After standard preparation, the ASM cells were fixed in acetone and then mounted on poly-L-lysine-coated slides. The cells were incubated with PBS buffer containing 10% human serum to suppress nonspecific staining and subsequently labeled overnight at 4°C with primary mouse antihuman CD23 anti-

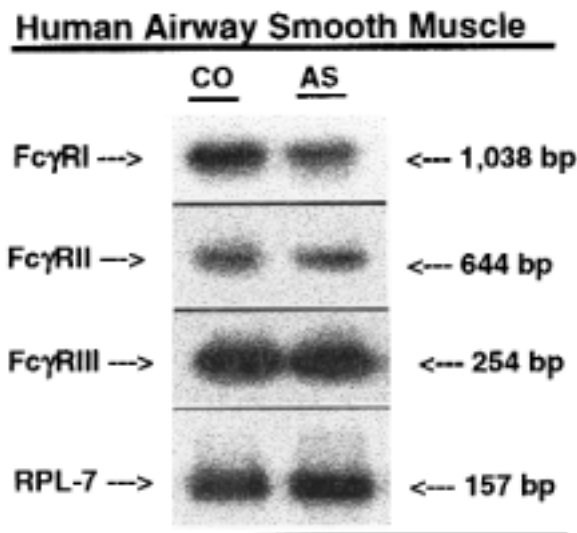


FIG 1. Comparison of expression of Fc γ RI (1038 bp), Fc γ RII (644 bp), and Fc γ RIII (254 bp) receptor mRNAs with use of RT-PCR in control (CO) and inherently asthmatic (AS) human ASM tissue. Expression of RPL7 was used to control for loading. Blots were probed with human specific Fc γ RI, Fc γ RII, and Fc γ RIII ^{32} P-labeled cDNA probes. Note: Human ASM tissue expresses mRNA for both the high-affinity Fc γ RI and the low-affinity Fc γ RIIAC and Fc γ RIII receptors, as determined by expected sizes of respective Fc γ RI, Fc γ RII, and Fc γ RIII cDNA fragments, and there were no differences in mRNA expression signals between control and asthmatic ASM. Data are representative of 3 separate experiments with ASM tissue isolated from patient 1. Comparable results were obtained with ASM tissue isolated from patient 2.

body in 1:250 dilution. In control sections the primary antibody was replaced by Igs of the same isotype according to the manufacturer's protocol (mouse IgG1 negative control). Parallel cell slides were also stained with an α -actin antibody to exclude contamination of the ASM cells with non-actin-containing cells. After subsequent repeat washing, FITC-labeled F(ab')₂ goat antimouse IgE or IgG fragments were used as secondary antibodies, as described above. After serial washing, the slides were examined under a fluorescent microscope, and quantitative analysis of the protein localization in the cell and tissue sections under the above experimental conditions was performed with use of the Metamorph Imaging System interfaced with a Nikon Diaphot 300 Image Analyzer that uses a Hamamatsu CCD camera.

ELISA measurements of IL-1 β protein

IL-1 β protein levels were measured in the culture media from microscopically isolated human ASM tissues at baseline and after a 24-hour exposure to maximum effective concentrations of either IgE alone, IgE immune complexes, human control serum, or human atopic asthmatic serum in the absence and presence of anti-CD23 mAb. IL-1 β protein was quantitatively assessed with an enzyme-specific immunoassay, as previously described.¹⁹ The latter assay was performed with a double-antibody sandwich strategy in which an acetylcholine esterase, Fab-conjugated IL-1 β -specific secondary antibody was targeted to a first cytokine-captured antibody. The enzymatic activity of the acetylcholine esterase was measured spectrophotometrically and, relative to a linear standard curve (range 0–250 pg/mL), the results were used to quantify the amount of the targeted IL-1 β present in the tissue culture media.

Statistical analysis

Statistical analysis was performed by means of the 2-tailed paired Student *t* test. *P* values <.05 were considered significant.

Reagents

The human airway tissue was provided by the Cooperative Human Tissue Network, which is funded by the National Cancer Institute. The human ASM cells and SMBM medium were obtained from Clonetics, Walkersville, Md. The Fc γ RI, Fc γ RIIAC, Fc γ RIIB, Fc γ RIII, Fc ϵ RI, Fc ϵ RII, and RPL7 primers were obtained from Integrated DNA Technologies, Coralville, Ia. The human myeloma IgE and the goat-antihuman IgE antibodies were purchased from Biodesign, Kennebunk, Me. The Fc γ RI, Fc γ RII, Fc γ RIII, and Fc ϵ RII mAbs and the F(ab')₂-FITC fragments used in protein assay studies were purchased from Serotec, Raleigh, NC. The anti-CD23 monoclonal blocking antibody (mAb135) was the generous gift of Dr M. Sarfati, Montreal, Quebec, Canada.

RESULTS

Fc receptor mRNA expression in inherently asthmatic and atopic asthmatic serum-sensitized ASM

To examine the expression of different Fc receptors in human ASM tissue and whether this expression is altered in inherently atopic asthmatic ASM, we used RT-PCR incorporating Fc receptor-specific primers for the different Fc γ and Fc ϵ receptor subtypes. Southern blots were then generated (see Methods) and probed with human cDNA probes specific for the individual Fc γ and Fc ϵ receptor genes. A 157-bp RPL7 probe was also used to control for gel loading, and the signals for the Fc γ , Fc ϵ , and RPL7 PCR products were quantitated on a Phosphor-Imager. As depicted in Fig 1, ASM tissue expressed mRNA for all 3 Fc γ receptor subtypes and there were no significant difference in the magnitudes of the signals for either Fc γ RI, Fc γ RII, or Fc γ RIII between control (nonatopic nonasthmatic) and inherently asthmatic ASM. In contrast to these observations, expression of the high-affinity IgE receptor, Fc ϵ RI, was undetectable in either the control or asthmatic ASM tissue specimens (Fig 2). mRNA expression of the low-affinity IgE receptor, Fc ϵ RII (ie, CD23), however, was present in both the control and asthmatic ASM tissues and, relative to the control ASM, the Fc ϵ RII signal was markedly up-regulated in the asthmatic ASM (Fig 2).

To determine whether the above selectively up-regulated expression of Fc ϵ RII mRNA in the inherently atopic asthmatic ASM tissues potentially represented an inducible phenomenon, similar to that recently reported in rabbit ASM passively sensitized with human atopic asthmatic serum,¹⁵ comparable experiments were conducted on adjacent segments of human control (nonatopic nonasthmatic) ASM tissue sections that were passively sensitized for 24 hours with either human control or atopic asthmatic serum. Subsequent Southern blot analysis demonstrated that, although the Fc ϵ RII signal was detectable in the control serum-treated tissues, expression of Fc ϵ RII was markedly enhanced in the atopic asthmatic serum-sensitized ASM. In contrast, the

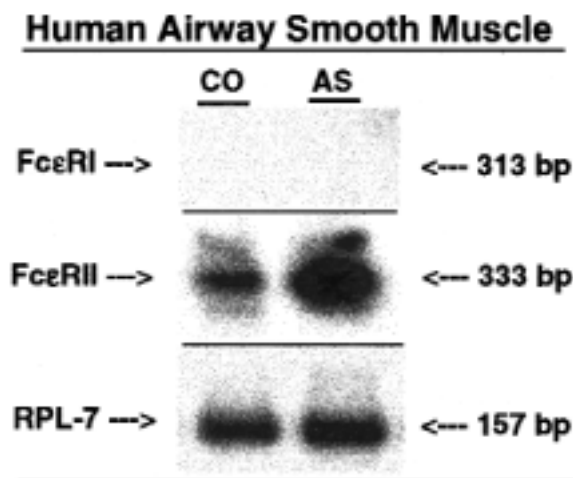


FIG 2. Comparison of expression of FcεRI (313 bp) and FcεRII (333 bp) receptor mRNAs with RT-PCR in control (CO) and inherently asthmatic (AS) human ASM tissue. Expression of RPL7 was used to control for loading. Blots were probed with human specific FcεRI and FcεRII ³²P-labeled cDNA probes. Note: In contrast to undetectable expression of FcεRI, human ASM tissue expresses mRNA for low-affinity FcεRII receptor, as determined by expected size of its FcεRII cDNA fragment, and expression of FcεRII mRNA signal is markedly increased in inherently asthmatic human ASM. Data are representative of 3 separate experiments with ASM tissue isolated from patient 1. Comparable results were obtained with ASM tissue isolated from patient 2.

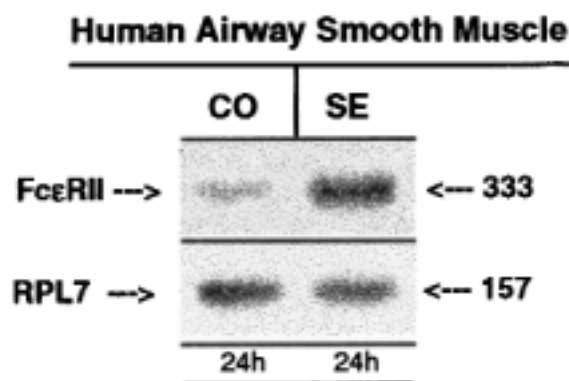
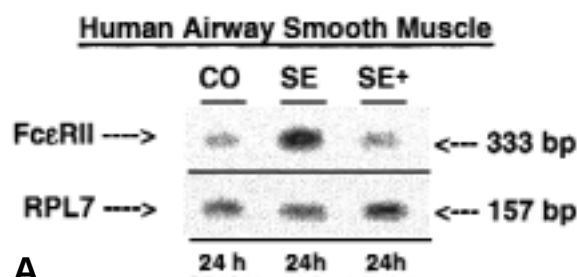


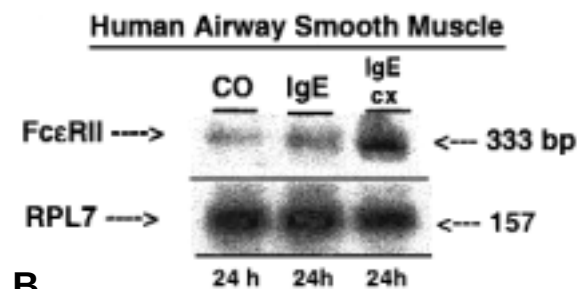
FIG 3. Representative Southern blot (n = 4) prepared from paired human ASM tissue samples incubated with control (CO) or atopic asthmatic (SE) serum for 24 hours. PCR products were amplified with human-specific FcεRII and RPL7 primers and probed with human-specific 333-bp FcεRII and 157-bp RPL7 human ³²P-labeled cDNA probes. Note: Marked induction of FcεRII expression in atopic asthmatic serum-sensitized sample.

constitutively expressed RPL7 signal was similar in both tissue preparations (Fig 3).

In light of the above results, together with our earlier pharmacodynamic evidence demonstrating a critical role for immune-complexed IgE/CD23 interaction in mediating proasthmatic changes in agonist responsiveness in atopic asthmatic serum-sensitized rabbit ASM tissues,¹⁵ we next examined whether our observed up-regulation of FcεRII expression in the atopic asthmatic serum-sensitized



A



B

FIG 4. A, Comparison of expression of FcεRII mRNA in human ASM tissue after 24-hour treatment with either control (CO) or atopic asthmatic serum in absence (SE) and presence (SE+) of anti-CD23 mAb. B, Comparison of FcεRII mRNA expression in human ASM treated for 24 hours with vehicle alone (CO) or maximal effective concentrations of IgE alone or IgE immune complexes (IgE cx). Products of RT-PCR reactions with human-specific primers for FcεRII receptor mRNA are shown. Expression of RPL7 was used to control for gel loading. Blots were probed with above ³²P-labeled human-specific FcεRII and RPL7 cDNA probes. Note: Anti-CD23 mAb significantly attenuated induction of FcεRII mRNA expression in atopic asthmatic-serum sensitized ASM (A). Comparably, treatment of human ASM with IgE immune complexes also significantly induced enhanced mRNA expression of FcεRII, whereas IgE alone had a lesser effect (B). Data are representative of 4 separate experiments.

state was associated with IgE-coupled activation of the FcεRII receptor itself. With use of ASM tissue sections microscopically isolated from the same human tracheal and bronchial specimens (see Methods) we found that this up-regulation of FcεRII expression was largely prevented by pretreating the atopic serum-sensitized tissues with a monoclonal anti-CD23 blocking antibody (SE⁺) (Fig 4, A), whereas the tissue samples exposed to atopic asthmatic serum (SE) displayed markedly induced FcεRII expression relative to the control (CO) serum-treated samples. Moreover, in complementary experiments examining the effects of exogenous administration of either human IgE alone or IgE immune complexes to naive ASM tissues, we found that treatment of the tissues for 24 hours with IgE immune complexes induced a pronounced up-regulated expression of FcεRII mRNA, whereas the corresponding RPL7 signal was unaffected (Fig 4, B). Comparably, exogenous administration of IgE alone had a qualitatively similar effect on FcεRII expression; however, the magnitude of this effect was relatively modest.

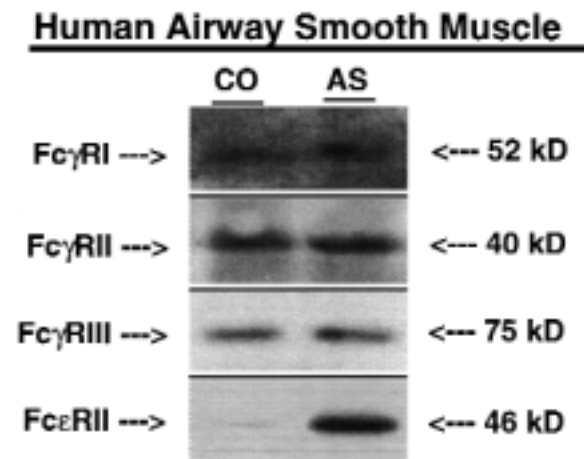


FIG 5. Representative Western blots depicting Fc γ RI (~52,000 kD), Fc γ RII (~40,000 kD), Fc γ RIII (~75,000 kD), and Fc ϵ RII (~46,000 kD) expression in membrane homogenates from human control (CO) and inherently asthmatic (AS) human ASM tissue. Fifty micrograms of membrane protein was loaded in each lane. Note: In contrast to unaltered Fc γ RI, Fc γ RII and Fc γ RIII expression, relative to control ASM, expression of Fc ϵ RII protein was significantly enhanced in inherently asthmatic ASM. Data are representative of 3 separate experiments with ASM tissue isolated from patient 1. Comparable results were obtained with ASM tissue isolated from patient 2.

Fc receptor protein expression in inherently asthmatic and atopic asthmatic serum-sensitized ASM

Cell surface expression of the Fc receptors Fc γ RI, Fc γ RII, Fc γ RIII, and Fc ϵ RII was also examined with use of Western immunoblot analysis of membrane protein samples isolated from human naive (control) and inherently asthmatic ASM tissues (see Methods). As demonstrated in Fig 5, protein expression signals were detected for the Fc γ RI, Fc γ RII, and Fc γ RIII receptors, and there were no discernible differences in these signals between the control and asthmatic ASM tissues. In contrast, although Fc ϵ RII protein expression was virtually undetectable in the control ASM, the expression of Fc ϵ RII was significantly up-regulated in the asthmatic ASM (Fig 5).

Given the above observations, in extended flow cytometric experiments we further examined whether cultured human ASM cells also express cell surface proteins for the Fc γ RI, Fc γ RII, Fc γ RIII, and Fc ϵ RII receptor subtypes and whether this expression is altered after passive sensitization of the cells with atopic asthmatic serum. As shown in Fig 6, ASM cells expressed surface proteins for the above Fc receptor subtypes (Fig 6, A), and there were no differences in the expression signals for the Fc γ receptors between cells that were exposed to control versus atopic asthmatic serum. In contrast to unaltered Fc γ receptor expression, relative to control cells, 24-hour exposure of the ASM cells to atopic asthmatic serum induced up-regulated cell surface expression of the Fc ϵ RII receptor (Fig 6, B). Finally, to further substantiate the latter observations, we also examined for altered Fc ϵ RII receptor expression in atopic asthmatic serum-

sensitized human ASM cells by immunofluorescence staining (see Methods). As shown in Fig 7, relative to the isotype-negative control antibody (upper panels), staining with a fluorescent-labeled anti-CD23 antibody was weakly positive in the control serum-treated cells (left panel). Comparatively, in accordance with the above flow cytometric analysis, immunofluorescence staining for the CD23 receptor protein was notably enhanced in the atopic asthmatic serum-sensitized ASM cells (right panel).

Taken together, the above observations demonstrate that (1) human ASM expresses the mRNAs and proteins for the Fc γ RI, Fc γ RII, and Fc γ RIII receptors for IgG, as well as for the Fc ϵ RII receptor for IgE, (2) relative to control ASM, the expression of Fc ϵ RII is selectively up-regulated in asthmatic ASM, and (3) up-regulated Fc ϵ RII receptor expression can be induced in naive ASM after exposure of the tissue or cultured cells to either human atopic asthmatic serum or exogenously administered IgE immune complexes.

Release of IL-1 β by atopic asthmatic sensitized ASM

We recently reported that IL-1 β protein is elaborated by rabbit ASM tissue or cultured cells passively sensitized with human atopic asthmatic serum.¹⁹ To the extent that the latter release of IL-1 β was found to act in an autocrine fashion to endogenously induce proasthmatic changes in ASM responsiveness,^{19,21} we next examined whether IgE immune complex-treated or atopic asthmatic serum-sensitized human ASM depicts altered IL-1 β protein release. The latter was analyzed with use of an enzyme-specific immunoassay (see Methods). There were no detectable levels of IL-1 β protein in the culture media of ASM tissues exposed for 24 hours to vehicle or IgE alone. In contrast, tissues that were exposed to immune-complexed IgE (Fig 8, A) or atopic asthmatic serum (Fig 8, B) depicted markedly enhanced elaboration of IL-1 β protein into the culture media at 8 and 24 hours. Moreover, this induced release of IL-1 β was inhibited by pretreating the atopic serum-sensitized tissues with an anti-CD23 monoclonal blocking antibody (Fig 8, B), implicating a role for Fc ϵ RII activation in mediating the release of IL-1 β in the sensitized state.

DISCUSSION

Bronchial asthma is characterized by exaggerated agonist-induced bronchoconstriction, attenuated β -adrenoceptor-mediated airway relaxation and airway inflammation, the latter most notably involving infiltration of the airways with eosinophils and lymphocytes.^{11,12,22} Although the mechanistic interplay between inflammation and the associated changes in airway responsiveness remains to be identified, it is relevant to note that the altered airway responsiveness in asthmatic individuals has been associated with elevations in total and antigen-specific serum IgE.^{23,24} Moreover, genetic analyses of family members with allergic asthma have demonstrated a significant correlation between altered airway respon-

Human Airway Smooth Muscle Cells

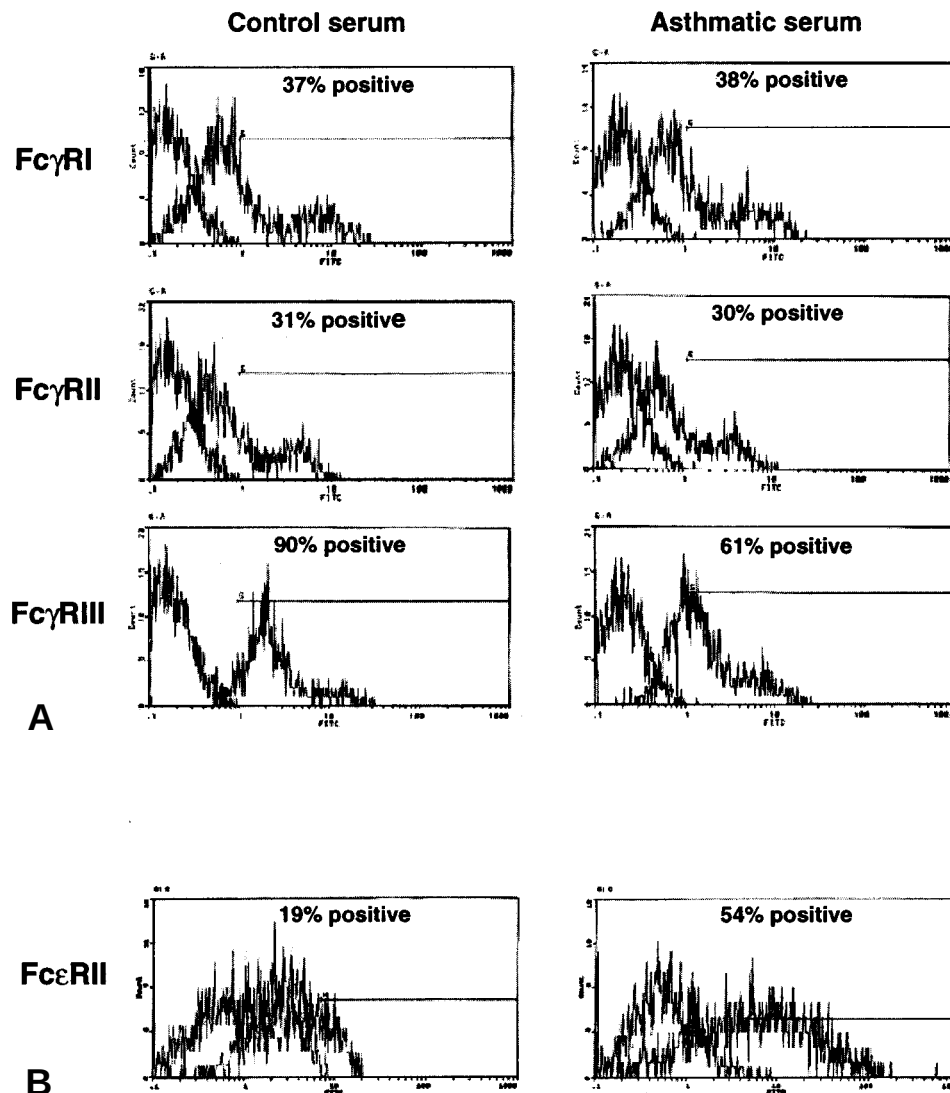


FIG 6. Representative flow cytometric analysis of FcγRI, FcγRII, and FcγRIII expression (**A**) and FcεRII cell surface protein expression (**B**) in human ASM cells treated for 24 hours with human control serum (*left*) and human atopic asthmatic serum (*right*). The ASM cells were stained with mouse antihuman mAbs specific for the individual FcγRI, FcγRII, FcγRIII, and FcεRII receptors. Levels of nonspecific background staining were measured by staining with isotype control IgG antibody (left histogram in each panel). Goat antimouse FITC-conjugated secondary antibody was used for detection of the signals. Note: Relative to cells exposed to control serum, there was significantly induced enhanced cell surface staining for FcεRII in cells that were exposed to atopic asthmatic serum ($P < .01$, $n = 3$). In contrast, atopic asthmatic serum had no appreciable effect on FcγRI, FcγRII, or FcγRIII expression.

siveness and serum IgE levels.^{25,26} As a first approximation, these findings suggest a mechanistic relationship between serum IgE levels and changes in airway responsiveness in atopic asthma. Indeed, it is well established that IgE can induce an immediate hypersensitivity reaction in the airways after allergen exposure and that this acute bronchoconstriction is initiated by degranulation of mast cells after activation of their high-affinity IgE receptor, FcεRI.⁴⁻⁷ Furthermore, progression of the ini-

tial IgE/FcεRI-coupled mast cell response into the late-phase asthmatic response has been largely attributed to the orchestrated extended release of various cytokines by other stimulated infiltrating inflammatory cell types.^{5,10,11,14} In this regard, it has been recently demonstrated that expression of the inducible form of the low-affinity IgE receptor (FcεRII) is up-regulated on monocytes and lung alveolar macrophages,^{27,28} as well as on circulating B lymphocytes²⁹⁻³¹ isolated from atopic asth-

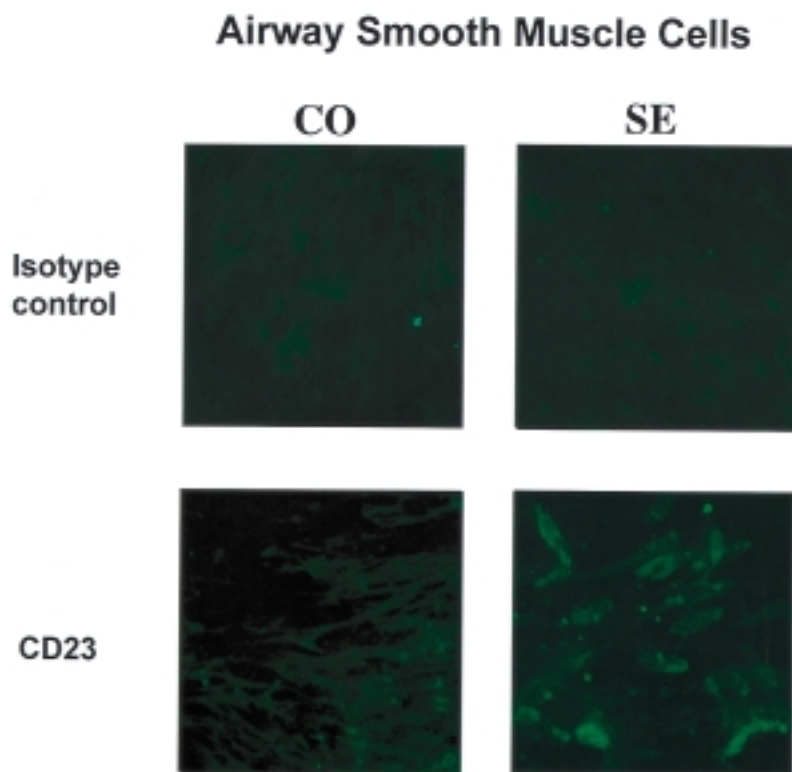


FIG 7. Representative immunofluorescence staining ($n = 4$) for CD23 surface receptor protein in control serum-treated (left) and atopic asthmatic serum-sensitized (right) ASM cells. (Original magnification $\times 100$). F(ab')₂-FITC conjugated fragments were used to detect the primary monoclonal anti-CD23 antibody. Isotype-negative control antibody was used to control for nonspecific staining (upper). Note: Significantly enhanced Fc ϵ RII (CD23) receptor staining in ASM cells that were treated with atopic asthmatic serum.

matic subjects. Thus, apart from mast cells per se, IgE-Fc ϵ receptor interactions involving other bone marrow-derived proinflammatory cells may also contribute to the allergic asthmatic pulmonary response.

Notwithstanding the above considerations, we recently identified the presence of Fc receptors in ASM tissue and cultured ASM cells and found that specific activation of Fc ϵ RII in rabbit ASM was associated with the induction of proasthmatic changes in agonist-mediated ASM constrictor and relaxant responsiveness.¹⁵ In light of these findings, the current study investigated whether expression of specific Fc receptors is altered in inherently asthmatic human ASM tissue and whether such altered Fc receptor expression represents a potentially inducible phenomenon in naive human ASM that is passively sensitized with human atopic asthmatic serum or IgE immune complexes. The results provided compelling new evidence demonstrating that (1) although Fc γ receptor subtype expression is present and similar in both isolated human control and inherently asthmatic ASM, expression of the low-affinity receptor for IgE (Fc ϵ RII) is relatively markedly up-regulated in the asthmatic tissue, (2) qualitatively comparable selective up-regulation of Fc ϵ RII expression is inducible in naive (control) ASM passively sensitized with human atopic asthmatic serum or IgE immune complexes, and (3) the latter inducible

phenomenon is associated with an autologous Fc ϵ RII-coupled release of the proinflammatory cytokine IL-1 β by the sensitized ASM. In light of these findings, the concept is raised that, apart from an implied role(s) for infiltrating leukocytes, the autologously up-regulated expression of Fc ϵ RII in ASM may also serve to foster the proasthmatic phenotype. Indeed, in extended support of this concept, we recently reported that the induced, IL-1 β -dependent, proasthmatic changes in constrictor and relaxant responsiveness in rabbit ASM passively sensitized with human atopic asthmatic serum or IgE immune complexes is preventable by pretreating the sensitized ASM with a monoclonal anti-CD23 blocking antibody.¹⁵

In addressing the mechanism underlying our observed increased expression of Fc ϵ RII in inherently atopic asthmatic ASM, our extended observations suggest that this may represent an inducible phenomenon in naive human ASM. Indeed, in agreement with our recent findings using rabbit ASM,¹⁵ we found that either exposure of naive human ASM tissue to atopic asthmatic serum or IgE immune complexes induced comparably increased Fc ϵ RII receptor expression in the sensitized ASM. Moreover, our findings demonstrated that this experimentally induced up-regulated expression of Fc ϵ RII was largely prevented by pretreating the human ASM tissue with an anti-CD23 blocking antibody (Fig 4, A and B). Thus our

findings support the presence of an IgE/CD23-dependent autocrine mechanism that serves to autologously up-regulate FcεRII expression in sensitized human ASM. This concept is in accord with a number of earlier studies that have reported a direct correlation between serum IgE levels and FcεRII and FcεRI expression in other cell types.³²⁻³⁴ Although these reports raised the notion of a possible common mechanism regulating the production of both IgE and its receptors, more recent evidence based on studies with different cell lines suggests that IgE itself may act to enhance FcεRI^{35,36} or FcεRII expression.³⁷⁻³⁹ Our current observations concur with the latter notion and, accordingly, suggest the presence of a positive feedback system in human ASM wherein IgE up-regulates the expression of its own low-affinity receptor.

In previously examining the mechanistic link between exposure of naive rabbit ASM tissue to atopic asthmatic serum and its resultant altered agonist responsiveness, with the same experimental model described here, we found that the induced proasthmatic changes in ASM responsiveness were initiated by IgE/FcεRII-coupled activation, associated with the induced autologous expression and autocrine action of IL-1β in the atopic sensitized ASM.^{15,19} Moreover, the mechanism of action of IL-1β in eliciting the proasthmatic changes in ASM responsiveness was attributed to its induced up-regulated expression of Gi protein, specifically Giα₂ and Giα₃, which inhibit intracellular cyclic AMP accumulation.²¹ Our current study did not allow for comparable examination of the above mechanism of altered responsiveness in human ASM tissue because of its limited availability in quantity sufficient for pharmacodynamic experiments. Nevertheless, consistent with the mechanism identified in rabbit ASM,¹⁵ we found that human ASM tissue passively sensitized with atopic asthmatic serum or IgE immune complexes depicts markedly enhanced release of IL-1β protein into the culture medium (Fig 8, A). In addition, we found that the latter induced release of IL-1β was largely prevented by pretreating the sensitized human ASM with an anti-CD23 blocking antibody (Fig 8, B). Thus, as a first approximation, it appears reasonable to suspect that sensitized human ASM tissue may undergo proasthmatic changes in its responsiveness that are comparable to those seen in sensitized rabbit ASM. This possibility, however, remains to be experimentally addressed.

In conclusion, the current study examined the presence and regulation of Fc receptors in inherently asthmatic human ASM tissue and in naive human ASM passively sensitized with human atopic asthmatic serum and IgE immune complexes. The results demonstrate that (1) human ASM tissue expresses mRNA and surface protein for the low affinity receptor for IgE, FcεRII, as well as for the FcγRI, FcγRII, and FcγRIII receptor subtypes that bind to IgG, whereas expression of the high-affinity receptor for IgE (FcεRI) is undetectable, (2) in contrast to unaltered Fcγ subtype expression, relative to naive (control) human ASM, expression of FcεRII mRNA and protein is significantly up-regulated in inherently asth-

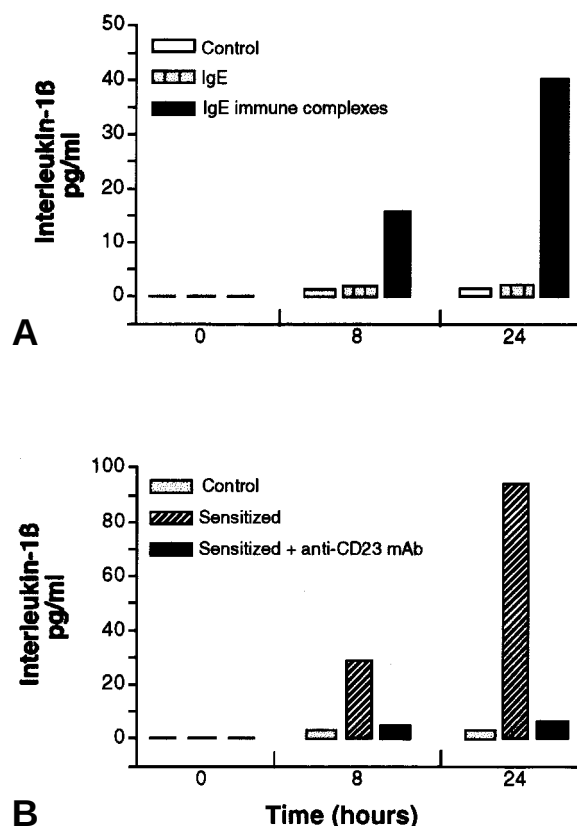


FIG 8. Comparison of mean levels of IL-1β protein released into culture media of ASM tissues after 0-, 8-, and 24-hour exposure to (A) SFM alone (open bars), IgE (hatched bars), or IgE immune complexes (filled bars) and (B) human control serum (open bars) or human atopic asthmatic serum in absence (hatched bars) and presence (filled bars) of anti-CD23 mAb. Note: Human ASM tissue exposed to IgE immune complexes or to atopic asthmatic serum released significantly increased ($P < .001$, $n = 5$) levels of IL-1β protein into tissue culture medium, whereas treatment with serum-free medium, human control serum, or IgE alone had no appreciable effect. Moreover, this effect of atopic asthmatic serum was largely prevented by pretreatment with anti-CD23 mAb. Data are representative of 3 separate experiments.

matic ASM tissues, (3) up-regulated expression of FcεRII represents, at least in part, an inducible phenomenon that is largely attributed to IgE-coupled activation of the FcεRII receptor itself, and (4) the latter action is associated with FcεRII-induced autologous elaboration of IL-1β protein by the sensitized ASM. Although comparably up-regulated expression of the FcεRII receptor has been demonstrated in different bone marrow-derived circulating leukocytes isolated from atopic asthmatic individuals,²⁷⁻³⁰ the current observations raise a novel consideration regarding the pathobiologic mechanisms of asthma. Accordingly, given the important immunologic, proinflammatory, and physiologic responses attributed to FcεRII receptor activation, the current findings support the concept that up-regulated FcεRII expression and action in human asthmatic ASM plays an important role in establishing its asthmatic phenotype.

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REFERENCES

- Bai TR. Abnormalities in airway smooth muscle in fatal asthma. *Am Rev Respir Dis* 1990;141:552-7.
- Goldie RG, Spina D, Henry PJ, Lulich KM, Paterson JW. In vitro responsiveness of human asthmatic bronchus to carbachol, histamine, beta-adrenoceptor agonists and theophylline. *Br J Clin Pharmacol* 1986;22:669-76.
- McFadden ER Jr. Asthma: morphologic-physiologic interactions. *Am J Respir Crit Care Med* 1994;150:523-6.
- Metzger H. The receptor with high affinity for IgE. *Immunol Rev* 1992;125:37-48.
- Beaven MA, Metzger H. Signal transduction by Fc receptors: the Fc RI case. *Immunol Today* 1993;14:222-6.
- Galli SJ. New concepts about the mast cell. *N Engl J Med* 1993;328:257-65.
- Matsumoto T, Takahashi H, Miike T. Increase of T cells bearing Fc epsilon R-associated antigen in patients with atopic asthma. *Ann Allergy* 1987;58:261-4.
- Watson ML, Smith D, Bourne AD, Thompson RC, Westwick J. Cytokines contribute to airway dysfunction in antigen-challenged guinea pigs: inhibition of airway hyperreactivity, pulmonary eosinophil accumulation and tumor necrosis factor generation by pre-treatment with an interleukin-1 receptor antagonist. *Am J Respir Cell Mol Biol* 1993;8:365-9.
- Capron M, Spiegelberg HL, Prin L, Bennich H, Butterworth AE, Pierce RJ, et al. Role of IgE receptors in effector function of human eosinophils. *J Immunol* 1984;132:462-8.
- Beasley R, Roche WR, Roberts JA, Holgate ST. Cellular events in the bronchi in mild asthma and after bronchial provocation. *Am Rev Respir Dis* 1989;139:806-7.
- Litchfield TM, Lee TH. Asthma: cells and cytokines. *J Asthma* 1992;29:181-91.
- Barnes PJ, Chung KF, Page CP. Inflammatory mediators and asthma. *Pharmacol Rev* 1998;40:49-84.
- Borish L, Mascali JJ, Rosenwasser LJ. IgE-dependent cytokine production by human peripheral blood mononuclear phagocytes. *J Immunol* 1991;146:63-7.
- Walker C, Bode E, Boer L, Hansel TT, Blaser K, Virchow JC. Allergic and non-allergic asthmatics have distinct pattern of T-cell activation and cytokine production in peripheral blood and bronchoalveolar lavage. *Am Rev Respir Dis* 1992;146:109-15.
- Hakonarson H, Grunstein MM. Autologously up-regulated Fc receptor expression and action in airway smooth muscle mediates its altered responsiveness in the atopic asthmatic sensitized state. *Proc Natl Acad Sci U S A* 1998;95:5257-62.
- Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987;162:156-9.
- Capel PJ, van de Winkel JG, van den Herik-Oudijk IE, Verbeek JS. Heterogeneity of human IgG Fc receptors. *Immunomethods* 1994;4:25-34.
- Kikutani H, Inui S, Sato R, Barsumian EL, Owaki H, Yamasaki K, et al. Molecular structure of human lymphocyte receptor for immunoglobulin E. *Cell* 1986;47:657-65.
- Hakonarson H, Herrick DJ, Gonzalez-Serrano P, Grunstein MM. Autocrine role of interleukin 1 β in altered responsiveness of atopic asthmatic sensitized airway smooth muscle. *J Clin Invest* 1997;99:117-24.
- Hakonarson H, Herrick DJ, Grunstein MM. Mechanism of impaired beta-adrenoceptor responsiveness in sensitized airway smooth muscle. *Am J Physiol* 1995;269:L645-52.
- Hakonarson H, Herrick DJ, Gonzalez-Serrano P, Grunstein MM. Mechanism of cytokine-induced modulation of beta-adrenoceptor responsiveness in airway smooth muscle. *J Clin Invest* 1996;97:2593-600.
- Kay AB. Asthma and inflammation. *J Allergy Clin Immunol* 1991;87:893-910.
- Sears MR, Burrows B, Flannery EM, Herbison GP, Hewitt CJ, Holdaway MD. Relation between airway responsiveness and serum IgE in children with asthma and in apparently normal children. *N Engl J Med* 1991;325:1067-71.
- Burrows B, Fernando D, Martinez MD, Barbee RA, Clive MG. Association of asthma with serum IgE levels and skin reactivity. *N Engl J Med* 1989;320:272-7.
- Burrows B, Martinez FD, Cline MA, Lebowitz MD. The relationship between parental and children's serum IgE and asthma. *Am J Respir Crit Care Med* 1995;152:1497-500.
- Burrows B, Sears MR, Flannery EM, Herbison GP, Holdaway MD. Relations of bronchial responsiveness assessed by methacholine to allergy skin test reactivity, lung function, respiratory symptoms, and diagnosis in thirteen-year-old New Zealand children. *J Allergy Clin Immunol* 1995;95:548-56.
- William J, Johnson S, Mascali JJ, Smith H, Rosenwasser LJ, Borish L. Regulation of low affinity IgE receptor (CD23) expression on mononuclear phagocytes in normal and asthmatic subjects. *J Immunol* 1992;149:2823-9.
- Joseph M, Tonnel AB, Torpier G, Capron A, Arnoux B, Benevise J. Involvement of immunoglobulin E in the secretory processes of alveolar macrophages from asthmatic patients. *J Clin Invest* 1983;71:221-30.
- Chihara J, Nakano N, Kurachi D, Seguchi M, Mouri T, Uenishi H, et al. Induction of IgE-Fc receptor (Fc epsilon R2/CD23) expression on lymphocytes from patients with mite-allergic bronchial asthma by mite allergen. *Ann Allergy* 1991;67:429-32.
- Gagro A, Rabatic S, Tressee A, Dekaris D, Medar-Lasic M. Expression of lymphocytes Fc-RII/CD23 in allergic children undergoing hyposensitization. *Int Arch Allergy Immunol* 1993;101:203-8.
- Rabatic S, Gagro A, Medar-Lasic M. CD21-CD23 ligand pair expression in children with allergic asthma. *Exp Immunol* 1993;94:337-40.
- Spiegelberg HL, Simon RA. Increase of lymphocytes with Fc receptors for IgE in patients with allergic rhinitis during the grass pollen season. *J Clin Invest* 1981;68:845-52.
- Conroy MC, Adkinson NF Jr, Lichtenstein LM. Measurement of IgE on human basophils: relation to serum IgE and anti-IgE-induced histamine release. *J Immunol* 1977;118:1317-24.
- Malveaux FJ, Conroy MC, Adkinson NF Jr, Lichtenstein LM. IgE receptors on human basophils: relationship to serum IgE concentration. *J Clin Invest* 1978;62:176-81.
- Lantz CS, Yamaguchi M, Oettgen HC, Katona IM, Miyajima I, Kinet JP, et al. IgE regulates mouse basophil Fc RI expression in vivo. *J Immunol* 1997;158:2517-21.
- Yamaguchi M, Lantz CS, Oettgen HC, Katona IM, Fleming T, Miyajima I, et al. IgE enhances mouse mast cell Fc RI expression in vitro and in vivo: evidence for a novel amplification mechanism in IgE-dependent reactions. *J Exp Med* 1997;663-72.
- Lee WT, Rao M, Conrad DH. The murine lymphocyte receptor for IgE, IV: the mechanism of ligand-specific receptor upregulation on B cells. *J Immunol* 1987;139:1191-8.
- Daeron M, Ishizaka K. Induction of Fc receptors on mouse macrophages and lymphocytes by homologous IgE. *J Immunol* 1986;136:1612-9.
- Lee WT, Conrad DH. Murine B cell hybridomas bearing ligand-inducible Fc receptors for IgE. *J Immunol* 1986;136:4573-80.