

Modulation of high-affinity IgE receptor expression in blood monocytes: Opposite effect of IL-4 and glucocorticoids

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Background: The expression of high-affinity IgE receptor (FcεRI) is increased in blood monocytes (BMs) from allergic patients compared with those of nonatopic subjects (NASs). **Objective:** We investigated the in vitro effect of cytokines involved in allergic diseases on the modulation of FcεRI expression in BMs from allergic asthmatic patients (AAPs) and NASs. The influence of in vitro and in vivo treatments with glucocorticoids (GCs) was also assessed.

Methods: FcεRI α-chain expression on BMs evaluated by flow cytometry analysis was studied ex vivo in AAPs treated or not with GCs and in NASs. IgE receptor expression was also evaluated in vitro with or without stimulation by IL-4, IL-13, GM-CSF, and/or GCs. Messenger (m)RNA expression was also analyzed with RT-PCR.

Results: The expression of the FcεRI α chain was significantly increased in BMs from untreated patients, with AAPs compared with NASs ($P < .05$). In steroid-treated AAPs FcεRI α-chain expression returned to the level found in BMs from NASs. In vitro addition of IL-4 induced a dose-dependent increase in FcεRI α-chain expression on BMs from NASs, and this effect was significantly enhanced with BMs from AAPs. FcεRI α-chain mRNA was significantly upregulated by IL-4, whereas the β chain was always undetectable. The γ chain was not modulated by IL-4. Similar findings were obtained with IL-13. In contrast with CD23 expression, GM-CSF alone or in coinubation with IL-4 had no effect on FcεRI α-chain expression in BMs. Lastly, GCs significantly inhibited in vitro the IL-4-induced FcεRI α-chain expression ($P < .05$).

Conclusion: Two different pathways by which FcεRI α-chain expression was modulated in BMs were identified: (1) the enhancing effect of IL-4 and IL-13 and (2) the inhibitory effect of GCs. Modulation of FcεRI α-chain expression on BMs may affect their capacity to regulate allergic inflammation. (J Allergy Clin Immunol 2001;107:114-22.)

Key words: Monocyte, IgE receptor, IL-4, GM-CSF, corticoid, asthma, allergy

Allergen-triggered IgE cross-linking induces receptor aggregation and then degranulation of mast cells and basophils, a process responsible for the development of

Abbreviations used

AAP:	Allergic asthma patient
BM:	Blood monocyte
FcεRI:	High-affinity IgE receptor
GAPDH:	6-Glyceraldehyde-phosphate dehydrogenase
GC:	Glucocorticoid
IL-4R:	IL-4 receptor
MFI:	Median fluorescence intensity
MP:	Methylprednisolone
NAS:	Nonatopic subject
STAT:	Signal transducers and activators of transcription

the allergic reaction. Two types of IgE receptors have been described: the high-affinity IgE receptor (FcεRI) and the low-affinity IgE receptor (CD23). In mast cells FcεRI is present as a tetrameric holoreceptor αβγ2. The α chain binds IgE, whereas the β chain and γ chain are implicated in signal transduction.¹

More recently, the high-affinity receptor has been described as a trimeric form αγ2 in other cell types, such as eosinophils, platelets, Langerhans cells, and blood monocytes (BMs).²⁻⁵ The absence of the β chain suggests that this form of receptor induces a mild activation in these cells compared with cells bearing the αβγ2 form. Nevertheless, FcεRI activation in BMs triggers Ca²⁺ mobilization and PGE2 production.⁶ In addition, the high-affinity IgE receptor mediates the IgE-dependent allergen presentation in antigen-presenting cells⁷ and cytokine production in mast cells.⁸ However, the secretion of proinflammatory mediators induced by IgE-dependent activation of monocytes-macrophages from allergic asthmatic patients (AAPs) is dependent on CD23.⁹⁻¹¹ Thus in BMs the relationship between FcεRI expression and allergic inflammation remains to be defined.

The expression of FcεRI is increased in BMs, as well as in eosinophils and platelets, from allergic patients compared with healthy subjects.^{3,4,12} In the lung the number of positive alveolar macrophages was significantly higher in patients with allergic asthma compared with control subjects.¹³ The mechanisms of this FcεRI overexpression are not clearly defined. Reischl et al¹⁴ showed that the presence of IgE in the cell environment led to cycloheximide-resistant FcεRI expression on BM membrane, a process also observed with basophils.¹⁵ The

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expression of FcεRI α chain in mast cells and eosinophils was found to be upregulated by IL-4.¹⁶⁻¹⁸ In the presence of IL-4 and GM-CSF, BMs differentiate into dendritic cells that express high levels of FcεRI.¹⁹ Both cytokines play an important role in the pathogenesis of the allergic reaction: IL-4 is mainly involved in IgE production and T_H2 differentiation, whereas GM-CSF is implicated in the inflammatory reaction and as a growth factor for hematopoietic progenitors.^{20,21} On the other hand, glucocorticoids (GCs) currently represent the most efficient anti-inflammatory therapy used in allergic patients.

Taking all these data into account, we developed this study to define pathways susceptible to modulate FcεRI expression in BMs and their relevance in allergic asthma. The effect of IL-4 and GM-CSF on FcεRI expression was measured in BMs from control subjects and allergic patients. The influence of an in vitro and in vivo treatment with GCs was also evaluated. Our results showed that IL-4 increased messenger (m)RNA and protein expression of FcεRI α chain by a tyrosine kinase-dependent mechanism. In contrast, in vitro exposure or treatment with systemic GCs decreased FcεRI expression on BMs.

METHODS

Patients

Fifteen patients with mild allergic asthma were included. Asthma was defined according to the American Thoracic Society criteria. Atopy was defined by the presence of 2 or more positive skin prick test responses to common aeroallergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat and dog dander, pollens, and mold). Exclusion criteria were infection, systemic or inhaled steroid use, or hospitalization during the previous 2 weeks. Mean FEV₁ (percent of predicted value) was 87.2% ± 6.4%. Six of the 15 patients received β₂-agonists. Asthma severity was determined according to the criteria of the National Heart, Lung, and Blood Institute/World Health Organization²² and classified into 4 categories: intermittent (n = 5), mild (n = 7), moderate persistent (n = 3), and severe persistent asthma (n = 0).

In addition, 7 patients with allergic asthma requiring oral corticosteroid therapy were also included to evaluate the FcεRI expression in a group of patients receiving GCs. Treatment was prescribed because of an acute attack of asthma. FcεRI expression was evaluated between 1 and 7 days after the beginning of the treatment. All patients received β₂-agonists. These patients presented with either intermittent (n = 1) or moderate persistent (n = 6) asthma.

Fifteen healthy nonsmoking nonatopic subjects (NASs) were included. None had history of asthma, rhinitis, or other allergic diseases. Lung function was in a normal range. The study was approved by the Ethics Committee of the University Hospital of Lille (CCPPRB LILLE: 9307).

Preparation of BMs

To avoid the contamination of BM preparations with other FcεRI-positive cells, such as basophils and dendritic cells, we purified BMs by means of positive selection with anti-CD14 magnetic beads. After gradient centrifugation, platelet-depleted mononuclear cells were incubated with anti-CD14 magnetic beads (Milteny Biotech, Bergisch Gladbach, Germany) for 30 minutes at 4°C in RPMI-1640 supplemented with 10% FCS and 2 mmol/L L-glutamine (complete RPMI; Life Tech, Paisley, UK). After passage through a column type VS (Milteny Biotech), the fraction containing the CD14-positive

cells was collected, centrifuged, and resuspended in complete medium. This positive fraction contained more than 95% CD14-positive cells and less than 0.5% basophil and dendritic cells.

BMs were cultivated in complete RPMI at 10⁶ cells/mL and 2 mL/well at 37°C and 5% CO₂. When indicated, human recombinant IL-4 (Genzyme, R&D, Minneapolis, Minn) and GM-CSF (Pepro-tech, Rocky Hill, NJ) were added alone or altogether at 200 U/mL (specific activity, 1 × 10⁷ U/mL) and 20 ng/mL (specific activity, 1 × 10⁷ U/mL), respectively. IL-13 was also tested (20 ng/mL; specific activity, 1 × 10⁷ U/mL; a generous gift of Dr A. Minty, Labège, France). In some experiments the effect of a GC, methylprednisolone (MP; 10⁻⁶ and 10⁻⁸ mol/L) was tested after addition with IL-4 either simultaneously or after a preincubation of 1 hour. The inhibitor of tyrosine kinase, genistein (5 and 25 μmol/L; Calbiochem, Schwalbach, Germany), was also added to some BM cultures 1 hour before addition of cytokines.

Flow cytometric analysis

Freshly purified monocytes and adherent monocytes were washed and resuspended in PBS with 1% normal human serum. They were incubated for 30 minutes at 4°C with the FITC-conjugated mouse mAb lineage cocktail 1 (lin-1; Becton-Dickinson, Le Pont de Claix, France) and the phycoerythrin-conjugated mouse mAb anti-CD14 (Coulter, Miami, Fla), anti-CD11c, anti-CD23, and anti-CD123 (Becton-Dickinson), as well as the peridinin chlorophyll protein-conjugated anti-HLA-DR (Becton Dickinson) and the corresponding isotype control. Indirect immunofluorescence was also performed with the anti-FcεRI α-chain mouse mAb (Clone 22E7, Dr J. P. Kochan, Nutley, NJ) followed by the addition of Alexa 488-conjugated or allophycocyanin-conjugated F(ab')₂ anti-mouse IgG (Molecular Probe, Eugene, Ore). Cells were washed, fixed with paraformaldehyde, and analyzed by means of flow cytometry (FacsCalibur, Becton Dickinson).

Evaluation of mRNA expression by using RT-PCR and Northern blot analysis

mRNA expression for CD23 and the α, β, and γ chains of FcεRI was evaluated by using a 2-step semiquantitative RT-PCR with 6-glyceraldehyde-phosphate dehydrogenase (GAPDH) mRNA as a reference. KU812 RNA was used as a positive control. As previously described, the sense and antisense primer sequences were as follows: CD23, sense CTG TGG CAC TGG GAC ACC ACA, antisense ACA CAC GTT GTG CAC GGG ACT T; α chain, sense CTG TTC TTC GCT CCA GAT GGC GT, antisense, TACCAACCTC-CTTGA CCC TAC ACA TGT TCC; β chain, sense GGA CAC AGA AAG TAA TAG GAG AG, antisense GAT CAG GAT GGT AAT TCC CGT T; γ chain, sense CCA GCA GTG GTC TTG CTC TTA C, antisense GCA TGC GGC ATA TGT GAT GCC; and GAPDH, sense GTC TTC ACC ACC ATG GAG A; antisense CCA GTA GGT ACT GTT GAA ACC.

To obtain a linear relationship between the quantity of mRNA and the RT-PCR product, the number of cycles was determined as 19, 25, 28, 30, and 22 for GAPDH, CD23, α chain, β chain, and γ chain, respectively. After gel electrophoresis and staining with Sybrgreen (FMC, Rockland, Me), the intensity of each band was measured with the Gel Analyst system (Clarusvision, Paris, France). Results were expressed as a ratio of GAPDH mRNA intensity.

For the Northern blot analysis, 10 μg of formaldehyde-denatured RNA was fractionated by means of electrophoresis through 1% agarose gels. After RNA transfer, nylon membranes were dried and exposed to UV irradiation. Prehybridization was performed at 42°C for 18 hours in prehybridization buffer containing 40% deionized formamide, 1× saline sodium citrate, 1% SDS, 50 μg/mL sperm salmon DNA, and 1× Denhardt's solution (Sigma, St Louis,

Mo). A full-length complementary (c)DNA encoding for α chain was obtained by using RT-PCR with mRNA from the KU812 cell line. The primers used were ATAGAATTTCGCACAGTAAG-CACCAGGAGTC for sense and ATACTCGAGTGAGTAG-CAATTGCTGATGCTG for antisense. The sequence of PCR product that corresponds to the published sequence of the α chain²³ was ligated into a p-Blue Script plasmid (Promega, Charbonnières, France). Phosphorus 33-labeled α -chain DNA probe was obtained by Klenow fragment transcription of the *EcoRI-Xho* fragment (Life Technologies) of Fc ϵ RI, a p-Blue Script plasmid. Hybridization was performed for 24 hours at 42°C with 2×10^6 cpm/mL labeled probe in prehybridization buffer. After washing, the blot was exposed to Kodak x-ray film (Amersham). Equivalent amounts of RNA were loaded in each lane as judged by 18S and 28S RNA spot intensity after staining by ethidium bromide.

Statistical analysis

Data from flow cytometry analysis were numbered as the difference between the percentage of positive cells or the median of fluorescence intensity (MFI) obtained either with the specific antibody or the isotype control. Results were expressed as mean $\pm$ SEM. Statistical analysis was performed by the use of the Wilcoxon test for paired data or the Mann Whitney *U* test for unpaired data. In some experiments the Kolmogorov-Smirnov test was also used to compare histograms of immunofluorescence analysis.

RESULTS

Expression of Fc ϵ RI and CD23 on blood monocytes from NASs and AAPs

Monocytes were specifically analyzed as CD14-positive cells, which allowed avoidance of contamination with basophils and dendritic cells. The percentage, as well as the MFI, of Fc ϵ RI α -chain-positive BMs were significantly increased in AAPs compared with NASs (Fig 1). Nevertheless, the expression of Fc ϵ RI was relatively low in BMs in comparison with dendritic cells (lin-1⁻, CD11c⁺, HLA-DR^{high}; 47% $\pm$ 9.6% [MFI, 14.2 $\pm$ 6]) and, mainly, with basophils (lin-1⁻, CD123⁺, HLA-DR^{low}; 90.7% $\pm$ 1.2% [MFI, 183 $\pm$ 37.5]; $P < .05$), as previously reported in allergic patients. The percentage of CD23-positive cells was higher in AAPs compared with NASs ($P < .05$), as previously demonstrated,²⁴ whereas the MFI was similar (data not shown).

The effect of GC therapy was evaluated on Fc ϵ RI α -chain expression by BMs from AAPs. Steroid-treated patients exhibited a lower expression of Fc ϵ RI α -chain expression than untreated patients ($P < .05$), which was similar to that of healthy subjects (Fig 1).

Effect of IL-4, IL-13, and GM-CSF on Fc ϵ RI α -chain and CD23 expression on BMs

Addition for 24 hours of IL-4 at 200 and 50 U/mL but not at lower concentrations significantly increased Fc ϵ RI α -chain expression on BMs from NASs and AAPs compared with cells in medium alone (Fig 2). Preincubation of IL-4 with neutralizing anti-IL-4 antibodies inhibited 90% of the effect obtained with IL-4 preincubated with an isotype control antibody. Fig 3 summarizes the data of CD23 and Fc ϵ RI α -chain expression in BMs from all NASs and AAPs incubated

or not with IL-4. In medium alone the expression of IgE receptors was similar to data obtained at day 0 (Fig 1), except for a decrease of MFI for Fc ϵ RI α -chain expression in BMs from AAPs ($P =$ not significant). Nevertheless, the percentage of Fc ϵ RI α -chain-positive BMs from AAPs was still higher compared with that of healthy subjects ($P < .05$). With IL-4 (200 U/mL), the response of BMs from AAPs was significantly higher for Fc ϵ RI α -chain expression compared with BMs from NASs, whereas concerning CD23, only the percentage of positive cells was significantly increased in AAPs. With BMs from NASs ($n = 5$), IL-13 also induced a significant increase of Fc ϵ RI α -chain expression (22.2% $\pm$ 11% [MFI, 1.2 $\pm$ 0.7]) compared with medium alone (8% $\pm$ 3.6% [MFI, 0.4 $\pm$ 0.2]). In contrast, GM-CSF alone had no significant effect on Fc ϵ RI α -chain expression on BMs from NASs ($n = 8$, Fig 4) and from 3 AAPs (data not shown). Moreover, costimulation with GM-CSF and IL-4 did not modulate the effect of IL-4 on Fc ϵ RI α ($P =$ not significant).

The effect of GM-CSF and IL-4 on mRNA expression was evaluated in BMs at day 1 by using Northern blot analysis showing that IL-4 increased Fc ϵ RI α -chain mRNA expression, whereas GM-CSF alone or with IL-4 had no effect (Fig 5, A). By using RT-PCR ($n = 10$), IL-4 (α chain/GAPDH ratio, 3.9 $\pm$ 1.4) and IL-13 (3.3 $\pm$ 1.3) significantly increased mRNA expression of Fc ϵ RI α chain in comparison with medium alone (1.2 $\pm$ 0.2), whereas GM-CSF had no effect (1.3 $\pm$ 0.4; Fig 5, B). β -Chain mRNA was always undetectable in BMs (data not shown), and γ -chain mRNA was not modulated by IL-4, GM-CSF, or both compared with medium alone (Fig 5, B).

Effect of MP on IL-4-induced Fc ϵ RI α -chain expression on BMs from NASs

In vitro, the effect of MP (10^{-6} mol/L) was evaluated after addition of IL-4 either simultaneously or after a 1-hour preincubation ($n = 3$). As for CD23 (data not shown), the IL-4-induced expression of Fc ϵ RI α chain significantly decreased in the presence of MP compared with IL-4 alone: at the level of positive cells, the percentages of inhibition were, for simultaneous addition and 1-hour preincubation, 71% and 64% for Fc ϵ RI α chain, respectively, and for MFI, they were 79% and 65% for Fc ϵ RI α chain, respectively. In a second set of experiments (Fig 6), 1-hour preincubation of BMs with 10^{-6} and 10^{-8} mol/L MP had no significant effect on spontaneous Fc ϵ RI α -chain expression. Whereas the effect of 10^{-8} mol/L MP on IL-4-stimulated BMs did not reach statistical significance (5% and 20% of inhibition), MP at 10^{-6} mol/L significantly decreased the percentage and the MFI of Fc ϵ RI α chain (41% and 52% of inhibition).

Similarly, Fc ϵ RI α -chain mRNA expression obtained in IL-4-stimulated BMs (α chain/GAPDH ratio, 4.3 $\pm$ 0.8) was downregulated by preincubation with 10^{-6} mol/L MP (1.3 $\pm$ 0.7), whereas the γ -chain mRNA expression was not significantly modified (6.8 $\pm$ 1.9 and 7.8 $\pm$ 2, respectively; $n = 4$).

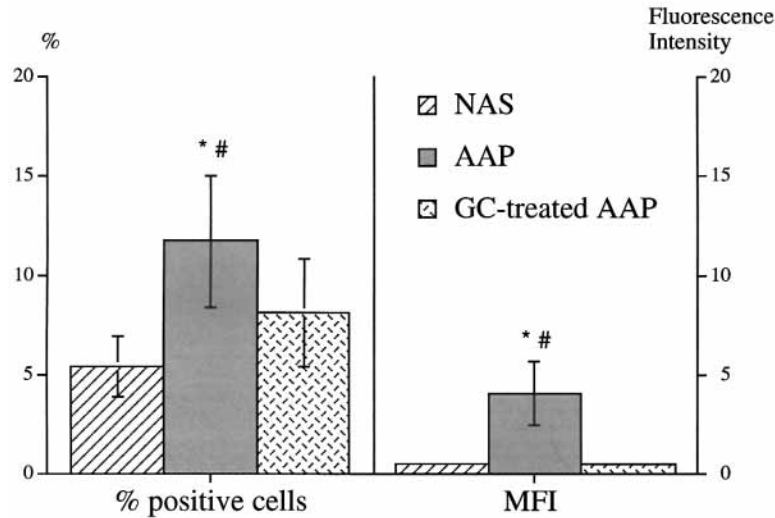


FIG 1. FcεRI α-chain expression evaluated by using immunofluorescence on freshly purified BMs from NASs (n = 11), AAPs (n = 12), and GC-treated AAPs (n = 7). These data are expressed as the difference between anti-FcεRI α-chain and IgG1 isotype control antibodies (mean ± SEM) of the percentage of positive cells and of variation in MFI between cells stained. **P* < .05 versus BMs from NASs; #*P* < .05 compared with BMs from AAPs treated with GCs.

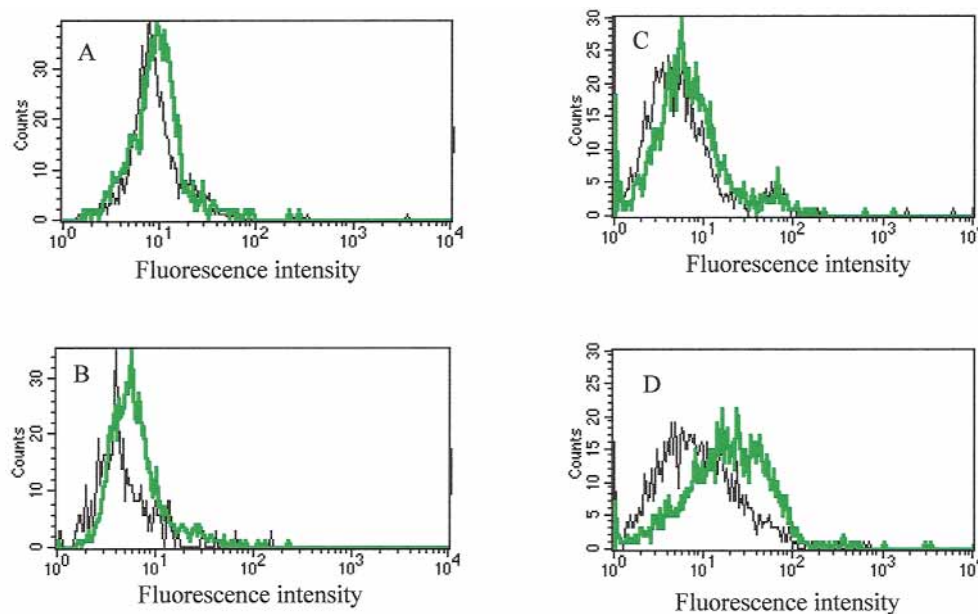


FIG 2. FcεRI α-chain expression evaluated by using immunofluorescence on BMs from an NAS (A and B) or from an AAP (C and D) incubated for 24 hours with medium alone (A and C) or with 200 U/mL IL-4 (B and D) and analyzed with flow cytometry. BMs were stained with anti-FcεRI α-chain antibodies (green line) or with mouse IgG1 isotype control antibodies (black line) and then with Alexa 488-conjugated anti-mouse IgG antibodies. Statistical analysis with the Kolmogorov-Smirnov test showed that IL-4 significantly increased FcεRI α-chain expression on BMs in both cases.

Involvement of tyrosine kinases in the effects of IL-4 on CD23 and FcεRI α-chain expression on BMs

IL-4-stimulated BMs from NASs were incubated in the presence of 5 and 25 μmol/L genistein for 24 hours (n = 5). Genistein at the concentration of 5 μmol/L had a weak inhibitory effect on IL-4-induced upregulation

of CD23 (20% inhibition, *P* < .05) and FcεRI α-chain (21% inhibition, *P* = not significant) expression. In contrast, a concentration of 25 μmol/L had a strong inhibitory effect on the expression of both receptors (76% and 70% inhibition, respectively). In parallel, the expression of CD23 and FcεRI α-chain mRNA was also inhibited by genistein at 25 μmol/L (79.8% and 95.5% inhibition).

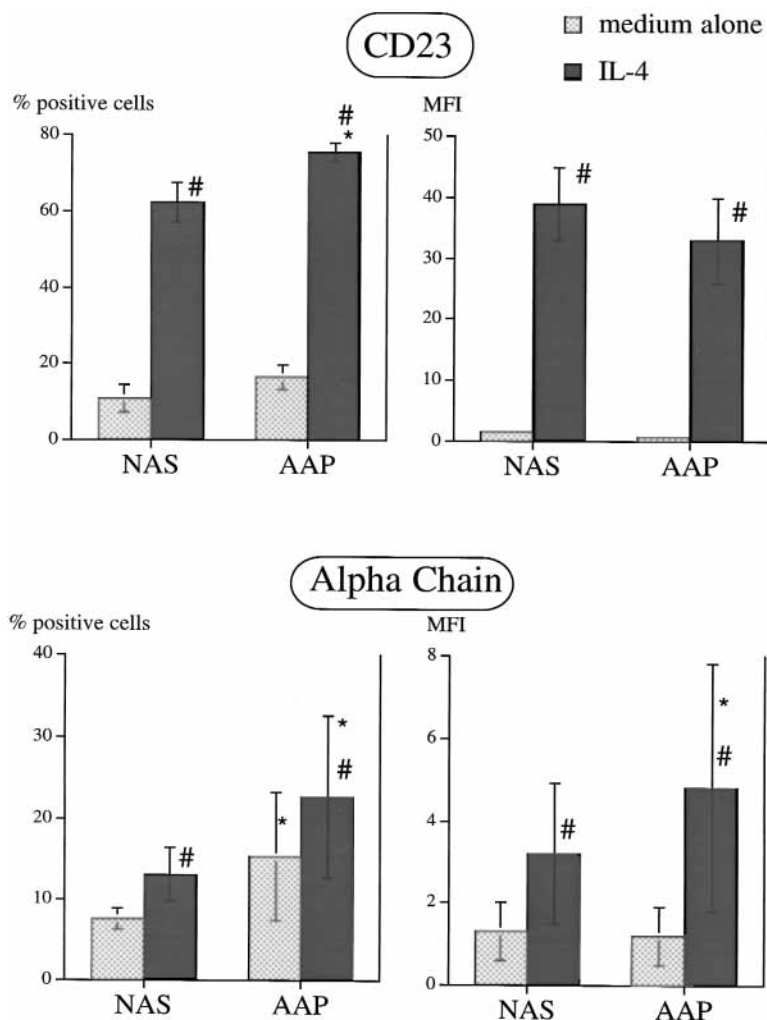


FIG 3. CD23 (upper) and FcεRI α-chain (lower) expression evaluated by using immunofluorescence on BMs from NASs (n = 11) and AAPs (n = 12) incubated for 24 hours with medium alone or with 200 U/mL IL-4. These results are expressed as the mean ± SEM of percentage of positive cells (left) and of variation in MFI (right) between cells stained with FcεRI α-chain antibodies and with IgG1 isotype control antibodies. #P < .05 versus BMs with medium alone; *P < .05 compared with BMs from NASs incubated with medium alone or with IL-4, respectively.

DISCUSSION

IgE-dependent stimulation induces the activation of monocytes-macrophages, leading to the secretion of several mediators and to exocytosis. Although these 2 phenomena seem to implicate mainly CD23,^{9-11,25-26} the involvement of FcεRI has been demonstrated by the measurement of Ca²⁺ influx,⁴ PGE2 production, and IgE-mediated antigen capture.^{6,7} Moreover, the expression of FcεRI is increased in allergic patients, as confirmed in this study in a group of AAPs. To elucidate the mechanisms leading to this enhanced expression in allergic patients, we analyzed the modulation of FcεRI expression in BMs by well-known cytokines and drugs involved in the pathogenesis and treatment of allergic asthma, respectively. Our results show that (1) IL-4, but not GM-CSF, upregulates FcεRI α-chain protein and

mRNA expression and (2) that GCs downregulate its expression.

The increased expression of FcεRI α chain induced by IL-4 seems to be quite specific because other cytokines, such as IL-3, IFN-γ, TNF-α, and IL-1β alone had no effect (data not shown). Although the action of IL-4 could seem of weak intensity by comparison to the modulation of CD23, it was statistically significant, and the level of enhancement induced by this cytokine when BMs from healthy subjects were used corresponded to the difference in FcεRI expression between BMs from patients with allergic asthma and those from healthy subjects. Moreover, IL-13, which has the ability to bind to the same receptor, also upregulated FcεRI α-chain expression with a similar intensity. The effect of IL-4 on FcεRI α-chain expression seemed more noteworthy in

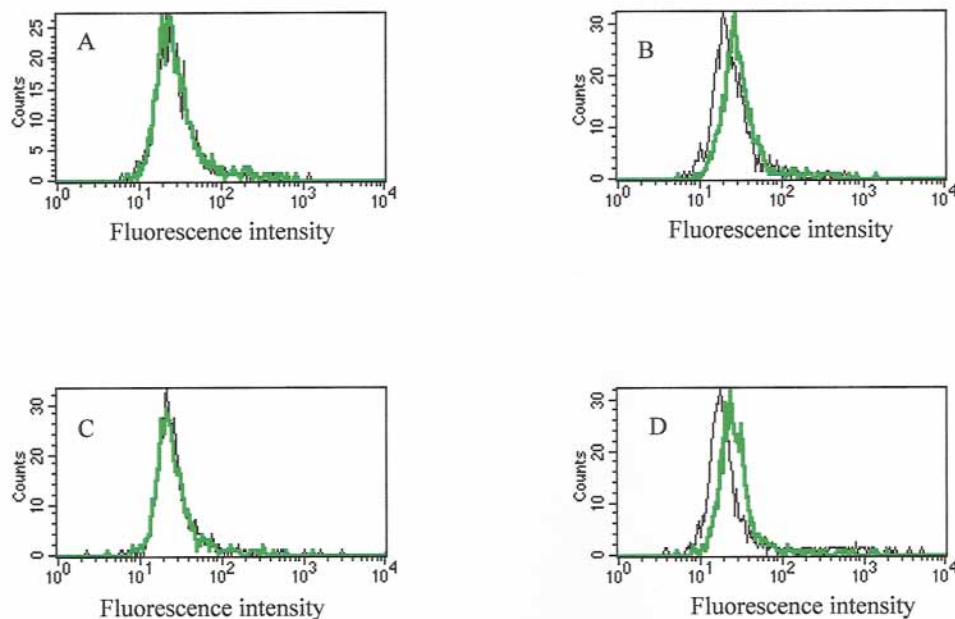


FIG 4. FcεRI α -chain expression evaluated by using immunofluorescence on BMs from an NAS incubated for 24 hours with medium alone (**A**), with 200 U/mL IL-4 (**B**), with GM-CSF (**C**), and with GM-CSF plus IL-4 (**D**) and analyzed by using flow cytometry. BMs were stained with anti-FcεRI α -chain antibodies (green line) or with mouse IgG1 isotype control antibodies (black line) and then with Alexa 488-conjugated anti-mouse IgG antibodies. Statistical analysis with the Kolmogorov-Smirnov test showed that IL-4 and GM-CSF plus IL-4 significantly increased FcεRI α -chain expression in BMs compared with medium alone; however, the levels of increase were similar between both conditions.

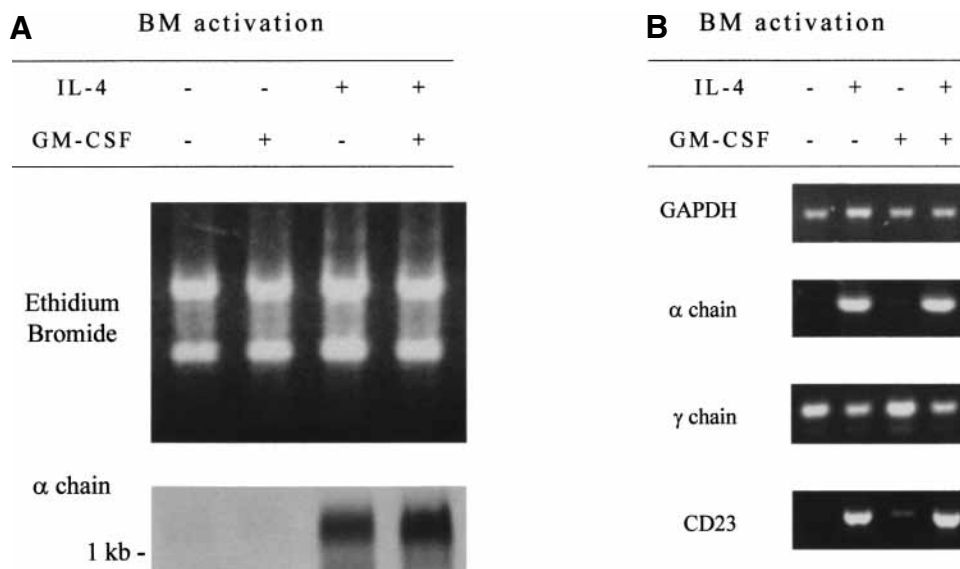


FIG 5. A, Northern blot analysis of mRNA expression for FcεRI α chain in BMs incubated with medium alone, GM-CSF, IL-4, and IL-4 plus GM-CSF. Specific hybridization signal was detected at 1.1 Kb. Similar quantities of RNA were loaded in each band, as shown with ethidium bromide staining. This is a representative experiment of 3 experiments. **B,** mRNA expression for GAPDH, FcεRI α and γ chains, and CD23 evaluated by RT-PCR in BMs from an NAS. BMs were incubated for 24 hours with medium alone, 200 U/mL IL-4, GM-CSF (25 ng/mL), and IL-4 plus GM-CSF. This is a representative experiment of 6 experiments.

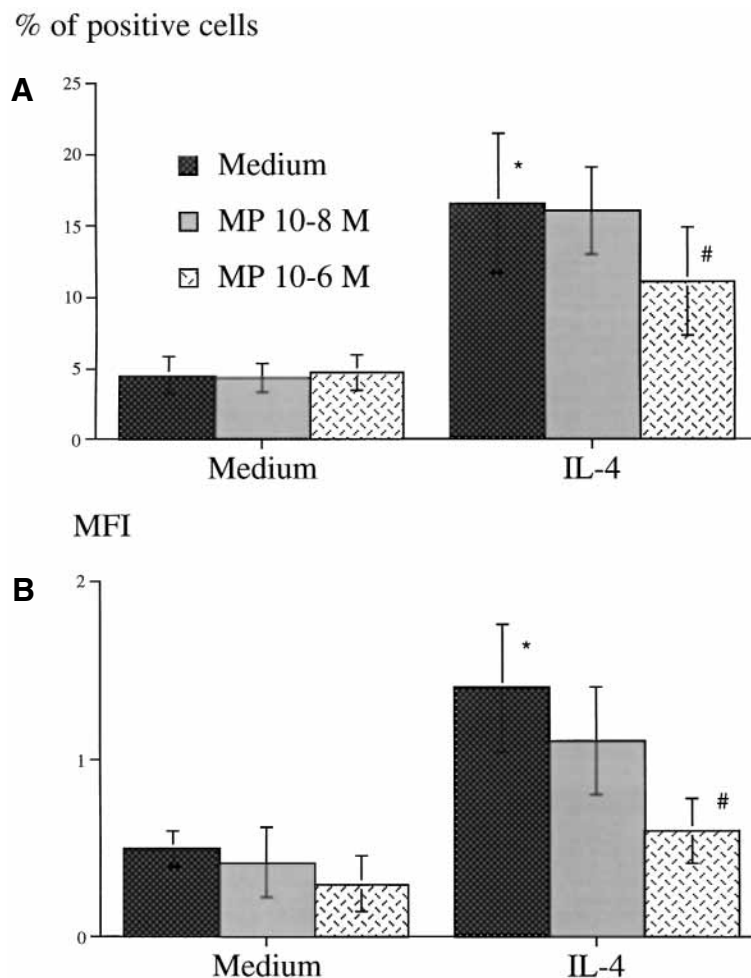


FIG 6. FcεRI α-chain expression evaluated by using immunofluorescence on BMs from NASs ($n = 7$) preincubated for 1 hour with medium alone and with 10^{-8} and 10^{-6} mol/L MP and then activated or not with 200 U/mL IL-4. These data represent the mean $\pm$ SEM of percentage of positive cells (**A**) and of variation in MFI (**B**) between cells stained with FcεRI α-chain antibodies and IgG1 isotype control antibodies. * $P < .05$ versus BMs in medium alone; # $P < .05$ compared with BMs incubated with IL-4 alone.

AAPs than in NASs. This might have been the consequence of an increased expression of IL-4 receptor (IL-4R) on BMs. We have therefore evaluated the expression of the α chain of IL-4R in our study population, but no difference was observed (data not shown). On the other hand, an increased production of IL-4, IL-13, or both in patients with allergic diseases has been widely reported.²¹ These data suggest that the enhancement of FcεRI α chain on BMs may be, at least in part, caused by an increased production of IL-4 in allergic patients.

Our results with cytokines and the tyrosine kinase inhibitor genistein suggest that CD23 and FcεRI α-chain expression can be regulated by linked mechanisms. Ligand binding on IL-4R induces the activation of tyrosine kinases, such as Janus tyrosine kinases, that can trigger the nuclear translocation of signal transducers and activators of transcription (STAT) 6 and, in this way, enhance transcription of several genes, including the gene encoding CD23.²⁷ However, no specific enhancer element for

STAT-6 has yet been identified in the gene encoding the FcεRI α chain. Other activation pathways can be involved: IL-4 induction of CD23 in BMs is dependent on p38 mitogen-activated protein kinase,²⁸ suggesting that the effect of genistein should implicate tyrosine kinases as targets other than the Janus tyrosine kinase-STAT pathway. Moreover, Nishiyama et al²⁹ showed that the transcription factors Elf-1 and GATA-1 enhanced transcription of the gene encoding FcεRI α chain in mast cells. There is evidence showing that Elf-1 promotes the initiation of macrophage-specific basal transcription³⁰ and that GATA-1 is involved in the development and maturation of myelomonocytic cells.³¹ Finally, our results demonstrate that IL-4 induced FcεRI α-chain expression in BMs in a transcriptional manner, mostly involving tyrosine kinase activity.

Previous reports showed that GM-CSF and IL-4 had a synergistic activity on CD23 expression by BMs.³² In contrast, we showed in this study that GM-CSF had no

effect on FcεRI α-chain expression. When coincubated with IL-4, no significant increase of FcεRI α-chain expression was observed after a 1-day incubation compared with IL-4 alone. All these data show that CD23 and FcεRI α-chain gene transcription are differentially regulated in response to IL-4 and GM-CSF.

The other important result obtained in this study concerns the *in vitro* and *in vivo* GC-induced inhibition of the FcεRI α-chain expression on BMs. This *in vitro* effect of GCs was observed at high concentrations and appeared to affect at least the IL-4-dependent activation, as previously shown for CD23.³³ Indeed, the IL-4-dependent increase of CD23 and FcεRI α-chain expression was reduced, whereas the spontaneous α-chain and γ-chain expression were not modulated. GCs directly interact with different transcription factors, including activating protein 1, nuclear factor κB,³⁴ GATA, and Ets.³⁵ This and the data reported by Nishiyama et al²⁹ suggest that these transcription factors may be the target of GCs for the modulation of the FcεRI α chain. An *in vivo* effect of GCs was also demonstrated in asthmatic patients. In additional experiments FcεRI α-chain expression was analyzed in 2 AAPs during an acute asthma attack treated with a bolus of GCs. A rapid decrease of CD23 and FcεRIα expression was observed in both patients, with a maximal activity at day 1, which returned to baseline 30 days later. This *in vivo* effect of GCs could be direct on monocytes, as shown in our *in vitro* experiments, or could implicate other targets. Indeed, GCs inhibit IL-4 production by T lymphocytes³⁶ and IL-4R α-chain expression in mononuclear cells³⁷ and therefore can block, by other mechanisms, the *in vivo* IL-4 induction of FcεRI α-chain expression.

In conclusion, we have demonstrated that the FcεRI α-chain expression on BMs is differentially regulated first by IL-4 and IL-13, which have enhancing effects, and second by GCs, which inhibit it. Additional experiments will be necessary to identify the transduction pathways regulating both activities and their involvement in the physiopathology of allergic diseases.

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