

CD203c expression on human basophils is associated with asthma exacerbation

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Background: CD203c is a basophil cell surface marker used to diagnose and monitor various allergic diseases, but its relationship to asthma is not clear.

Objective: We determined whether CD203c expression levels are associated with stable and exacerbated asthma.

Methods: We used flow cytometry to compare spontaneous expression levels of surface markers on basophils from patients with stable or exacerbated asthma and from healthy subjects. Longitudinal changes in these expression levels were measured after basophil stimulation by IgE-dependent or IgE-independent mechanisms and compared with patients' asthma status.

Results: Spontaneous expression levels of CD203c were significantly higher on basophils from patients with asthma exacerbation than patients with stable asthma or healthy subjects.

In contrast, no differences in spontaneous expression levels of CD63 or CD69 were observed among the 3 groups. Anti-IgE-induced expression of CD203c significantly increased in basophils during asthma exacerbation ($P = .005$). Low concentrations of *Dermatophagoides pteronyssinus* or IL-3 induced higher expression levels of CD203c during asthma exacerbation than during clinical improvement; induction of CD203c expression by these antigens therefore correlates with asthma control. In the patients with clinical improvement, there was a correlation between spontaneous CD203c expression levels and the percent predicted values of FEV₁ ($r = -0.761$; $P = .022$).

Conclusion: Asthma exacerbation was accompanied by increased expression of CD203c on basophils that decreased significantly during remission. Basophil expression levels of CD203c might therefore be used to monitor asthma in patients. (J Allergy Clin Immunol 2010;125:483-9.)

Key words: Airway inflammation, asthma exacerbation, basophil activation, basophil surface marker, biomarker, CD203c

Airway inflammation is an important feature of bronchial asthma. Various kinds of cells and their mediators are involved in

Abbreviations used

ENO: Exhaled nitric oxide

MFI: Mean fluorescence intensity

PEF: Peak expiratory flow

PGD2: Prostaglandin D2

15R-MePGD2: 15(R)-methyl prostaglandin D2

the pathogenesis of asthma. Mast cells and basophils can have an important role in the development of allergic inflammation through IgE-initiated and chemokine-initiated mediator release, although eosinophils are the effector cells most often associated with airway inflammation in people with asthma. Basophils secrete 3 key classes of inflammatory mediators in allergic diseases: vasoactive amines, lipid metabolites, and cytokines. It has been reported that the number of basophils in the airways is higher in patients with asthma than in normal subjects¹ and that this number increases during asthma exacerbation.² It was also found that human basophils produce high levels of IL-4 in the lung after segmental allergen challenge.³ Oosaki et al⁴ reported that in patients with spontaneous asthma attacks, urinary excretions of eosinophil protein X, leukotriene E4, and 11-dehydrothromboxane B2 significantly increase during the attack and return to control levels in the improved phase. In addition, Nakano et al⁵ showed that patients admitted to a hospital because of non-resolving asthma exacerbation had higher plasma levels of C3a, a potent proinflammatory mediator, than patients discharged immediately after the exacerbation or patients with stable asthma. However, the changes in each mediator and the relationship among mediators during asthma exacerbations have not been well documented. It is important to improve our understanding of the mechanisms of asthma exacerbation and develop more effective, specific therapies.

Various surface markers expressed on human basophils, such as CD63, CD69, and CD203c, have been used to demonstrate the role of basophils *in vivo* and *in vitro*.^{6,7} CD203c is considered to be the most useful marker of basophil activation and differentiation. The functional role of CD203c in basophil differentiation and maturation is not clear. Upregulation of CD203c on the basophil cell surface is the result of an early FcεRI-mediated event and has a role in late, cytokine-mediated activation of basophils.⁸ Because of the increased cell surface levels of CD203c in response to IgE-dependent stimuli, this protein is also a promising marker of activation that can be used in flow cytometry-based diagnosis of allergy. Flow cytometry-based basophil tests have been used to diagnose anaphylaxis,⁷ chronic urticaria,⁹ and other allergic diseases, as well as to confirm the sensitization of patients to food, drugs, or insects.¹⁰ These tests appear therefore to be

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suitable for detecting immediate-type hypersensitivity and to monitor the success of hyposensitization protocols in patients with allergy.

On the basis of these studies, we propose that CD203c expression on basophils is involved in the pathogenesis of asthma exacerbation. However, changes in the CD203c expression levels on basophils have not been examined in patients with asthma. We investigated the relationship between CD203c expression and the pathological condition of asthma. We determined whether the CD203c expression on basophils increases in patients with asthma exacerbation and returns to control levels after improvement with treatment. In addition, we examined the extent of change in CD203c expression levels after *in vitro* stimulation via IgE-dependent and IgE-independent mechanisms.

METHODS

Subjects

We conducted a hospital-based, prospective study from August to December 2008 in 28 patients with asthma exacerbation. They were recruited from the emergency or outpatient department of the Pulmonology-Allergy Department at Sagami National Hospital. The diagnoses of asthma and asthma exacerbation were based on clinical history, symptoms, examination findings, and pulmonary function parameters, in accordance with international guidelines.¹¹ Inclusion criteria were an asthma history of at least 12 months, peak expiratory flow (PEF) of <60% of the normal predicted value, no smoking history, and meeting criteria for discharge after 120 minutes of therapy. Patients were excluded from this study if they had chronic obstructive pulmonary disease, definite bacterial airway infection, pneumonia, or autoimmune disease; needed immediate resuscitation or airway intervention; or had received systemic corticosteroids or allergen immunotherapy in the preceding 4 weeks. Nonresponders (basophils did not release histamine in response to anti-IgE or failed to upregulate CD203c) were also excluded. Sixteen patients with stable asthma without exacerbation in the preceding 3 months and 11 healthy control subjects were enrolled for comparative analyses. Sample size was calculated assuming a power of 0.8. Permission to conduct this study was obtained from the Ethics Committee of the Sagami National Hospital; all the participants gave their informed consent.

Study design

At the time of asthma exacerbation, all patients were given 2.5 mg salbutamol (Ventolin; GlaxoSmithKline, Tokyo, Japan) to inhale through a nebulizer and 125 mg methylprednisolone (Solu-medrol; Pfizer, Tokyo, Japan) intravenously. Subsequently, patients were given a single, daily, oral dose of 20 mg methylprednisolone (prednisolone; Shionogi, Osaka, Japan) for 5 days. All other asthma therapies were unchanged for each patient during the study. PEF was measured at baseline, after inhalation of the bronchodilator, 120 minutes after therapy, and on clinical improvement. Predicted values for PEF were based on data reported by Tsukioka.¹² Blood samples for flow cytometry were collected as soon as possible before systemic corticosteroid therapy.

Four weeks after asthma exacerbation it was confirmed that the patients met the definition of clinical improvement—that they no longer had symptoms, did not need reliever therapy, and had PEF of approximately 80% of the predicted values. All the patients were evaluated by chest radiograph and spirometry analyses, and exhaled nitric oxide (ENO) and blood samples were collected. Spirometry, including FEV₁, was carried out by using Minato Autospiro AS-303 (Osaka, Japan) in accordance with the American Thoracic Society criteria.¹³ ENO was collected and measured in accordance with the recommendation of American Thoracic Society/European Respiratory Society (Sievers Instrument, Boulder, Colo).¹⁴

Reagents

Reagents used in the study included the allergenicity kit for quantification of basophil CD203c expression (Beckman Coulter, Fullerton, Calif);

phycoerythrin-cyanine 7-conjugated anti-CD3 antibody; phycoerythrin-conjugated antibodies against CD63, CD69, and CD203c (Beckman Coulter); fluorescein isothiocyanate-conjugated anti-CRTH2 (Santa Cruz Biotechnology, Santa Cruz, Calif); recombinant human IL-3 (R&D Systems, Minneapolis, Minn); and 15(R)-15-methyl prostaglandin D2 (15R-MePGD2; Cayman Chemical, Ann Arbor, Mich). *Dermatophagoides pteronyssinus* 1 (Der p 1) was supplied by H. Yasueda at Sagami National Hospital, Sagami, Japan.¹⁵

Measurement of CD203c surface expression by flow cytometry

Boumiza et al¹⁰ developed a 3-color flow-cytometric protocol to monitor basophil activation on the basis of identification of cells that are CD3⁺, CRTH2⁺, and CD203c⁺; it was used to distinguish basophils from other cells in whole-blood samples. Expression of CD203c on basophils was analyzed by flow cytometry using the allergenicity kit according to the manufacturer's instructions. Briefly, after EDTA-containing peripheral blood (7 mL) was centrifuged at 260g for 10 minutes, the cells isolated from the buffy coat were incubated with serial dilutions of Der p 1, IL-3, 15R-MePGD2, or mouse monoclonal antihuman IgE for 15 minutes at 37°C in the dark after addition of a calcium solution, to override the chelating capacity of EDTA. Phycoerythrin-cyanine 7-conjugated anti-CD3, fluorescein isothiocyanate-conjugated anti-CRTH2, and phycoerythrin-conjugated anti-CD203c antibodies were also added during the reaction. The reactions were stopped, and erythrocytes were removed by adding the lysis solution included in the kit. After incubation, the cells were washed once with 2 mL PBS and fixed in 0.5 mL 0.1% formaldehyde. Expressions of CD63 and CD69 were analyzed in a manner similar to that of CD203c.

Samples were analyzed by using a FACSCalibur with CellQuest software (Becton Dickinson, Franklin Lakes, NJ). Basophils were detected on the basis of forward side scatter characteristics, lack of CD3 expression, and expression of CRTH2 (see this article's Fig E1). In each assay, an isotype control was used for negative staining. Data was expressed as net mean fluorescence intensity (MFI) (actual MFI – MFI of isotype IgG control).

Histamine release assay

To evaluate the IgE-dependent reactivity of basophils *in vitro*, parallel blood samples were collected from patients, incubated with heparin, and analyzed by a histamine release test, as previously described.¹⁶

Statistical analysis

Data were analyzed by using SPSS for MS Windows, version 12.0 (SPSS, Inc, Chicago, Ill). Data are presented as median values with ranges or mean values ± SEMs. Comparisons of paired data were carried out by using the Wilcoxon signed-rank test for calculation of differences. In all the other calculations, an unpaired *t* test or the Mann-Whitney test was used. When more than 2 groups were compared, Kruskal-Wallis ANOVA followed by the Mann-Whitney test with Bonferroni correction or the Friedman repeated-measures ANOVA followed by the Wilcoxon *t* test with Bonferroni correction was carried out. Correlation was evaluated by the Spearman rank test. Differences were considered significant if *P* values were less than .05.

RESULTS

Patient characteristics

Background data from the subjects enrolled in this study are summarized in Table I. Among the 28 patients with asthma exacerbation, 4 were excluded: 2 had bacterial pneumonia, 1 had eosinophilic pneumonia, and 1 had prolonged asthma exacerbation after routine therapy. None were excluded based on failed upregulation of CD203c expression. In total, 24 patients with asthma exacerbation, 16 patients with stable asthma, and 11 healthy control

TABLE I. Clinical characteristics

	Healthy controls (n = 11)	Stable asthma (n = 16)	Asthma exacerbation (n = 24)
Age (y)	42 (21-60)	48 (25-72)	51 (19-65)
Male:female	5:6	5:11	11:13
Atopy:nonatopy	NA	6:10	14:10
Duration of asthma (y)	NA	20 (2-42)	18 (1-37)
Inhaled steroid dose (µg/d)	NA	800 (400-2000)	800 (200-2000)
Total serum IgE (IU/mL)	NA	655 (12-8261)	532 (8-3258)
Histamine PC ₂₀ (mg/mL)	NA	2.3 (0.1-5.8)	2.8 (0.2-7.5)

		Exacerbation	Improvement	
PEF		76*	48†	73*
(% predicted)	96 (78-127)	(61-98)	(28-58)	(58-91)
FEV ₁		72*		65*
(% predicted)	102 (81-138)	(60-121)	NA	(55-111)
		25.1**		38.1‡
Exhaled NO (ppb)	12.2 (8.1-18.3)	(12.3-55.2)	NA	(15.5-45.2)

NA, Not applicable; NO, nitric oxide.

Data are expressed as medians (ranges).

* $P < .05$.** $P < .01$ vs healthy controls.† $P < .001$ vs healthy controls, patients with stable asthma, and patients with clinical improvement.‡ $P < .05$ vs healthy controls and patients with stable asthma.

subjects were enrolled in this study. We measured FEV₁ and ENO only during the clinical improvement phase because of challenges in obtaining reliable measurements.

Relationship between asthma control and expression levels of CD63, CD69, and CD203c on basophils

We used 3-color flow cytometry to compare spontaneous *in vivo* expression levels of surface markers on basophils isolated from patients with asthma exacerbation, patients with stable asthma, and healthy control subjects. As shown in Fig 1, A, the level of spontaneous CD63 expression was slightly higher on basophils from patients with asthma exacerbation than from those with stable asthma or healthy subjects, although the differences were not statistically significant. In contrast, no differences in spontaneous CD69 expression level were observed among the 3 groups (Fig 1, B). On the other hand, basophils from patients with asthma exacerbation showed significantly higher levels of spontaneous CD203c expression than those from the patients with stable asthma ($P = .023$) or healthy subjects ($P = .018$; Fig 1, C).

To examine whether CD203c expression was induced after IgE receptor activation, leukocytes were incubated with 10 μ g/mL anti-IgE; this concentration was chosen because it induced maximum levels of CD203c expression in a preliminary study. The expression pattern of surface markers induced was similar to spontaneous expression patterns on unstimulated leukocytes. The level of anti-IgE-induced CD63 expression on leukocytes from patients with asthma exacerbation was significantly higher than on leukocytes from healthy subjects ($P = .007$; Fig 1, D). There were no significant differences in anti-IgE-induced CD69 expression levels among the 3 groups (Fig 1, E). On the other hand, the anti-IgE-induced CD203c expression level was significantly higher on

leukocytes from patients with asthma exacerbation ($P < .001$) and those with stable asthma ($P = .031$) than from healthy subjects (Fig 1, F). The anti-IgE-induced CD203c expression level was also significantly higher on leukocytes from patients with asthma exacerbation than from patients with stable asthma ($P = .015$). These observations suggest that the spontaneous and anti-IgE-induced expression levels of CD203c are superior to those of CD63 or CD69 as parameters for monitoring the pathophysiology of asthma.

Expression levels of CD63 and CD203c on basophils after *in vitro* stimulation with anti-IgE

Expression levels of anti-IgE-induced CD63 correlated with levels of CD203c on basophils from patients with asthma exacerbation ($r = 0.683$; $P = .035$); the correlation was observed even among basophils isolated from patients with atopic ($r = 0.762$; $P = .012$) and nonatopic ($r = 0.591$; $P = .039$) asthma. Similarly, in basophils from patients with clinical improvement, there was a correlation between anti-IgE-induced CD63 and CD203c expression levels ($r = 0.615$; $P = .0036$). However, in basophils from patients with asthma exacerbation or clinical improvement, there was no correlation between spontaneous CD63 and CD203c expression levels, suggesting that anti-IgE-induced CD203c expression correlates with asthma control.

Changes in CD203c expression levels on basophils from patients with asthma exacerbation after therapy

Spontaneous and anti-IgE-induced expression of CD203c. We measured the expression levels of basophil surface markers isolated from patients at different time points to associate longitudinal changes in expression levels with asthma status.

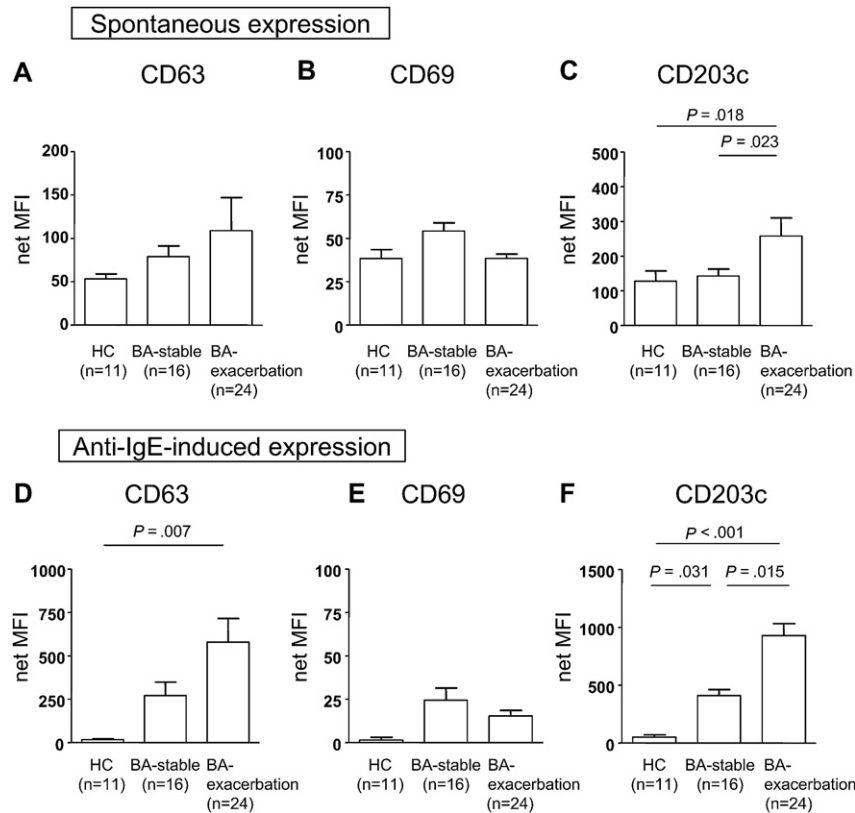


FIG 1. Relationship between asthma control and expression levels of CD63, CD69, and CD203c on basophils. Comparison of the expression levels of CD63, CD69, and CD203c on basophils from patients with asthma exacerbation (*BA-exacerbation*; $n = 24$), patients with stable asthma (*BA-stable*; $n = 16$), and healthy controls (*HC*; $n = 11$). The spontaneous and anti-IgE-induced expression levels are shown in 1 and 2, respectively. Data are expressed as net MFI, and error bars indicate SEMs.

Fig 2 shows the changes in the spontaneous and anti-IgE-induced CD203c expression levels, before and after therapy, in basophils from patients with asthma exacerbation. The spontaneous expression levels of CD63, CD69, and CD203c were not correlated with asthma condition. However, levels of anti-IgE-induced CD63 ($P = .016$) and CD203c ($P = .005$) decreased significantly with asthma improvement.

Patients with asthma exacerbation were classified as atopic or nonatopic; atopy was diagnosed on the basis of positive skin test results and the presence of IgE antibodies against mite allergens in serum. We examined CD203c expression levels on basophils from patients with atopic ($n = 14$) and nonatopic ($n = 10$) asthma after *in vitro* stimulation with IL-3 or 15R-MePGD2; we also examined Der p 1 levels in cells from patients with atopic asthma. Anti-IgE-induced CD203c expression levels were significantly higher on basophils from patients with exacerbated asthma that was atopic ($n = 14$) than nonatopic ($n = 10$; $P = .028$; see this article's Table E1 in the Online Repository at www.jacionline.org). Similarly, among patients with clinical improvement, anti-IgE-induced CD203c expression levels were significantly higher in atopic than in nonatopic patients with asthma ($P = .041$). However, there were no significant differences in spontaneous CD203c expression levels between atopic and nonatopic patients with asthma. We classified patients with asthma exacerbation into 2 groups, mild and severe, based on PEF 120 minutes after intensive therapy. PEF values were more than 80% and 50% to 80% of

the predicted values in patients with mild ($n = 13$) and severe asthma ($n = 11$), respectively. Basophils from patients with severe asthma had significantly higher levels of spontaneous CD203c expression than those from patients with mild asthma during asthma exacerbation ($P = .012$).

CD203c expression after *in vitro* stimulation with Der p 1, IL-3, or 15R-MePGD2. We examined CD203c expression levels on basophils from patients with asthma who were sensitized with Der p 1; cells were stimulated *in vitro* with various concentrations of Der p 1 ($n = 14$). Fig 3, A and B shows that the Der p 1-induced CD203c expression levels increased in a dose-dependent manner on cells from patients with asthma exacerbation and those with clinical improvement. In the patients with clinical improvement, the dose response curves for Der p 1-induced CD203c expression shifted to the right; maximum CD203c expression levels were significantly lower than those from patients with asthma exacerbation. Basophil sensitivity was defined as the minimum concentration required for a 50% increased expression level (EC_{50}) of CD203c in response to Der p 1 stimulation. Based on estimates from graphed data, the EC_{50} values were 0.1 ng/mL and 1 ng/mL for asthma exacerbation and clinical improvement, respectively; therefore, basophil sensitivity is approximately 10-fold lower in patients with clinical improvement. The EC_{50} values for IL-3 stimulation were 0.1 ng/mL and 1 ng/mL for asthma exacerbation and clinical improvement, respectively (Fig 3, C

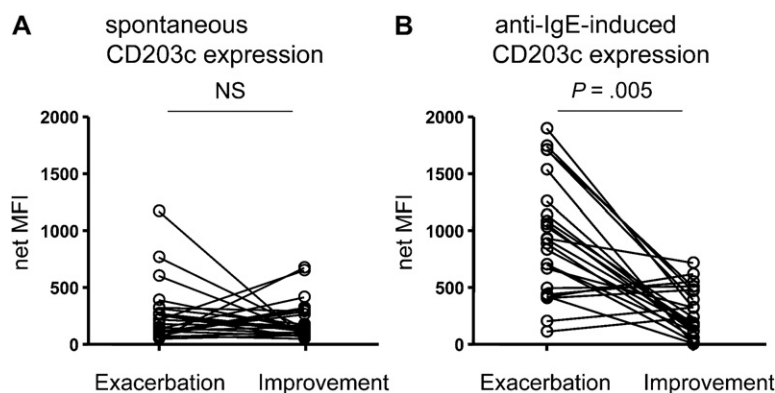


FIG 2. Changes in CD203c expression levels in on basophils from patients with asthma exacerbation after therapy. Changes in spontaneous (**A**) and anti-IgE-induced (**B**) expression levels of CD203c in the patients with asthma exacerbation, before and after therapy. NS, Not significant.

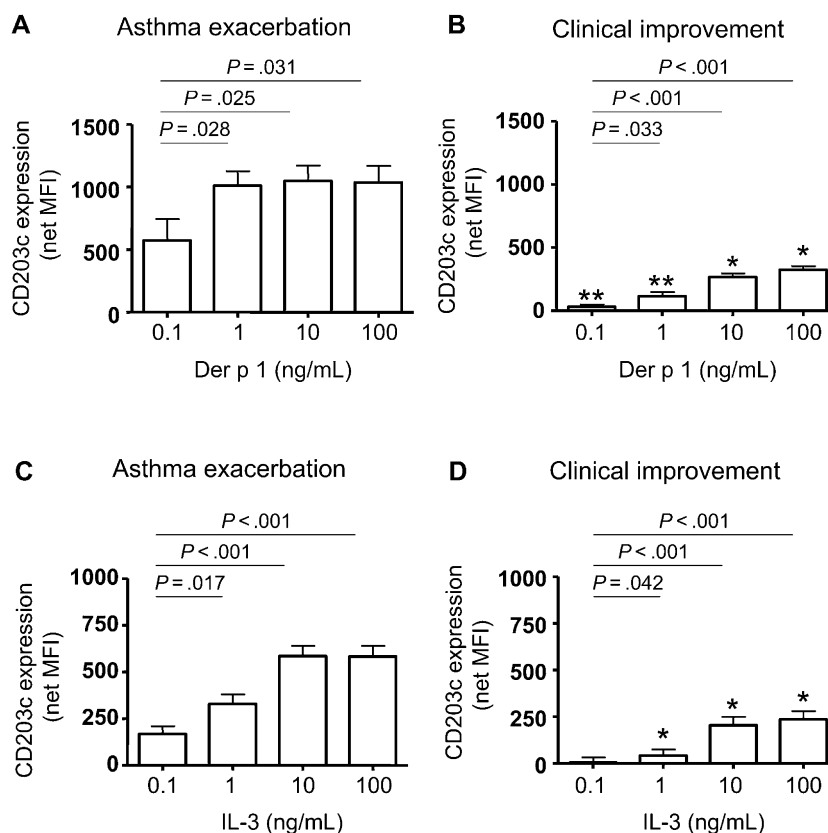


FIG 3. CD203c expression after *in vitro* stimulation of basophils with Der p 1 or IL-3. CD203c expression levels induced by *in vitro* stimulation of basophils (from patients with asthma exacerbation or clinical improvement) with various concentrations of Der p 1 (**1**, $n = 14$) or IL-3 (**2**, $n = 24$). * $P < .05$, ** $P < .001$ compared with the CD203c expression levels for the same concentrations in the patients with asthma exacerbation.

and *D*). Because CD203c expression levels increase after stimulation with IL-3 or 15R-MePGD2, we examined the effect of IL-3 or 15R-MePGD2 on CD203c expression in the patients with asthma exacerbation and those with clinical improvement ($n = 24$). The effect of IL-3 on CD203c expression was similar to that of Der p 1, although there were no significant differences in IL-3-induced CD203c expression levels between atopic and nonatopic patients with asthma (Fig 3-2). The patterns of

CD203c expression induced by Der p 1 or IL-3 were similar to those of anti-IgE-induced CD203c expression; the expression levels in the patients with stable asthma were also significantly lower than those in the patients with asthma exacerbation. However, no significant difference in induction of CD203c expression by 15R-MePGD2 was found between patients with asthma exacerbation and those with clinical improvement (see this article's Fig E2 in the Online Repository at www.jacionline.org).

Histamine release and CD203c expression after anti-IgE stimulation. When basophils were stimulated with anti-IgE, there was no correlation between histamine release and CD203c expression in patients with asthma exacerbation or in patients with clinical improvement. In the same patients, the maximum level of histamine release during asthma exacerbation was the same as during clinical improvement; maximum histamine release levels were correlated with asthma exacerbation and remission ($r = 0.795$; $P = .002$), suggesting that clinical condition does not affect anti-IgE-induced histamine release.

Relationship between CD203c expression levels on basophils and clinical parameters

In patients with asthma exacerbation, spontaneous expression levels of CD203c correlated with the PEF (% predicted) measured at baseline ($r = -0.513$; $P = .037$). In patients with clinical improvement, there was an inverse correlation between spontaneous CD203c expression level and FEV₁ (% predicted; $r = -0.761$; $P = .022$; Fig 4) but not between spontaneous CD203c expression and PEF measured on clinical improvement. There was no correlation between spontaneous CD203c expression levels and dose of inhaled corticosteroids, total level of IgE, RAST score, histamine PC₂₀, or arterial oxygen saturation in patients with asthma exacerbation or in patients with clinical improvement. Moreover, anti-IgE-induced CD203c expression levels were not correlated with any clinical parameters.

DISCUSSION

CD203c, a multifunctional ectoenzyme, belongs to the type II transmembrane protein family.⁷ Because the CD203c expression level rapidly increases on the basophil membrane after cross-linking of FcεRI, it is assumed that CD203c is stored intracellularly and transported to the cell surface after activation. The biological significance and potential functions of CD203c are unknown; it has not been established whether CD203c expression is an essential event or a secondary episode of basophil activation.

We measured spontaneous and induced expression levels of CD203c on basophils after *in vitro* stimulation to determine how inflammatory stimuli modulate the functions of basophils. Basophils were incubated with allergen, IL-3, or 15R-MePGD₂, which is an agonist for the prostaglandin D₂ (PGD₂) receptor subtype CRTH2. Various cytokines, including IL-3, IL-5, and GM-CSF, increase the level of histamine release from human basophils. The order of potency of histamine release is IL-3 > GM-CSF > IL-5,¹⁷ and human basophils express higher levels of the IL-3 receptor than IL-5 and GM-CSF receptors,¹⁸ so we used IL-3 to activate basophils. The CD203c expression level significantly increased from baseline, in a dose-dependent manner, after incubation with IL-3 in our study and others.^{8,10} However, the mechanisms of basophil IL-3 priming are unknown. Human mast cells generate a large amount of PGD₂ and a small amount of thromboxane A₂ through IgE-mediated stimulation.¹⁹ The effects of PGD₂ are mediated *in vivo* by 2 types of G-protein-coupled receptors for PGD₂, DP1, and CRTH2. CRTH2 has an important role in eosinophil recruitment into the airways and was shown to mediate PGD₂-induced eosinophilic airway inflammation.²⁰ 11-Dehydro-thromboxane B₂ is an agonist of CRTH2,²¹ so CRTH2 might be stimulated by PGD₂ as well as 11-dehydro-thromboxane B₂ *in vivo*. For this reason, we used a

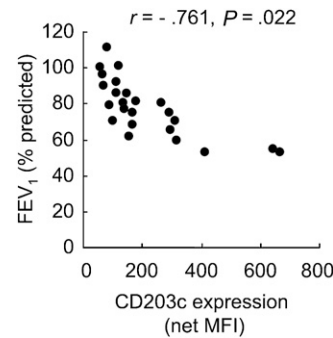


FIG 4. Relationship between expression levels of CD203c on basophils and FEV₁ in pulmonary function tests. In patients with clinical improvement, spontaneous CD203c expression levels correlated with the percent predicted values for FEV₁.

CRTH2 agonist, 15R-MePGD₂, to examine the effects of asthma exacerbation on CRTH2 receptors.

As shown in Fig 1, basophil spontaneous and anti-IgE-induced expression levels of CD203c are associated with asthma status and are better markers of disease severity than CD63 or CD69. The CD203c expression level also increased in a dose-dependent manner after stimulation with Der p 1 (which activates basophils by cross-linking of FcεRI), IL-3 (which has a priming effect to increase the level of histamine release from basophils), or 15R-MePGD₂ (which is an agonist of PGD₂ receptor subtype CRTH2). IL-3 is released mostly from activated T cells, and PGD₂ is a dominant prostaglandin generated by human mast cells.²² These results indicate that these mediators induce CD203c expression *in vivo* on basophils in the patients with asthma exacerbation.

There are several explanations for the decrease in CD203c expression level during clinical improvement of asthma. A recent study by Schroeder et al²³ demonstrated that human basophils themselves generate IL-3 and that an antibody against the IL-3 receptor partially suppresses IL-3 generation by basophils,²³ suggesting that IL-3 regulates basophil functions in an autocrine manner. In addition, they reported that CD69 expression on basophils is regulated indirectly by autocrine IL-3, suggesting that CD203c expression is also regulated by autocrine IL-3. Agonists to CRTH2 might also be released during asthma exacerbation. Thus, the decrease in the CD203c expression level after stimulation with agonists might result from the autologous desensitization of the receptors. However, the decrease in the CD203c expression level after the basophils were stimulated with anti-IgE or Der p 1 could preclude this possibility. IL-3 or 15R-MePGD₂ induced an increase in the CD203c expression level in a pattern similar to that observed with Der p 1, suggesting that the signaling pathways involved in the induction of CD203c expression are similar for IgE-dependent and IgE-independent stimuli. The signaling pathways that control CD203c expression might be affected by asthma control, although the regulatory mechanism remains to be elucidated. However, we cannot exclude the possibility that the decrease in CD203c expression during remission is associated with steroid therapy.

Steroid administration is likely to affect CD203c expression because of the following

1. Although Schleimer et al²⁴ reported that the inhibitory effect of dexamethasone on histamine release was abrogated by

incubation with IL-3, IL-3-induced CD203c expression levels decreased in patients with clinical improvement (Fig 3-2).

2. When we compared the extent of histamine release from basophils before and after steroid administration, the extent of anti-IgE-induced histamine release during exacerbation was not significantly different from that during remission ($72.2\% \pm 8.5\%$ vs $76.8\% \pm 6.1\%$; $n = 24$). However, there is little information on the effect of steroids on CD203c expression; further studies are required to clarify the relationship between CD203c expression and steroid administration.

Although the spontaneous CD203c expression did not predict asthma status, it is possible that with a larger sample size, a statistically significant decrease in spontaneous CD203c expression level would be observed during clinical improvement. During asthma exacerbation, patients with severe asthma showed significantly higher spontaneous expression levels of CD203c than patients with mild asthma. It is noteworthy that the degree of airflow limitation correlated with the spontaneous CD203c expression levels during both the exacerbation and clinical improvement phases. In addition, levels of exhaled NO were significantly higher in patients during clinical improvement than in those with stable asthma, suggesting that potential airway inflammation exists not only during asthma exacerbation but also during clinical improvement. The data on exhaled levels of NO support our results regarding CD203c expression in patients with asthma.

In conclusion, our study indicates that asthma exacerbation is accompanied by increased levels of CD203c that significantly decrease during remission and that spontaneous CD203c expression levels correlate with airflow limitation during asthma exacerbation. These observations suggest that basophils are activated by a number of factors during asthma exacerbation and that changes in CD203c expression levels might be monitored to determine asthma status.

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Clinical implications: Changes in expression levels of CD203c on basophils correlate with asthma status and might be used as biomarkers for disease monitoring.

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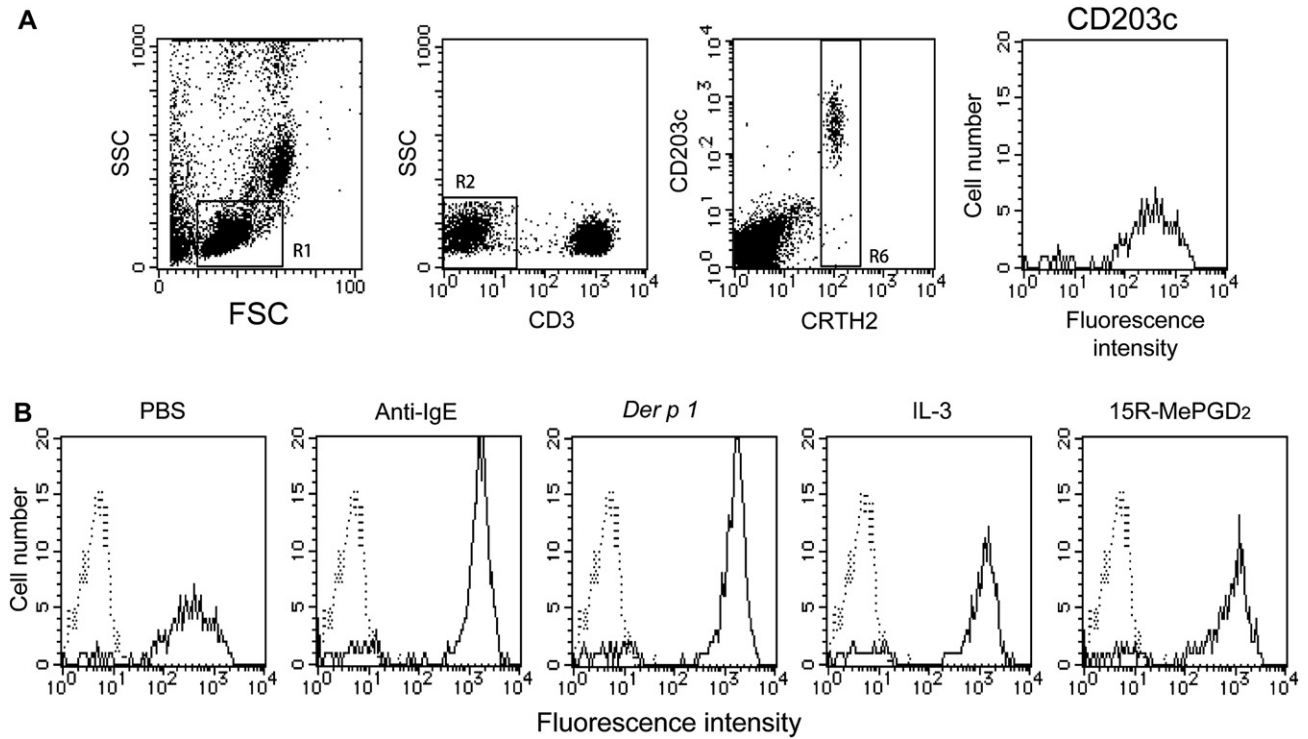


FIG E1. Example of optimal basophil gating in the CD203c protocol (**A**) and induced CD203c expression by stimulation with anti-IgE, Der p 1, IL-3, or 15R-MePGD2 (**B**). Basophils were detected on the basis of forward side scatter characteristics and negative expression of CD3 and positive expression of CRTH2. In each assay, isotype control was used for negative staining. Data were expressed as net MFI (actual MFI – MFI of isotype IgG control).

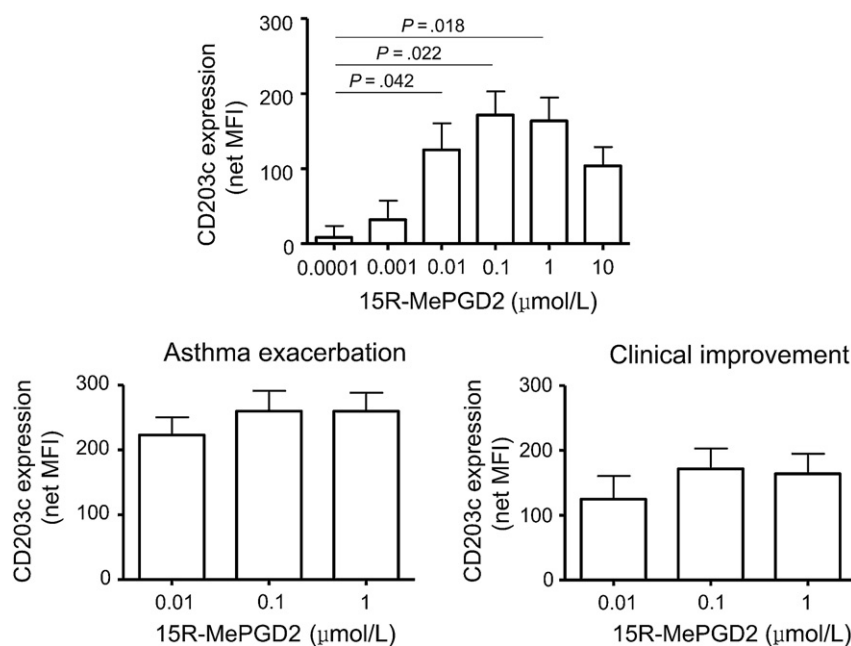


FIG E2. Induced CD203c expression by *in vitro* stimulation with 15R-MePGD2. No significant difference was found between the patients with asthma exacerbation and those with clinical improvement in the extent of CD203c expression induced by 15R-MePGD2. We examined the effect of 15R-MePGD2 on CD203c expression in both the atopic (n = 14) and nonatopic (n = 10) patients with asthma.

TABLE E1. CD203c expression levels and clinical state of patients with asthma

		Atopy (n = 14)	Nonatopy (n = 10)	Mild (n = 13)	Severe (n = 11)	Total (n = 24)
Spontaneous	Exacerbation	344 (55)	205 (88)	156 (46)*	302 (65)	258 (85)
	Improvement	218 (75)	189 (56)	213 (56)	195 (33)	210 (87)
Anti-IgE- induced	Exacerbation	1022 (188)**†	733 (161) ††	930 (232) ‡	982 (127) ‡‡	929 (130) ¶
	Improvement	352 (76) ***	126 (47)	280 (81)	213 (48)	276 (78)

Data are expressed as means (SEMs).

In spontaneous expression.

In anti-IgE-induced expression.

* $P = .012$ vs severe asthma group with exacerbation.

** $P = .028$ vs nonatopic asthma group with exacerbation.

*** $P = .041$ vs nonatopic asthma group with clinical improvement.

† $P = .002$ vs atopic asthma group with clinical improvement.

†† $P = .005$ vs nonatopic asthma group with clinical improvement.

‡ $P = .031$ vs mild asthma group with clinical improvement.

‡‡ $P = .023$ vs severe asthma group with clinical improvement.

¶ $P = .005$ vs all the patients with clinical improvement.

Boldface indicates basophils were detected on the basis of forward side scatter characteristics and negative expression of CD3 and positive expression of CRTH2.