Recombinant allergens promote expression of CD203c on basophils in sensitized individuals

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Mechanisms of allergy

Background: Traditionally, the diagnosis of type I allergies is based on clinical data, skin test results, and laboratory test results with allergen extracts. During the past few years, several attempts have been made to refine diagnostic assays in clinical allergy by introducing recombinant allergens and novel markers of IgE-dependent cell activation.
Objectives: We have identified the ectoenzyme CD203c as a novel basophil antigen that is upregulated on IgE receptor cross-linkage. In this study we applied CD203c and a panel of recombinant allergens to establish a novel basophil test that allows for a reliable quantification of IgE-dependent responses at the effector cell level.
Methods: Patients allergic to birch (Bet v 1, n = 15; Bet v 2, n = 8) and grass (Phl p 1, n = 15; Phl p 2, n = 10; Phl p 5, n = 14) pollen allergens, as well as 10 nonallergic donors, were examined. Basophils were exposed to various concentrations of recombinant allergens for 15 minutes and then examined for expression of CD203c by means of flow cytometry. CD203c upregulation was correlated with the increase in CD63.
Results: Exposure to recombinant allergens resulted in a dose-dependent increase in expression of CD203c on peripheral blood basophils in sensitized individuals, whereas no increase was seen in healthy control subjects. The effects of the recombinant allergens on CD203c expression were also time dependent. There was a good correlation between allergen-induced upregulation of CD203c and upregulation of CD63 (R = 0.76).
Conclusion: Flow cytometric quantitation of CD203c on blood basophils exposed to recombinant allergens is a useful approach to determine the allergic state in sensitized individuals and represents a basis for a sensitive novel allergy test. (J Allergy Clin Immunol 2002;110:102-9.)

Key words: *Basophils, allergy, recombinant allergens, diagnostic test, E-NPP3, CD203c*

Abbreviations used
CSA: Cyclosporin A
E-NPP3: Ectonucleotide pyrophosphatase/phosphodiesterase 3
MFI: Mean fluorescence intensity
MNC: Mononuclear cell

IgE-dependent allergies represent a significant medical problem in industrialized countries worldwide.[1,2] Notably, an increasing proportion (about 15%-30%) of the urban population has symptoms of IgE-mediated reactions to environmental allergens. Symptoms include rhinitis, conjunctivitis, asthma, and atopic dermatitis.[1,2] In many patients these symptoms require continuous medical care and treatment, and sometimes allergic reactions might be grave or even life threatening.[2]

Allergens are widely distributed throughout the environment. Recent progress has led to the characterization and cloning of various allergens.[3,4] Allergens are (glyco)proteins exposing multiple IgE-binding epitopes.[5] Sequence homologies of allergens in different species and cross-sensitization have been described.[3,4] Today, recombinant allergens serve as a useful tool for in vitro studies of anaphylactic reactions at the effector cell level.[5,6] Thus, we and others have shown that recombinant allergens are able to induce histamine release from basophils in allergic donors.[6] In contrast, allergen fragments that cannot bind IgE are unable to elicit mediator secretion.[7] The availability of recombinant allergens has also led to the development of specific (component resolved) diagnostic tests.[8] However, still many questions remain to be answered. Likewise, in individual patients striking differences are sometimes noted when biologic skin test results (prick test) are compared with serology test results.[9-12] This notion points to an important role of tissue-specific reactions and effector cell activation in the various clinical manifestations of allergy.

Thus far, only a few effector cell–based in vitro tests that might help in defining the allergic state in sensitized individuals have become available. The measurement of allergen-induced histamine release from the patients' blood basophils is a powerful approach.[6,13,14] However, histamine-release measurements are expensive and time consuming and therefore cannot be used as a widely

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TABLE I. Patients' characteristics

No.	Age (y)	Sex	Symptoms	Therapy	Allergen source	Skin prick test	Allergen-induced histamine release
Allergic patients							
1	32	F	RC, AA	AH	B, T	B+/T+	T+
2	26	M	RC, AA	β2, C	B, T	B+/T+	B+/T+
3	33	M	Conjunctivitis	None	B	B+/T–	B+
4	46	F	RC, AA	AH	B, T	B+/T+	NT
5	48	M	RC	None	B, T	B+/T+	B+/T+
6	26	F	RC	AH	T	B–/T+	T+
7	48	M	RC	AH	B, T	B+/T+	B+
8	27	M	RC, AA	AH	T	B–/T+	NT
9	25	M	RC, AA	AH, β2	B, T	B+/T+	B+/T+
10	25	M	RC	AH	B, T	B+/T+	B+/T+
11	36	F	RC	None	B, T	B+/T+	NT
12	31	M	RC	AH	B, T	B+/T+	NT
13	31	M	RC, AD	AH, TC	B, T	B+/T+	NT
14	42	M	RC, AA	β2, C, AH	B, T	B+/T+	NT
15	28	F	RC	None	B, T	B+/T+	B+/T+
16	52	F	RC, AA	None	B, T	B+/T+	NT
17	58	M	RC, AD	None	B, T	B+/T+	B+/T–
18	27	M	RC	None	NT	NT	NT
19	39	M	RC	None	B, T	B+/T+	B+/T+
20	30	F	RC, AD	AH	B, T	B+/T+	B–/T+
Nonallergic donors							
1	35	M	—	—	—	B–/T–	B–/T–
2	33	F	—	—	—	B–/T–	NT
3	38	M	—	—	—	B–/T–	B–/T–
4	27	M	—	—	—	B–/T–	NT
5	31	F	—	—	—	B–/T–	B–/T–
6	50	F	—	—	—	B–/T–	B–/T–
7	27	F	—	—	—	B–/T–	B–/T–
8	34	F	—	—	—	B–/T–	B–/T–
9	30	F	—	—	—	B–/T–	B–/T–
10	42	F	—	—	—	B–/T–	B–/T–

Skin prick tests were performed with allergen extracts, and histamine release experiments were performed with recombinant allergens.
RC, Rhinoconjunctivitis; *AA,* allergic asthma; *AH,* antihistamines; *B,* birch; *T,* timothy grass; β_2, β_2-mimetica; *C,* inhaled corticosteroids; *NT,* not tested; *TC,* topical steroids.

applied test. In recent years, the flow cytometric determination of CD63 and its allergen-induced upregulation on basophils in sensitized individuals has been introduced as a novel assay.[15-18] Thus, by using CD63 basophil responses can be measured in allergic patients. However, the CD63-based test (Baso-test) has several limitations. One limitation is that CD63 is not a basophil-specific marker. Therefore, this test requires the additional use of an IgE-dependent reagent for detection of IgE receptor cross-linked basophils.

We have recently identified the ectoenzym ectonucleotide pyrophosphatase/phosphodiesterase 3 (E-NPP3; CD203c) as a novel, basophil-specific activation antigen.[19,20] In particular, this antigen is expressed on blood basophils but not on other blood leukocytes and is upregulated in response to IgE receptor cross-linking similar to CD63.[19,21] In the present study, we have examined the effects of 5 different recombinant allergens on CD203c expression in blood basophils obtained from sensitized individuals. Our results suggest that allergen-induced upregulation of CD203c on basophils can be used as a powerful approach to establish an allergen-specific effector cell–based test for the diagnosis of grass and birch pollen allergies.

METHODS

Patients

Twenty patients (7 female and 13 male patients) allergic to one or more grass pollen or birch pollen allergens were examined. Patients were allergic to Bet v 1 (n = 15), Bet v 2 (n = 8), Phl p 1 (n = 15), Phl p 2 (n = 10), or Phl p 5 (n = 14). Allergy diagnosis was based on skin tests, specific IgE measurements (RAST), and clinical history. Levels of IgE were expressed as kilounits of antigen per liter. The patients' characteristics are shown in Table I. The control group consisted of 10 healthy nonallergic donors.

Recombinant allergens and other reagents

Recombinant allergens (rPhl p 1, rPhl p2, rPhl p 5, rBet v 1, and rBet v 2) were expressed in *Escherichia coli* and purified as previously described.[13,14,22,23] The FITC-labeled mAb CLB-gran12 (CD63), the anti-IgE mAb E-124-2-8 (Dε2), and the phycoerythrin-labeled mAb 97A6 (CD203c) were from Immunotech (Marseille, France).

Determination of specific IgE levels and skin test results

The levels of IgE antibodies specific for rBet v 1, rBet v 2, rPhl p 1, rPhl p 2, and rPhl p 5 were quantified by means of CAP fluoroenzyme immune assay (Pharmacia, Uppsala, Sweden). Skin prick tests were performed as previously described[24] by using commercially available allergen extracts (timothy grass pollen and birch pollen), histamine (positive control), and 0.9% sodium chloride solution (negative control) (all from Allergopharma, Reinbeck, Germany). Respective results are shown in Table I.

Basophil activation and flow cytometry

Heparinized peripheral blood was obtained from 20 allergic and 10 healthy subjects after informed consent was given. Blood aliquots (100 μL) were incubated with serial dilutions of recombinant allergens (10^{-7} to 10 μg/mL), anti-IgE antibody (1 μg/mL), or PBS for 15 minutes (37°C). In time-course experiments cells were incubated with allergens for 0.5 to 180 minutes. After incubation, cells were washed in PBS-EDTA and then incubated with 10 μL of phycoerythrin-labeled CD203c mAb 97A6 and 20 μL of FITC-labeled CD63 mAb CLB-gran 12 for 15 minutes at room temperature. Thereafter, samples were subjected to erythrocyte lysis with 2 mL of FACS Lysing Solution (Becton Dickinson, San Diego, Calif). Cells were then washed, resuspended in PBS, and analyzed by means of 2-color flow cytometry on a FACScan (Becton Dickinson). Basophils were detected on the basis of forward side-scatter characteristics and expression of CD203c and analyzed with Paint-a-gate (Becton Dickinson). Peripheral blood mononuclear cells (MNCs) of 2 allergic individuals were sorted as $CD203c^+$ and $CD203c^-$ cells by using a FACS-Vantage (Becton Dickinson) to confirm specificity of CD203c for basophils. Allergen-induced upregulation of CD203c and CD63 was calculated from mean fluorescence intensities (MFIs) obtained with stimulated (MFI_{stim}) and unstimulated ($MFI_{control}$) cells and expressed as stimulation index (MFI_{stim} /$MFI_{control}$). An SI of 2.0 or greater (≥2-fold upregulation) was considered to be indicative of a specific (diagnostic) response.

Desensitization experiments

In 2 allergic patients blood MNCs were separated from whole blood by means of Ficoll density centrifugation. MNCs (10^6 per tube) were preincubated with allergens (rPhl p 1, rPhl p 2, rPhl p 5, and rBet v 1, each at 0.1 μg/mL) or anti-IgE (0.1 μg/mL) in desensitizing PIPES buffer containing EDTA (4 μmol/L), NaCl (110 μmol/L), KCl (5 μmol/L), glucose (1 μmol/L), and 0.003% BSA at 37°C for 45 minutes. After incubation, cells were washed in PBS (4°C) and then exposed to recombinant allergens (1 μg/mL of each allergen) or anti-IgE (1 μg/mL) in PIPES buffer containing Ca^{2+} (37°C for 15 minutes). Thereafter, cells were washed in PBS-EDTA and stained with mAbs against CD203c and CD63, as described above.

Modulation of CD203c expression by cyclosporin A

Cyclosporin A (CSA) is well known to inhibit allergen-induced (FcεRI-dependent) histamine release in human basophils.[25,26] To examine whether allergen-induced upregulation of CD203c on basophils can be inhibited by CSA, we performed experiments with blood MNCs of 3 allergic individuals. MNCs were preincubated with control buffer or CSA (0.01-1 μg/mL) at room temperature for 15 minutes. Then, cells were washed and exposed to recombinant allergens (1 μg/mL) or anti-IgE (1 μg/mL). Thereafter, cells were washed and then stained with CD203c mAb and CD63 mAb.

Histamine release assay

Basophils were enriched from allergic patients by Dextran sedimentation. Granulocyte isolation and histamine release were performed essentially as described.[27] In brief, isolated cells were washed, resuspended in histamine release buffer, and exposed to recombinant allergens (1 μg/mL) or anti-IgE mAb E-124-2-8 (1 μg/mL) in 96-well microtiter plates (TPP, Trasadingen, Switzerland) for 30 minutes at 37°C. After incubation, cells were centrifuged. Cell-free supernatants were recovered and analyzed for histamine content by using a commercial radioimmunoassay (Immunotech). Histamine release was expressed as a percentage of total histamine measured in cell lysates.

Statistical evaluation of data

Appropriate statistical tests, including the Student *t* test, were applied to evaluate statistical significance of allergen-induced upregulation of CD antigens and histamine release. In case of multiple comparisons, data were corrected according to the method of Bonferroni. A *P* value of less than .05 was considered to indicate statistical significance.

RESULTS

Recombinant allergens promote expression of CD203c on basophils in sensitized individuals in a dose-dependent manner

Recent data suggest that CD203c is a specific marker for human basophils and is upregulated in response to FcεRI cross-linkage.[19] In this study, unstimulated basophils obtained from patients with grass or birch pollen allergy expressed detectable levels of CD203c similar to those in normal basophils, whereas other blood leukocytes appeared to be CD203c negative. The specificity of CD203c for basophils in allergic donors was confirmed by sorting experiments (Fig 1). In sensitized donors exposure of basophils to recombinant allergens resulted in a significant upregulation of CD203c ($P < .05$). In initial time-course experiments it was found that incubation with allergens causes a rapid increase in expression of CD203c, with a clear upregulation occurring after only 1 minute. Maximum upregulation was seen after 15 minutes (Fig 2). Allergen-dependent upregulation of CD203c was also dose dependent and did not occur in the absence of Ca^{2+}. Fig 3 shows the dose-dependent effect of rBet v 1 in a sensitized individual. In the same patient no effect of rBet v 2 was seen corresponding to a negative RAST. In general, incubation of basophils with the reactive allergens (1 μg/mL) resulted in significant upregulation of CD203c (SI ≥ 2.0) in almost all donors with a positive RAST ($P < .05$), whereas no upregulation was obtained with allergens to which allergic donors were not sensitized (negative RAST, $P > .05$, Table II). In nonallergic donors no upregulation was seen after stimulation with allergens, whereas anti-IgE stimulation resulted in a significant increase of CD203c ($P < .05$).

Desensitization of basophils and effects of CSA

To further demonstrate the specificity of allergen-induced upregulation of CD203c, we carried out desensitization experiments. In these experiments preincubation of basophils with recombinant allergens (rPhl p 1, rPhl

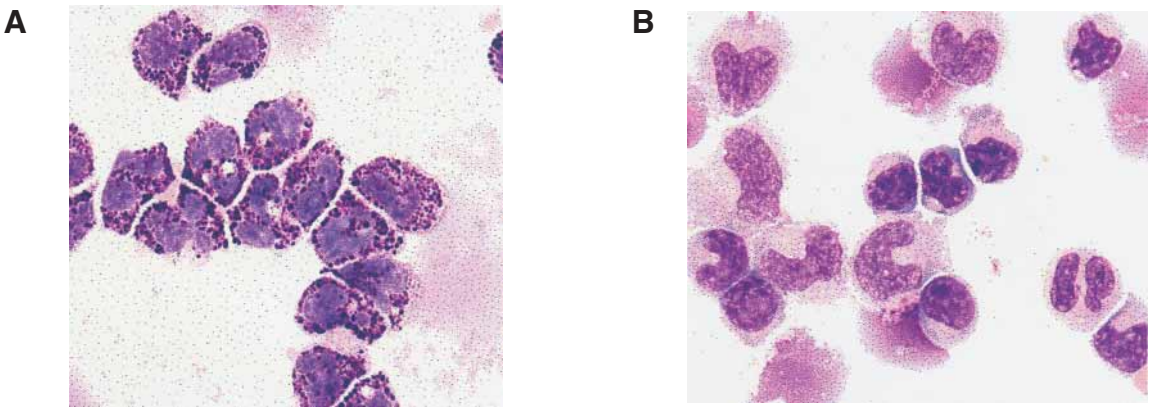

FIG 1. Morphology of CD203c+ and CD203c– blood cells. Peripheral blood cells obtained from a patient with birch pollen allergy were sorted for CD203c+ cells **(A)** and CD203c– cells **(B)** on a FACS-Vantage system. Separated cells were spun on Cytospin slides and stained with Wright-Giemsa.

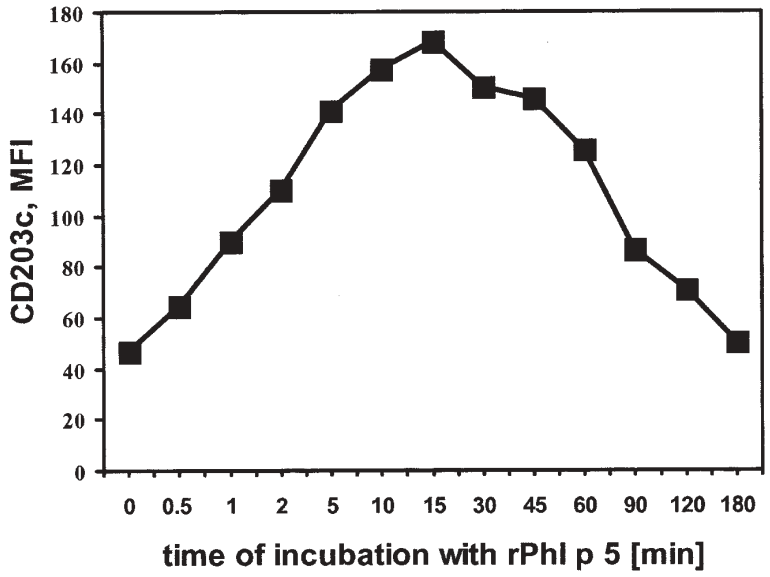

FIG 2. Time-dependent upregulation of CD203c by recombinant rPhl p 5 on basophils in a sensitized individual. Blood basophils were incubated with recombinant Phl p 5 (1 μg/mL) at 37°C for various time periods, as indicated, and then analyzed for expression of CD203c by flow cytometry.

p2, rPhl p 5, and rBet v 1, each at 0.1 μg/mL) in Ca^{2+}-free PIPES buffer resulted in desensitization. Thus, these basophils were less capable of upregulating CD203c on their surface when rechallenged with the same allergen in the presence of Ca^{2+} compared with control cells (Fig 4).

We next asked whether CSA inhibits allergen-induced upregulation of CD203c on basophils in sensitized individuals. The results of our experiments show that CSA is indeed capable of counteracting allergen-induced upregulation of CD203c on basophils in allergic patients. The inhibitory effects of CSA were dose dependent, with marked inhibition occurring at concentrations of 0.01 μg/mL or greater (Fig 5).

Correlation between CD203c upregulation and CD63 upregulation

Recent studies have shown that incubation of basophils with allergens is followed by an increase in expression of CD63.[16-18] In this study, we found that allergen-induced upregulation of CD203c is always accompanied by upregulation of CD63. Comparing respective stimulation indices (CD203c and CD63), a significant correlation ($R = 0.76$, $P < .05$) was found. Fig 6 shows a flow cytometric demonstration of rPhl p 1–induced upregulation of CD63 and CD203c on basophils in an allergic individual.

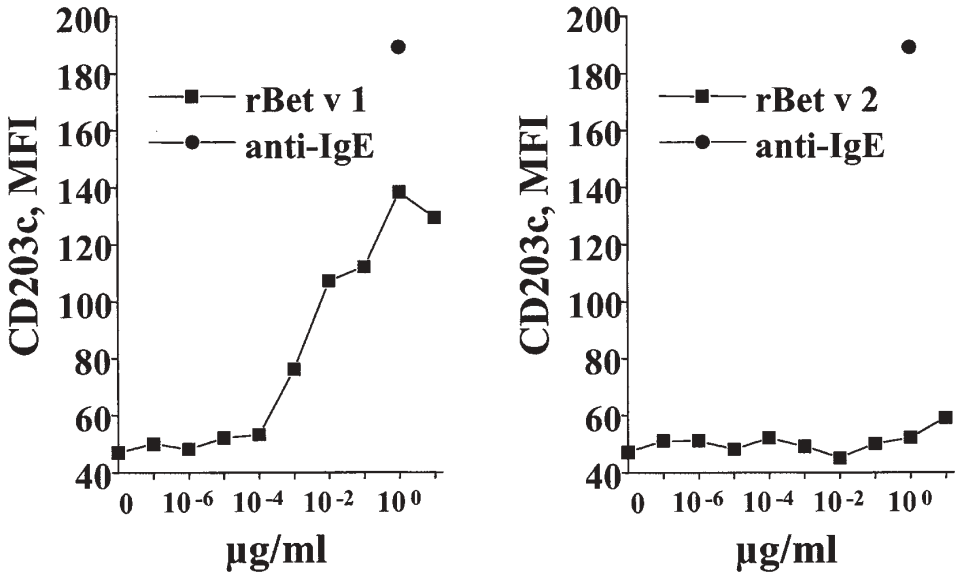

FIG 3. Dose-dependent effect of recombinant allergens on expression of CD203c in a sensitized individual. Whole blood was obtained from a patient allergic to Bet v 1 but not Bet v 2. Cells were incubated with serial dilutions of recombinant allergens or anti-IgE at 37°C for 15 minutes, as indicated. Cells were then stained with CD203c mAb and analyzed on a FACScan.

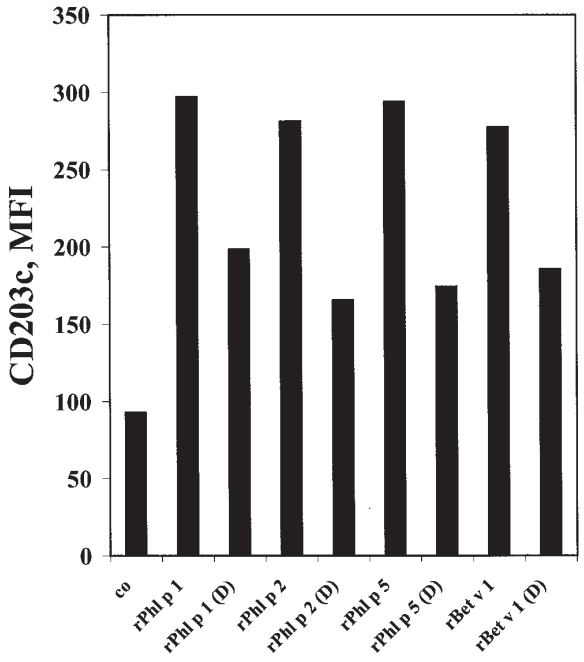

FIG 4. Desensitization of basophils. Peripheral blood MNCs of an allergic patient were preincubated with allergens (rPhl p 1, rPhl p 2, rPhl p 5, and rBet v 1, each at 0.1 µg/mL) in desensitizing PIPES buffer *(D)* or control medium for 45 minutes. After washing, MNCs were exposed to recombinant allergens (1 µg/mL each) in PIPES buffer containing Ca^{2+} for 15 minutes. After washing, cells were stained with the phycoerythrin-labeled CD203c mAb 97A6.

Correlation between CD203c upregulation and other parameters

In all allergic patients tested who showed a response to recombinant allergens in the CD203c test, the same allergens also induced significant release of histamine from blood basophils ($P < .05$, Tables I and II). Moreover, in all patients who showed upregulation of CD203c on their basophils in response to grass or birch pollen allergen, the same allergen type was found to produce a positive skin test response (Table I).

DISCUSSION

A number of previous studies have shown that flow cytometric quantitation of basophil activation can be used to measure IgE-dependent responses to allergens in sensitized individuals.[15-18,21] Thus far, most tests have used antibodies against CD63 and crude allergen extracts.[15-18] In this study, we have used recombinant allergens and the novel basophil marker CD203c[19,20] to measure and quantify allergen-induced activation of basophils. Our data show that CD203c represents a novel sensitive marker of allergen-induced activation of basophils and thus might represent a useful basis for the development of an effector cell-based allergy test.

In sensitized individuals exposure of basophils to recombinant allergens resulted in a time- and dose-dependent increase in CD203c. The allergen-induced increase of CD203c occurred after 30 seconds, with maximum stimulation seen after 15 minutes. Furthermore, because of the cell specificity of CD203c, the assay can be performed in a single-step fashion without any preactivation or additional basophil reagent. All in all, the CD203c-based assay provides a sensitive, specific, and rapid test to quantify the allergic response at the effector cell level.

Recent studies have shown that IgE-dependent activation of basophils is associated with upregulation of

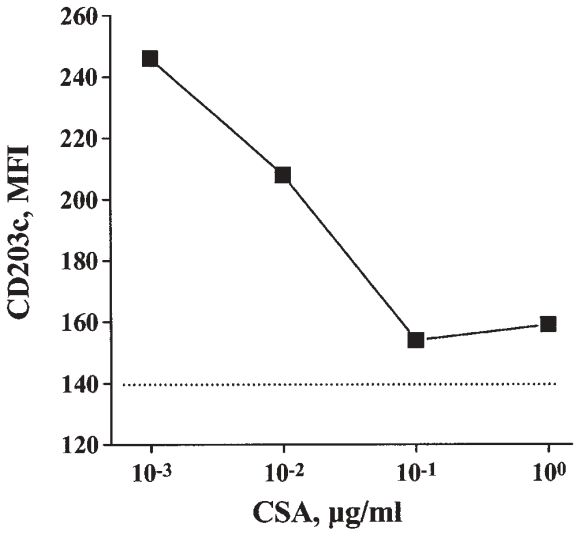

FIG 5. Influence of CSA on allergen-induced upregulation of CD203c. Peripheral blood MNCs of patients with Phl p 1 allergy were preincubated with control buffer or CSA (0.01-1 µg/mL) for 15 minutes. Then cells were washed and exposed to rPhl p 1 (1 µg/mL). After washing, cells were stained with CD203c mAb 97A6 and analyzed by means of flow cytometry.

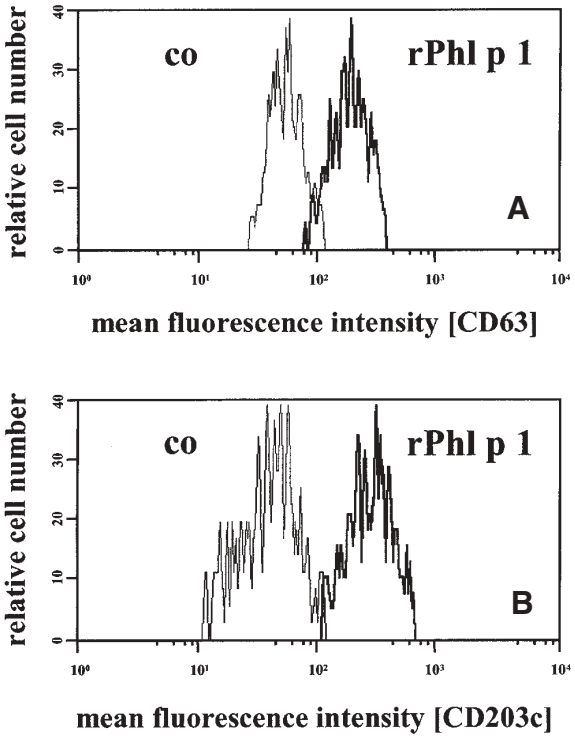

FIG 6. Comparison of CD203c and CD63 upregulation on stimulated basophils. Whole blood cells obtained from a patient with Phl p 1 allergy were incubated with recombinant Phl p 1 (1 µg/mL) for 15 minutes. Then cells were washed and labeled with a FITC-conjugated CD63 mAb **(A)** and a phycoerythrin-conjugated CD203c mAb **(B)**. Expression of CD63 and CD203c on basophils was analyzed by flow cytometry.

CD63.[15-18] However, the CD63-based test appears to have several limitations. First, CD63 is not a basophil-specific marker.[28,29] Therefore, the CD63 Baso-test is using an additional IgE-based reagent for basophil detection.[18] However, it is well known that nonbasophil cells in allergic patients also express FcεRI.[29-32] Moreover, exposure of basophils to an IgE-based reagent with subsequent activation or modulation of the IgE receptor might interfere with the test. These disadvantages can clearly be overcome by using CD203c mAb in such an assay.

Thus far, effector cell–based allergy tests have used impure allergen extracts.[15-18,21] In the present study, we used a panel of recombinant allergens to establish a novel allergy test. In particular, the results of our study show that recombinant allergens promote expression of CD203c in the same way as has been described for impure allergen extracts.[21] Thus, by using recombinant allergens and CD203c, it will be possible to establish and apply a highly sensitive assay, both in terms of effector cells and in terms of component-resolved diagnosis of allergies.[3,8]

In almost all cases examined, incubation of basophils with recombinant allergens resulted in upregulation of CD203c in sensitized individuals (ie, those with a positive RAST result and positive case history), whereas no effects were seen in healthy control subjects. However, in a few sensitized patients, negative results were obtained with the CD203c assay. Such "false-negative" results might also occur in skin prick tests and might be explained by a recent contact with allergen, resulting in desensitization. Alternatively, these patients might exhibit blocking IgG antibodies interfering with IgE-dependent responses. A third possibility could be that the levels of allergen-specific, basophil-bound IgE was too low to mediate allergic reactions. A general defect in releasability could be excluded since the other allergens as well as anti-IgE induced an upregulation of CD203c in the same donors. In 3 allergic donors tested, a high constitutive expression of CD203c, as well as a high spontaneous histamine release, were found. In these patients effector cells might have been in a preactivated state. It is of interest in this regard that all 3 donors had severe atopic disease. Nevertheless, in all 3 donors we found a marked diagnostic (allergen-induced) upregulation of CD203c. A number of other factors might also interfere with the CD203c test. Likewise, the upregulation of CD203c might be influenced by previous drug intake. Specifically, a number of drugs, such as corticosteroids or CSA, might counteract effector cell responses in vivo.[25,26] In this regard it is noteworthy that in vitro preincubation of basophils with CSA resulted in a partial inhibition of allergen-induced upregulation of CD203c in this study.

CD203c has recently been characterized as E-NPP3.[20] This enzyme is expressed on basophil progenitors as well as on mature blood basophils. Other blood leukocytes do not express detectable amounts of CD203c.[19,20] In the present study, we were able to confirm the specificity of CD203c for basophils derived from allergic individuals. In fact, sorting of peripheral blood cells for CD203c in

TABLE II. Recombinant allergen–induced upregulation (SI) of CD203c: Comparison with RAST

	Anti-IgE	rPhl p 1		rPhl p 2		rPhl p 5		rBet v 1		rBet v 2	
	SI	SI	RAST	SI	RAST	SI	RAST	SI	RAST	SI	RAST
Allergic patients											
1	2.5	2.8	2.58	NT	<0.35	NT	2.26	NT	9.58	NT	<0.35
2	4.0	4.0	17.8	3.1	5.1	3.9	14.6	3.0	0.43	1.1	<0.35
3	3.7	NT	<0.35	NT	<0.35	NT	<0.35	3.4	1.2	NT	<0.35
4	4.9	NT	<0.35	NT	<0.35	NT	<0.35	NT	<0.35	4.6	2.61
5*	1.1*	2.2	87.8	NT	15.3	2.2	68.3	2.0	92.8	NT	0.43
6	2.5	NT	3.12	NT	1.09	3.6	5.1	NT	<0.35	NT	<0.35
7	4.4	NT	38.8	NT	10.7	NT	36.1	6.1	77.1	5.7	7.07
8	3.2	6.0	22.9	6.2	14.2	4.2	20.9	NT	<0.35	NT	<0.35
9	2.4	3.5	12.8	1.2	1.3	3.0	23	4.0	66.2	NT	<0.35
10	2.9	4.3	18.7	3.8	1.86	3.8	8.91	3.7	41.1	1.5	0.4
11*	1.8*	2.2	7.6	NT	0.74	2.0	14.4	NT	24.9	2.0	5.06
12	2.0	2.2	15.7	2.4	11.2	2.3	37.3	2.6	17.5	1.0	1.8
13*	1.7*	2.7	38.9	1.4	3.3	2.4	20.6	2.7	63.8	1.0	0.6
14	2.3	2.8	10.2	2.9	12.9	2.7	20.3	3.0	71.1	2.1	3.2
15	3.1	3.5	>100	4.6	17.1	3.7	>100	3.2	11.7	1.0	<0.35
16	3.0	2.1	1.6	1.1	0.53	1.9	<0.35	2.0	2.4	1.4	<0.35
17	2.2	1.1	<0.35	1.2	<0.35	1.1	<0.35	4.3	>100	1.4	<0.35
18	3.3	4.8	22.5	4.9	9.2	4.2	37.1	5.0	85.4	2.9	2.5
19	2.6	3.1	4.9	1.1	<0.35	3.1	10.4	3.3	18.2	1.3	<0.35
20	3.3	3.8	1.5	1.1	<0.35	3.8	2.1	1.4	<0.35	1.1	<0.35
Nonallergic donors											
1	5.7	1.5	<0.35	1.1	<0.35	1.4	<0.35	1.5	<0.35	1.3	<0.35
2	3.7	1.1	<0.35	1.1	<0.35	1.1	<0.35	1.0	<0.35	1.1	<0.35
3	7.2	1.1	<0.35	1.1	<0.35	1.0	<0.35	1.6	<0.35	1.6	<0.35
4	1.3	1.0	<0.35	1.1	<0.35	1.0	<0.35	1.0	<0.35	1.1	<0.35
5	3.9	1.1	NT	1.1	NT	1.0	NT	1.1	NT	1.2	NT
6	2.9	1.0	NT	1.0	NT	1.0	NT	1.0	NT	1.0	NT
7	2.0	1.0	NT	1.1	NT	1.0	NT	1.0	NT	1.0	NT
8	3.2	1.0	NT	1.0	NT	1.0	NT	1.0	NT	1.0	NT
9	3.7	1.0	NT	1.0	NT	1.0	NT	1.0	NT	1.0	NT
10	2.1	1.0	NT	1.0	NT	1.0	NT	1.0	NT	1.0	NT

RAST data are expressed as kilounits of antigen per liter. Basophils were incubated with recombinant allergens (each at 1 μg/mL), anti-IgE mAb E-124-2-8 (1 μg/mL), or control medium (15 minutes for 37°C) and then analyzed for expression of CD203c by flow cytometry. The stimulation index was calculated from the MFI obtained with stimulated cells and cells kept in control medium ($MFI_{stim}/MFI_{control}$).

SI, Stimulation index; *NT,* not tested.

*In 3 atopic subjects constitutive expression of CD203c on unstimulated cells was higher compared with that seen in other patients.

these patients resulted in a pure population of basophils. The biologic significance of E-NPP3 expression on basophils remains unknown. It also remains unknown whether upregulation of CD203c is a secondary phenomenon occurring after IgE receptor cross-linking or an initial event required for IgE receptor activation. From the rapid upregulation of CD203c after FcεRI cross-linking, it is tempting to speculate that the increase is due to a translocation of the antigen to the cell surface. The consecutive decrease in CD203c to pretreatment levels after 180 minutes might also point to a transient translocation of CD203c to the surface membrane. Alternatively, the decrease to normal levels of CD203c after 180 minutes is due to (or associated with) desensitization. All in all, it seems likely that CD203c upregulation and degranulation (histamine release) occur simultaneously and might represent associated events in allergen-activated basophils. In line with this notion, CSA was found to downregulate allergen-induced upregulation of CD203c in a similar way, as has been described for histamine release.[26] In addition, basophils could be desensitized for allergen-induced upregulation of CD203c in the same way, as has been described for allergen-dependent histamine release.[6,33]

In summary, our data show that CD203c can be used as a novel specific marker of IgE-dependent activation of basophils. Using recombinant allergens and basophils of sensitized individuals, upregulation of CD203c might provide a reliable basis for a sensitive and specific allergy test.

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