



Letter

Increased basophil activation in adult patients with anaphylaxis

Anaphylaxis is a severe, life-threatening allergic reaction primarily mediated through IgE antibodies that interact with basophils and mast cells to release vasoactive and proinflammatory mediators.¹ Basophils comprise less than 1% of circulating leukocytes in humans; however, in recent years, it has drawn considerable attention because of involvement in various allergic diseases.² Like mast cells, basophil activation and degranulation induced by allergens lead to a release of various mediators that can generate anaphylaxis.³ Along with release of mediators, activation of human basophils results in the expression of surface proteins and the upregulation of expressed proteins. CD203c and CD63 are surface markers of basophils that are upregulated or expressed on activated basophils.² Basophil activation tests (BATs) using the activation markers CD203c and CD63 have been widely used to assess basophil activation status and as an important tool for checking IgE-mediated allergic reactions.⁴ There have been many reports regarding increased basophil activation in anaphylactic patients after allergen exposure,³ but the baseline status of these patients has not yet been studied. In the present study, we evaluated basophil activation status using CD203c and CD63 expressions in adult patients with anaphylaxis for a further understanding of pathogenic mechanisms of adult-onset anaphylaxis.

A total of 41 patients with various allergic diseases, including asthma and/or rhinitis, chronic urticaria, and anaphylaxis, and 23 healthy controls were recruited from Ajou University Hospital, Suwon, South Korea. Allergic patients were divided into 2 groups according to the diagnosis of adult-onset anaphylaxis to various drugs, including nonsteroidal anti-inflammatory drugs and antibiotics; foods, including wheat and seafood; and diagnostic reagents, such as radiocontrast media: the anaphylaxis ($n = 13$) and nonanaphylaxis ($n = 28$) groups. All participants provided written informed consent, and this study was approved by the institutional review board of Ajou Medical Center.

Basophil CD203c and CD63 expressions were measured by flow cytometry. Blood samples were collected in the stable state of each patient, at least 2 months after the anaphylactic event in cases of anaphylaxis. Participants did not take any medication or stopped taking medications for at least 1 week before blood sampling. The whole blood was collected in an EDTA tube, and red blood cells were lysed with a red blood cell lysis buffer (0.154 M NH_4Cl , 10 mM KHCO_3 , 0.1 mM EDTA, pH 7.2–7.4). Basophils were incubated with anti-IgE antibody (1 $\mu\text{g}/\text{mL}$) or calcium ionophore (1 $\mu\text{g}/\text{mL}$; Sigma-Aldrich Co, St Louis, MO) as positive controls or left untreated for 30 minutes. After being washed with a phosphate-buffered saline, the resuspended cells were stained with phycoerythrin-conjugated

antihuman CD203c or CD63 (Beckman Coulter, Marseille, France), fluorescein isothiocyanate-conjugated antihuman CD123 (BD Pharmingen, San Jose, CA), and allophycocyanin-conjugated anti-human human leukocyte antigen DR (BD Pharmingen), or isotype-matched controls on ice in the dark for 30 minutes. After washing once with phosphate-buffered saline, the cells were analyzed on a FACS Canto II flow cytometer (Becton Dickinson, San Jose, CA).

The Mann-Whitney U test was used to compare CD203c and CD63 expression levels between the groups. Spearman correlation analysis was used for the correlation analysis. $P < .05$ was considered to indicate statistical significance. All statistical analyses were performed using the SPSS statistical software for Windows, version 22 (SPSS Inc, Chicago, IL).

Mean (SD) baseline expression levels of CD203c and CD63 were significantly higher in the anaphylaxis group than in the healthy control group (30.28 [23.10] vs 13.76 [14.60], $P = .04$; 17.67 [20.48] vs 3.31 [3.73], $P = .03$, respectively; Fig 1) and tended to be higher in the anaphylaxis group than in the nonanaphylaxis group, although statistical significance was not reached. When the positive cutoff value for a positive BAT result was defined as the mean value plus 2 SDs of the CD203c expression levels in the healthy control group, positive BAT rates tended to be higher in the anaphylaxis group than in the nonanaphylaxis group (38.46% vs 17.85%, $P = .20$; eFig 1), although no differences were noted in CD63 expression levels. No significant differences were found in baseline percent expressions of CD203c and CD63 or those induced by anti-IgE or calcium ionophore according to age, sex, atopic status, and total IgE level. In addition, no significant correlations were found between the expressions of CD203c and CD63 with age and total IgE (data not shown).

Increased baseline expressions of CD203c and CD63 in the anaphylaxis group suggest that basophils in anaphylactic patients may be in a primed state and thus have hyperreactive responses to specific allergens. Lie et al⁵ found that basophils have primed phenotypes in patients with allergic asthma that cause the release of high levels of histamine when exposed to stimuli. The high-affinity IgE receptor ($\text{Fc}\epsilon\text{RI}$) that plays an important role in basophil degranulation has been found to be upregulated on basophils in patients with chronic urticaria with CD203c and CD63 expressions compared with healthy controls.² An in vitro study found that mast cells expressing enhanced levels of $\text{Fc}\epsilon\text{RI}$ are more sensitive to activation when stimulated with IgG anti- $\text{Fc}\epsilon\text{RI}$.⁶ Therefore, basophils of anaphylactic patients may have increased $\text{Fc}\epsilon\text{RI}$ expression in the basal state that enhances its sensitivity toward specific allergens.

Cytokines, such as interleukin (IL) 3, IL-33, and thymic stromal lymphopoietin, can activate basophils and enhance IgE-mediated degranulation.^{7,8} Patients with anaphylaxis have been reported to have high serum levels of IL-33 during anaphylactic shock, suggesting that IL-33 plays an important role in anaphylaxis.⁷ In the present study, baseline CD203c and CD63 expressions were significantly higher in the anaphylaxis group than in the

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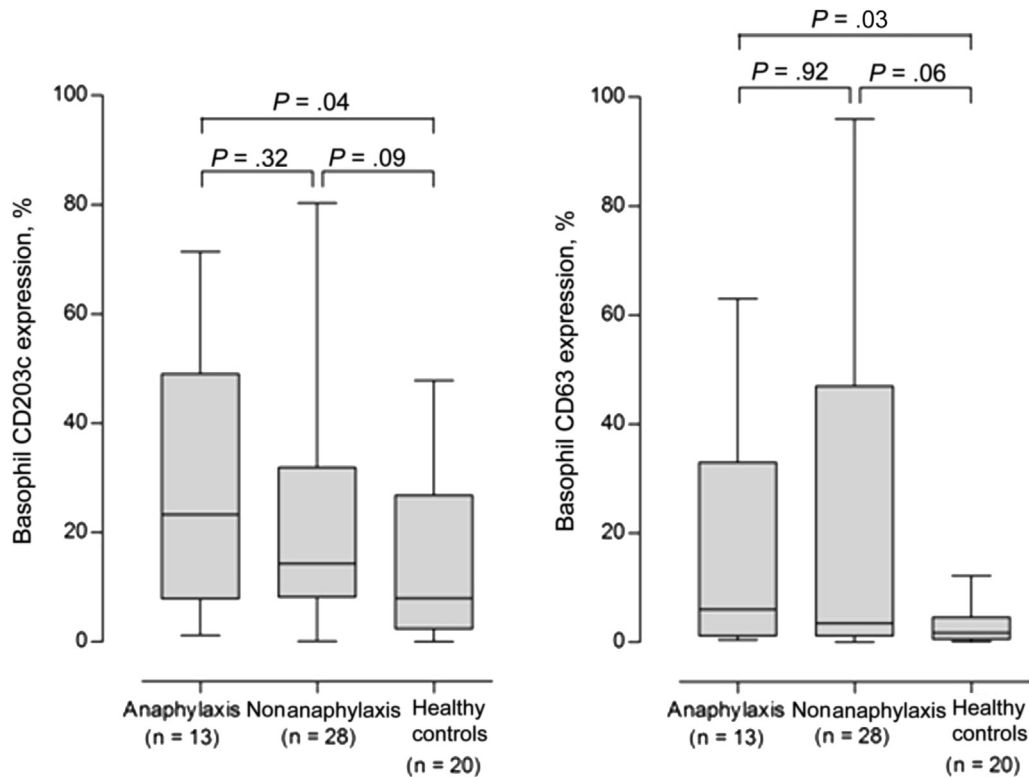


Figure 1. Mean percentages of basophil CD203c and CD63 expression at baseline in the anaphylaxis and nonanaphylaxis groups compared with the healthy control group. *P* values were determined using the Mann-Whitney *U* test.

nonanaphylaxis group and tended to be higher in the anaphylaxis group than in the healthy control group without statistical significance. Gernez et al⁹ also found an increased baseline CD203c expression in nut allergic patients compared with healthy controls. Therefore, altered cytokine milieu in the baseline condition may exist in patients with anaphylaxis that can induce basophil activation.

In the present study, we used 2 surface markers of basophils to assess basophil activation. Although CD203c is thought to be the most useful marker of basophil activation because of basophil-specific and high sensitivity,¹⁰ both markers are elevated in patients with anaphylaxis. These findings suggest that implication of these 2 activation markers will be more useful for evaluating basophil activation status in patients with anaphylaxis. More studies are needed to understand why basophil activation status, even in asymptomatic patients with anaphylaxis, can be a target for new therapeutic interventions.

These findings suggest that the increased basal activation status of basophils may contribute to the development of anaphylaxis in adult patients.

Supplementary Data

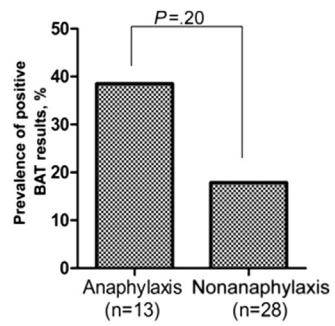
Supplementary data related to this article can be found online at <http://dx.doi.org/10.1016/j.anai.2015.08.014>.

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References

- [1] Ye YM, Kim MK, Kang HR, et al. Predictors of the severity and serious outcomes of anaphylaxis in Korean adults: a multicenter retrospective case study. *Allergy Asthma Immunol Res.* 2015;7:22–29.
- [2] Lourenco FD, Azor MH, Santos JC, et al. Activated status of basophils in chronic urticaria leads to interleukin-3 hyper-responsiveness and enhancement of histamine release induced by anti-IgE stimulus. *Br J Dermatol.* 2008;158:979–986.
- [3] Apostolou E, Deckert K, Puy R, et al. Anaphylaxis to Gelofusine confirmed by in vitro basophil activation test: a case series. *Anaesthesia.* 2006;61:264–268.
- [4] Hagau N, Longrois D, Petrisor C. Threshold for positivity and optimal dipyrone concentration in flow cytometry-assisted basophil activation test. *Allergy Asthma Immunol Res.* 2013;5:383–388.
- [5] Lie WJ, Knol EF, Mul FP, Jansen HM, Roos D, van der Zee JS. Basophils from patients with allergic asthma show a primed phenotype. *J Allergy Clin Immunol.* 1999;104:1000–1007.
- [6] Gomez G, Jogie-Brahim S, Shima M, Schwartz LB. Omalizumab reverses the phenotypic and functional effects of IgE-enhanced Fc epsilonRI on human skin mast cells. *J Immunol.* 2007;179:1353–1361.
- [7] Pushparaj PN, Tay HK, H'Ng SC, et al. The cytokine interleukin-33 mediates anaphylactic shock. *Proc Natl Acad Sci U S A.* 2009;106:9773–9778.
- [8] Siracusa MC, Saenz SA, Hill DA, et al. TSLP promotes interleukin-3-independent basophil haematopoiesis and type 2 inflammation. *Nature.* 2011;477:229–233.
- [9] Gernez Y, Tirouvanziam R, Yu G, et al. Basophil CD203c levels are increased at baseline and can be used to monitor omalizumab treatment in subjects with nut allergy. *Int Arch Allergy Immunol.* 2011;154:318–327.
- [10] Bühring HJ, Streble A, Valent P. The basophil-specific ectoenzyme E-NPP3 (CD203c) as a marker for cell activation and allergy diagnosis. *Int Arch Allergy Immunol.* 2004;133:317–329.



eFigure 1. The prevalence of basophil activation test (BAT) positive results based on CD203 expression between the anaphylaxis and nonanaphylaxis groups. Cutoff values were calculated as mean $\pm$ 2 SDs.