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Original Article

Decreased intracellular histamine concentration and basophil activation in anaphylaxis

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Abbreviations:
AUC, area under the curve; CI, confidence interval; EDTA, ethylenediaminetetraacetic acid; FITC, fluorescein isothiocyanate; HPLC, high performance liquid chromatography; ICU, intensive care unit; IQR, interquartile ranges; PBS, phosphate-buffered saline; PC7, phycoerythrin covalently linked to cyanine 7; PE, phycoerythrin; ROC, receiver operating characteristic; SD, standard deviation

ABSTRACT

Background: Histamine is a crucial mediator in the development of anaphylaxis. Although histamine is promptly degraded because of its short half-life in plasma, basophils, which release histamine, remain in the blood for days. To explore basophils as a potential marker and their involvement in the pathogenesis of anaphylaxis, we evaluated the intracellular histamine concentration and the degree of basophil activation in anaphylaxis patients.
Methods: We conducted a case–control study enrolling anaphylaxis patients and healthy controls. Basophil activation was evaluated by flow cytometry using up-regulation of CD203c expression.
Results: We enrolled 23 patients and measured their blood histamine concentration. Basophil activation was analyzed in seven of 23 patients. The median intracellular histamine concentrations at admission were significantly lower in patients compared with controls (16.4 ng/mL [interquartile range {IQR}, 2.70 to 34.0] vs. 62.3 ng/mL [IQR, 46.0 to 85.1]; $p < 0.0001$). The median basophil number at admission was also significantly lower in patients compared with controls (2.21 cell/μL [IQR, 0.75 to 12.3] vs. 21.0 cell/μL [IQR, 19.5 to 28.9]; $p = 0.027$). CD203c expression was not up-regulated in any of the seven patients *in vitro*, but it was up-regulated in response to anti-IgE stimulation *in vitro* in two patients at admission and four patients at follow-up.
Conclusions: Anaphylaxis is associated with a decrease in intracellular histamine, and a reduced number and reactivity of peripheral basophils. Impaired basophil function and a decrease in their number and intracellular histamine levels in the circulation may reflect the underlying mechanism, suggesting that basophils may be a marker of anaphylaxis.

Introduction

Anaphylaxis is an acute life-threatening allergic response.[1] It may be caused either by immune mechanisms including type I allergy, which is mediated by IgE, or a non-immune mechanism, which directly activates mast cells or basophils.[2] Regardless of the trigger or mechanisms, chemical mediators released from mast cells in tissues and basophils in the bloodstream are suspected to play important roles in the development of anaphylaxis.[3] Among the mediators, histamine induces vasodilatation, increases vascular permeability,[4,5] causes tachycardia, and decreases blood pressure.[6] Moreover, the plasma concentration of histamine may reflect the severity of anaphylaxis.[7] However, histamine released into the peripheral blood circulation is rapidly hydrolyzed by histamine methyltransferase and its half-life in plasma is only a few minutes.[8]

Laroche *et al.*[8] demonstrated that the increase in plasma histamine level can be detected in 10 out of 19 patients with anaphylaxis. Robert *et al.*[9] showed that the increase in plasma histamine level was observed in 47% of patients who presented to the emergency department with acute allergic reactions. However, the half-life of basophils is 2–3 days, and the decrease in the amount of intracellular histamine caused by basophil degranulation is not restored immediately. Thus, the amount of intracellular histamine may reflect basophil degranulation in the blood for a few days after

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anaphylaxis occurs. Moreover, basophil activation may be evaluated by the level of cell surface protein, such as CD203c and CD63. In this study, we investigated the level of intracellular histamine and cell surface CD203c in basophils and their reactivity compared with clinical characteristics of basophil donors, to explore the potential of basophils as a marker and their involvement in the pathogenesis of anaphylaxis.

Methods

Study design

We conducted a case–control study in the emergency and critical care center and intensive care unit (ICU) of Hiroshima University Hospital. We recruited patients with anaphylaxis whose plasma and total histamine concentrations could be measured. The criteria for anaphylaxis was adopted from the 2006 National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network meeting.[1] The severity of anaphylaxis was classified based on the classification recommended by Brown *et al.*[10] Patient information including causes of anaphylaxis, medical history, clinical symptoms, plasma and total histamine concentrations in blood, disease severity, medications, and outcomes were collected and analyzed together with the number, activity, and activation capacity of basophils in blood. We also collected blood samples from age and sex-matched controls, who visited outpatient clinic or worked in the hospital for the levels of plasma and whole blood histamine. This study was approved by the ethics committee for epidemiology of Hiroshima University. All the participants provided written informed consent to enroll in this study and to obtain blood samples.

Measurement of histamine concentrations in whole blood and plasma

Blood samples were collected from patients with anaphylaxis and healthy controls at admission and on the next day (follow up). Ethylenediaminetetraacetic acid (EDTA)-treated peripheral whole blood was immediately stored in a refrigerator (4 °C) until analysis. The samples of whole blood and plasma were mixed with $HClO_4$ to a final concentration of 2.5% $HClO_4$ to denature excess protein, followed by incubation for 30 min, and then histamine was extracted by centrifugation. The amounts of histamine were measured using high performance liquid chromatography (HPLC), as described previously.[11] The HPLC system consisted of an LC-4A (Shimadzu, Kyoto, Japan) with an RF-535 fluorescence monitor (Shimadzu) operating at an emission wavelength of 450 nm and an excitation wavelength of 350 nm. The levels of intracellular histamine were determined as the difference between the levels of histamine in whole blood and those in plasma.

Flow cytometry analysis for evaluating basophil count and activity

Basophil counts and its activity were evaluated by flow cytometry using the Allergenicity® kit (Beckmann Coulter, Brea, CA, USA), in accordance with the manufacturer's instructions. The cells in EDTA-treated peripheral whole blood obtained from patients with anaphylaxis were stained with fluorescein isothiocyanate (FITC)-labeled anti-CRTH2 antibody, phycoerythrin (PE)-labeled anti-CD203c antibody, and phycoerythrin covalently linked to cyanine 7 (PC7)-labeled anti-CD3 (Beckmann Coulter) for 15 min at 37 °C while being protected from light on water bath. Erythrocytes were lysed with lysing solution provided in the kit. After washing twice with phosphate-buffered saline (PBS), the stained cells were analyzed using an Attune® acoustic focusing cytometer (Thermo Fisher Scientific, Waltham, MA, USA). Basophils can be identified by CRTH2 and CD203c double-positive and CD3 negative cells. The basophil activation level was evaluated by analyzing the increase in CD203c expression. To evaluate the capacity of basophils to respond, we stimulated basophils with anti-human IgE reagent, which was prepared using the Allergenicity® kit. We also measured the number of basophils in healthy controls using the same protocols. The measurement of plasma and whole blood histamine and flow cytometry analysis were conducted at admission and the next morning as a follow-up.

Statistical analysis

We reported continuous variables as the mean and standard deviation (SD) or the median and interquartile range (IQR), and categorical variables as proportions. We used the Wilcoxon rank-sum test to compare continuous variables. Analysis of the receiver operating characteristic (ROC) curve and calculation of the area under the curve (AUC) was used to assess the diagnostic accuracy of plasma and intracellular histamine levels for anaphylaxis. All analyses were conducted using JMP 12 software (SAS Institute, Cary, NC, USA). A two-sided p-value <0.05 was considered to indicate statistical significance.

Results

Study subjects

We enrolled 23 patients with anaphylaxis who had their levels of plasma and whole blood histamine were measured. Table 1 shows the baseline characteristics of the patients enrolled. Drugs are the most frequent cause of anaphylaxis (74%). The top two drugs that were suspected to cause anaphylaxis were radiocontrast media in seven cases, and anticancer drugs in four cases. Twenty-one (91%) patients were classified with severe anaphylaxis (Table 2).

Table 1
Clinical characteristics of the enrolled patients.

Variables	Anaphylaxis	Control
Number of patients	23	23
Age, yr	56.5 ± 16.4	56.9 ± 17.3
Male sex, n (%)	16 (70)	16 (70)
Out of hospital/In hospital	9/14	
Past history of anaphylaxis, n (%)	2 (10)	
Cause of anaphylaxis, n (%)		
Drugs	17 (74)	
Foods	4 (17)	
Insect sting	1 (4)	
FDEIA	1 (4)	
Risk Factors, n (%)		
Comorbidities		
Cancer	11 (48)	
Cardiovascular disease	4 (17)	
Acute infection	3 (13)	
COPD	1 (4)	
Drug		
NSAIDs	1 (4)	
Allergic predisposition	9 (39)	
Treatments, n (%)		
Adrenaline (intramuscular injection)	18 (78)	
H1 and/or H2 antihistamines	22 (96)	
Corticosteroids	17 (74)	
Vasopressors	11 (48)	
Mechanical ventilation	2 (9)	

FDEIA, food dependent exercise induced anaphylaxis; COPD, chronic obstructive pulmonary disease; NSAIDs, non-steroidal anti-inflammatory drugs.

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Table 2
Clinical symptoms at the onset of anaphylaxis.

	n (%)
Skin, subcutaneous tissue, and mucosa	
Erythema	19 (83)
Urticaria	6 (26)
Periorbital edema	3 (13)
Swelling of lips and/or tongue	3 (13)
Pruritus	3 (13)
Respiratory	
Dyspnea	10 (43)
Hypoxemia (SpO_2<92%)	8 (35)
Edema of larynx	2 (9)
Cough	1 (4)
Hoarseness	1 (4)
Stridor	1 (4)
Cardiovascular and central nervous system	
Hypotension	21 (91)
Decrease levels of CNS	12 (52)
Syncope	5 (22)
Convulsion	1 (4)
Abdominal	
Diarrhea	4 (17)
Nausea	3 (13)
Vomiting	3 (13)
Cardiopulmonary arrest	1 (4)
Severity of anaphylaxis	
Mild	0 (0)
Moderate	2 (9)
Severe	21 (91)

CNS, central nervous system.

Plasma and intracellular histamine levels

The median plasma histamine level in patients with anaphylaxis at admission was 8.24 ng/mL (IQR, 3.06 to 27.7), which tended to be higher than that in healthy controls (5.02 ng/mL; IQR, 2.32 to 8.45; $p = 0.0588$; Fig. 1A). The median plasma histamine level in patients with anaphylaxis at follow-up was 0.19 ng/mL (IQR, 0 to 1.83), which was significantly lower than that in healthy controls ($p = 0.0005$; Fig. 1A). The median intracellular histamine level was significantly lower in patients with anaphylaxis at admission compared with healthy controls (16.4 ng/mL [IQR, 2.70 to 34.0] vs. 62.3 ng/mL [IQR, 46.0 to 85.1]; $p < 0.0001$; Fig. 1B). The median intracellular histamine level in patients with anaphylaxis at follow-up (9.44 ng/mL; IQR, 2.29 to 24.3) was also significantly lower compared with controls ($p < 0.0001$; Fig. 1B). The ROC curves for intracellular and plasma histamine levels for detecting anaphylaxis revealed sensitivities of 78.3% (95% confidence interval [CI], 61.4 to 95.1) and 60.9% (95% CI, 40.9 to 80.8) and specificities of 87.0% (95% CI, 73.2 to 100) and 65.2% (95% CI, 45.8 to 84.7) at cutoff values of 34.0 ng/mL for intracellular histamine and 5.35 ng/mL for plasma histamine, respectively. The AUC of intracellular histamine levels was significantly higher than that of plasma histamine (0.93 [95% CI, 0.83 to 0.97] vs. 0.66 [95% CI, 0.49 to 0.80]; $p = 0.0043$; Fig. 2).

Number of basophils

Basophil counts and its activity were evaluated in seven of 23 patients. The median number of basophils was significantly lower in patients with anaphylaxis at admission (2.21 cell/μL; IQR, 0.75 to 12.3) compared with controls (21.0 cell/μL; IQR, 19.5 to 28.9; $p = 0.0266$; Fig. 3A). A further decline in the median number of basophils in patients with anaphylaxis was observed at follow-up (1.00 cell/μL; IQR, 0.75 to 5.37), which was also lower than that in controls ($p = 0.0034$, Fig. 3A).

Intracellular histamine per cell and basophil activation

There was no significant difference between the median intracellular histamine per cell in patients with anaphylaxis at admission (1.34 pg/cell; IQR, 0 to 4.37) and that in controls (2.95 pg/cell; IQR, 2.76 to 3.10; $p = 0.224$; Fig. 3B). There was also no significant difference between the median intracellular histamine levels per cell in patients with anaphylaxis at follow-up (1.89 pg/cell; IQR, 0 to 2.91) and that in controls ($p = 0.0999$; Fig. 3B).

We conducted flow cytometry analysis to evaluate CD203c expression, which is a marker of basophil activation, in seven patients (Table 3). There was no up-regulation of CD203c in all seven patients at admission. After anti-IgE stimulation, an increase in CD203c levels was observed in two patients (29%) at admission and four patients (57%) at follow-up. Representative dot plots of CD203c expression levels are shown in Supplementary Figure 1.

Four patients with anaphylaxis who showed an increase in CD203c levels at follow-up in response to anti-IgE antibody stimulation also showed an increase in intracellular histamine per cell.

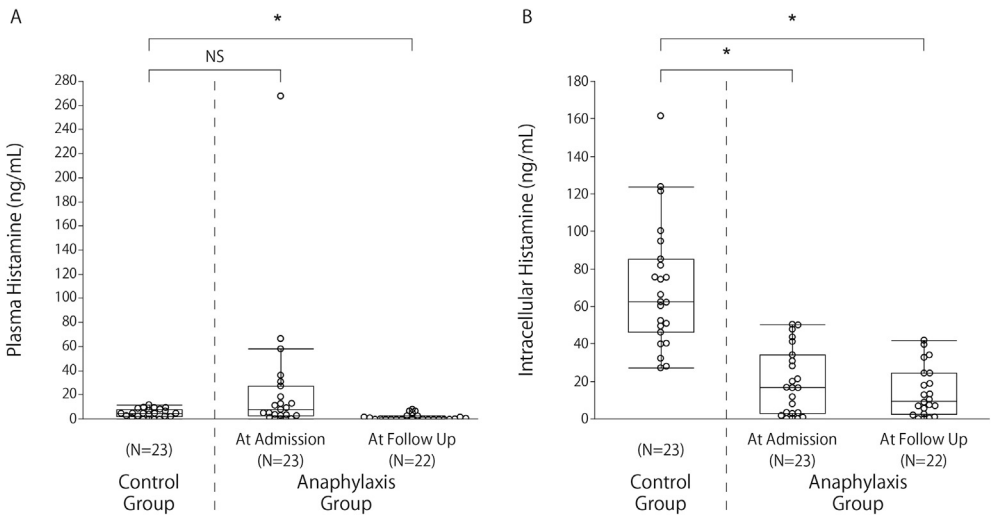

Fig. 1. Histamine levels. Box plot graphs showing the plasma histamine ranges **(A)**; intracellular histamine **(B)** in patients with anaphylaxis at admission and at follow-up (next morning), and in controls. NS, nonsignificant; $^*p < 0.05$ (significantly different from control by Wilcoxon rank-sum test).

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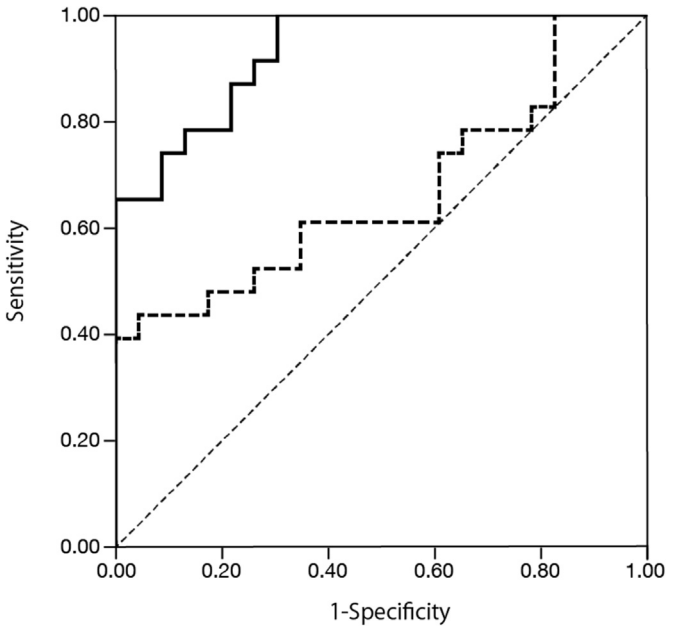

Fig. 2. Receiver operating characteristic curves for detecting anaphylaxis. The solid line indicates intracellular histamine levels with an area under the curve (AUC) of 0.93 [95% confidence interval (CI), 0.83–0.97], and the dotted line indicates plasma histamine levels with an AUC of 0.66 [95%CI, 0.49–0.80] for detecting anaphylaxis.

Conversely, the intracellular histamine levels per cell decreased further in three patients who did not respond to anti-IgE antibody (Table 3). The intracellular histamine levels per cell in these patients also remained low at follow-up compared with those at admission (Table 3).

Discussion

To the best of our knowledge, this is the first study that showed decreased intracellular histamine levels and decreased basophil counts in peripheral blood in patients with anaphylaxis compared with controls. The number of basophils remained decreased during follow-up the next day. However, there was no significant difference in plasma histamine levels between patients with anaphylaxis and healthy controls. Additionally, no patients with anaphylaxis demonstrated basophil activation in the peripheral blood, regardless of the amount of intracellular histamine per cell.

Van der Linden *et al.*[12] demonstrated that the plasma histamine levels in patients with anaphylaxis were significantly higher than those in healthy controls. However, plasma histamine levels in association with symptoms should be measured within 1 h after the onset of the symptoms because of the short half-life of histamine in circulation.[3,8] They included anaphylaxis in patients in response to the insect-sting challenge test, and strictly controlled the timing of blood sampling from the onset of anaphylaxis. However, in real-world clinical practice, it is difficult to define blood sample timing, which may largely affect the measured plasma histamine level. In this study, we found no statistical difference in the levels of plasma histamine between patients and healthy control subjects. Because approximately 40% of patients analyzed in this report were outpatients, it is likely that a large part of histamine in the blood circulation had been degraded before sampling.

The intracellular histamine level in patients with anaphylaxis was significantly lower, both at admission and during the follow-up period compared with healthy controls. The causes of anaphylaxis varied in patients in this study, including non-immunologic agents, such as radiocontrast medium and anticancer drugs, which suggests that intracellular histamine can be a useful biomarker regardless of the patient's allergic predisposition.

Previous studies demonstrated that the number of basophils in the peripheral blood also decreased in chronic idiopathic/spontaneous urticaria (CSU).[13,14] There was a significant negative correlation between basophil numbers and urticarial activities.[14] Moreover, the decrease in the peripheral basophil numbers was reversed in association with clinical improvement by treatments with corticosteroids[14] or omalizumab[15] in CSU. However, no studies have investigated the kinetics of peripheral blood basophils in anaphylaxis. Both anaphylaxis and urticaria develop erythema and wheals, which are induced by the release of chemical mediators including histamine from basophils and mast cells. In type I allergy, basophils and mast cells are sensitized by IgE binding to their high affinity IgE receptors (FcεRI) on the cell surface causing the release mediators, such as histamine, leukotriene, and tryptase in response to cross-linkage of FcεRI by the binding of specific antigens to IgE. The CD63 level, another marker of basophil activation, was upregulated in the blood of patients with CSU.[16] However, no patients

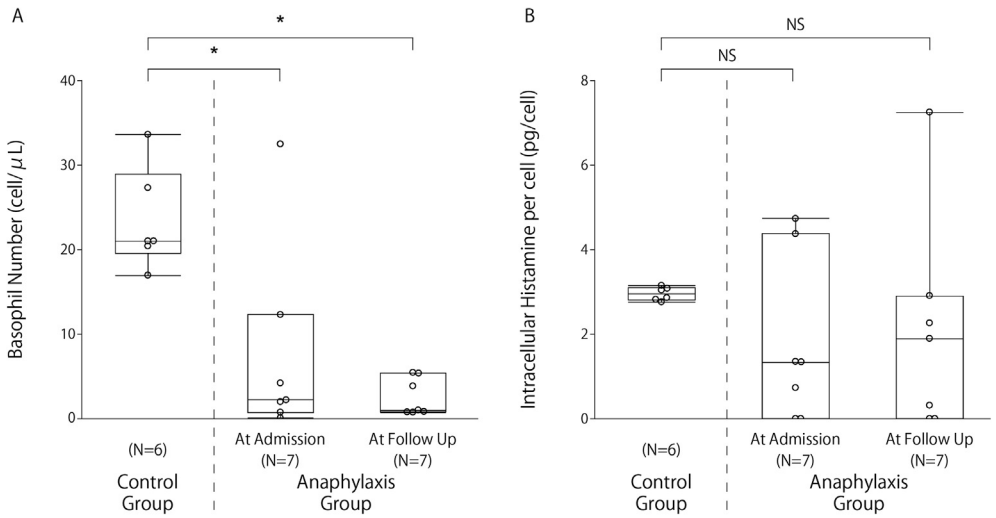

Fig. 3. Basophil number and intracellular histamine per cell. Box plot graphs showing basophil number **(A)**, and intracellular histamine per cell **(B)** in patients with anaphylaxis at admission and at follow-up (next morning), and in controls. NS, nonsignificant; $^{*}p < 0.05$ (significantly different from control by Wilcoxon rank-sum test).

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Table 3
Intracellular histamine concentration and basophil activation.

Patient No	Cause of anaphylaxis	Intracellular histamine per cell (pg/cell)		Basophil number (/μL)		Activation of peripheral blood basophils (up-regulation of CD203c expression)			
		At admission	Follow -up	At admission	Follow -up	At admission vehicle	At admission anti-IgE	Follow-up vehicle	Follow-up anti-IgE
1	Anticancer drug	4.73	0	4.20	0.75	non-reactive	non-reactive	non-reactive	non-reactive
2	Anticancer drug	4.37	0.31	0.75	0.78	non-reactive	non-reactive	non-reactive	non-reactive
3	Radiocontrast media	1.34	0	32.5	1.00	non-reactive	non-reactive	non-reactive	non-reactive
4	Radiocontrast media	0	7.25	1.99	5.45	non-reactive	non-reactive	non-reactive	reactive
5	Radiocontrast media	0	1.90	0.07	5.37	non-reactive	non-reactive	non-reactive	reactive
6	Radiocontrast media	1.35	2.26	12.3	3.85	non-reactive	reactive	non-reactive	reactive
7	Radiocontrast media	0.73	2.91	2.21	0.83	non-reactive	reactive	non-reactive	reactive

IgE, immunoglobulin E.

with anaphylaxis showed basophil activation in the peripheral blood in this study. Moreover, only two of seven patients showed the potential for basophil activation in response to anti-IgE stimulation. The discrepancy between basophil activation in CSU and that in anaphylaxis in this study remains controversial. This may be because of the low sensitivity of CD203c compared with CD63. There was no difference in CD203c expression between basophils from patients with CSU and those from healthy controls.[16] The most possible explanation is that the activated basophils had migrated out of the peripheral blood into the tissue, and that residual immature basophils that contain only a small amount of histamine and cannot respond to crosslinkage of FcεRI by anti IgE in the peripheral blood of patients with anaphylaxis. Mukai *et al.* demonstrated, using a mouse model of chronic allergy, that peripheral basophils can migrate to the tissue.[17] Increased basophil infiltration was also observed in lungs and bronchial tissue of postmortem patients with fatal asthma compared with non-asthmatic fatal cases.[18] The increase in the basophil activation capability on follow-up in two patients (Numbers 4 and 5) might have been associated with the new recruitment of mature basophils, possibly from bone marrow, which was suggested by the increase in basophil number. Although there is a possibility that the basophils of patients with anaphylaxis have been already activated enough before the basophil activation test, the result that there was no patient with an increase in CD203c levels at admission may suggest that immature basophils, which were not capable of responding to anti-IgE stimulus, remained in the peripheral blood.

There are several limitations in our study. First, flow cytometry analysis should be performed within a few hours after blood sampling. There could be a selection bias toward patients in whom flow cytometry analysis was immediately possible. All patients in whom flow cytometry analysis could be performed were inpatients. Second, previous studies showed that anaphylactic patients with hypotension and impaired consciousness accounted for 9.2–21.6% and 2.8–15.2% of patients, respectively.[19,20] In our study, patients with hypotension and impaired consciousness accounted for more than 90% and 50% of patients, respectively, suggesting that the patients' symptoms in this study are more severe than those in previous studies. Third, we assigned healthy volunteers to the control group. To determine whether intracellular histamine levels are useful to differentiate anaphylaxis from non-anaphylactic patients, further studies including a control group comprising non-anaphylactic patients with similar symptoms to anaphylaxis, such as hypotension or impaired consciousness, should also be studied. Fourth, up-regulation of CD203c on the surface of basophils of each patient was evaluated manually, because we enrolled the patients who already developed anaphylaxis and could not analyze unstimulated cells before the onset of anaphylaxis to define the threshold of CD203c up-regulation. Finally, we did not analyze our results based on stratification by the severity of anaphylaxis because of the small sample size in this study. The analysis of basophil counts in peripheral blood were performed only in seven patients with anaphylaxis. Further prospective studies with a larger sample size are needed to validate our results.

In conclusion, we found a decrease in the level of intracellular histamine and the number of basophils and their reactivity to an anti-IgE stimulus in the blood of patients with anaphylaxis. These results suggest a crucial role for basophils in the mechanism of anaphylaxis, regardless of the involvement of type I allergy.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.alit.2019.05.009.

Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

MH conceived of the study, participated in its design. SY, YY, and KI performed the experiments and contributed to data collection. SY performed the statistical analysis and drafted the manuscript. SO, NS, and MH contributed to interpretation of data and revised the manuscript.

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