

Elevated Ca^{2+} influx–inducing activity toward mast cells in pretransfusion sera from patients who developed transfusion-related adverse reactions

Hiroshi Azuma, Miki Yamaguchi, Daisuke Takahashi, Mitsuhiro Fujihara, Shinichiro Sato, Toshiaki Kato, and Hisami Ikeda

BACKGROUND: Type I allergic reactions such as urticaria-like manifestations constitute a large percentage of transfusion-related adverse events. Along with donor factors, patient factors might be involved in these reactions. Sera from some patients with chronic idiopathic urticaria show histamine-releasing activity (HRA). Sera from patients who develop Type I allergic reaction might possess HRA.

STUDY DESIGN AND METHODS: Pretransfusion serum samples were collected. Mast cells were cultured from peripheral blood CD34+ cells and mixed with the serum samples. Cells with elevated intracytoplasmic Ca^{2+} concentrations were monitored using flow cytometry to evaluate Ca^{2+} influx–inducing activity (CaIA) in serum. The amount of histamine released into the supernatant was measured using an enzyme immunoassay kit to evaluate HRA. In some assays, cells were incubated with pertussis toxin (Ptx).

RESULTS: CaIA values were higher ($p < 0.05$) in sera from patients with reactions (27.68 ± 20.38 [$n = 145$]; range, 0.49–84.90) than in sera of patients without reactions (10.70 ± 6.50 [$n = 54$]; range, 2.67–36.97) and control sera (9.67 ± 5.88 [$n = 107$]; range, 1.11–25.68). No difference in CaIA was found between patients with (27.38 ± 20.46 [$n = 88$]) and without (28.38 ± 20.59 [$n = 57$]) skin manifestations. However, CaIA was higher ($p < 0.05$) in patients with pure urticaria (mean, 30.58 ± 21.3 [$n = 30$]; range, 70.43–4.1) than in patients with fever alone (mean, 18.61 ± 14.71 [$n = 18$]; range, 45.94–3.19). Levels of HRA were higher ($p < 0.001$) in CaIA-positive sera than in CaIA-negative sera. Both CaIA and HRA were blocked by Ptx.

CONCLUSION: Elevated CaIA and HRA in pretransfusion sera might be attributable to adverse reactions, especially to urticaria-like manifestation. The Gi protein–coupled receptor complexes on mast cells and its ligand must be involved.

Transfusion-related adverse reactions manifest in various ways with Type I allergic reactions (from mild urticaria to anaphylaxis), accounting for a substantial percentage of such reactions. Factors present in blood products, such as antibodies to alloantigens or biologic response modifiers (RANTES, interleukin-6 [IL-6], etc.), are suggested to be involved in adverse reactions.¹ However, patient-related factors might also play a role. For example, bacterial infections are proposed as a predisposing factor for transfusion-related acute lung injury.² However, in most cases of adverse reactions including urticaria, little is known about patient factors.

Among various adverse reactions, urticaria-like manifestations are a primary concern because degranulation of mediators such as histamine from activated mast cells is regarded as a key event in the occurrence of urticaria. Degranulation is known to be initiated either by immunological triggers, a process that involves the aggregation of high-affinity receptors (FcεRI) for immunoglobulin E (IgE) by the corresponding antigens³ or by nonimmunologic triggers, which bind Gi-protein–coupled receptors (GPCRs) and activate pertussis toxin

ABBREVIATIONS: CaIA = Ca^{2+} influx–inducing activity; CRA1 = anti-human Fcε monoclonal antibody; GPCR(s) = Gi-protein–coupled receptor(s); HRA(s) = histamine-releasing activity(-ies); PC = platelet concentrate; Ptx = pertussis toxin

From the Research Division, Hokkaido Red Cross Blood Centre, Sapporo, Japan.

Address reprints requests to: Hiroshi Azuma, Hokkaido Red Cross Blood Centre, 2-2 Nishi-ku, Sapporo 063-0002, Japan; e-mail: azuma@hokkaido.bc.jrc.or.jp.

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TRANSFUSION **,***.**.

(Ptx)-sensitive Gi protein.⁴ The latter include substance P, bradykinin, and various amines such as the synthetic compound 48/80.⁵ In the case of urticaria-like reactions after blood transfusion, it is generally assumed that an antibody that is specific to some plasma protein (possibly IgE) exists in the patient and causes degranulation of mast cells when the patient undergoes transfusion. However, little clear evidence of this exists, except in particular cases, such as patients with IgA deficiency.

A substantial percentage (30%) of serum samples from patients with chronic idiopathic urticaria tested positive in an intracutaneous autologous serum skin test; some sera were shown to have histamine-releasing activity (HRA) *in vitro*.^{6,7} The possible association of these activities in sera with disease manifestation has been discussed. Based on these findings, we hypothesized that sera from patients who develop transfusion-related adverse reactions might display similar activity.

An influx of Ca^{2+} (CaI) precedes the degranulation of mast cells and can be monitored using flow cytometry.^{8,9} We recently developed a culture system for expansion of functional human mast cells from CD34+ cells.¹⁰ We decided to examine the effect of patients' sera on CaI in mast cells and compared it with that of sera derived from healthy donors or patients without adverse reactions. We then addressed whether sera that were positive for CaI activity (CaIA) have HRA. Herein, we report that a substantial percentage of pretransfusion serum samples from patients who developed transfusion-related adverse reactions did include CaIA and confirmed that the sera that were positive for CaIA had HRA.

MATERIALS AND METHODS

Collection of sera and patient characteristics

Doctors in Japan are expected to collect and store a patient's serum (pretransfusion serum) before carrying out blood transfusion. Detailed information is reported to a regional blood center if an adverse reaction should occur and if the doctor judges that it should be reported. The pretransfusion serum is sent to a blood center to determine the cause of the reaction. Fundamentally, antibodies to alloantigens or serum proteins in the serum are examined.

Pretransfusion sera derived from 145 patients (Group A; age, 1-94 years; mean age, 59 years; 86 male, 59 female) who reportedly developed febrile nonhemolytic type adverse reactions after allogeneic blood transfusions were collected. Details of those 145 patients were the following: hematologic disorder (mostly leukemia or myelodysplastic syndrome), 74; nonhematologic malignancy, 24; and other, 47. In fact, 21 of 74 patients had received pretransfusion medication such as hydrocortisone, methylprednisolone, polaramine, or glycyrrhizin. Of 24

patients, 4 had received polaramine, and 3 of 47 patients had received nonantihistaminics such as antibiotics. No record was found indicating that any patient was allergic to some substance. Among 145 patients, 130 patients received only one type of blood product (platelet concentrate [PC], 64; red blood cells [RBCs], 50; fresh-frozen plasma [FFP], 16); 2 patients received FFP and RBCs; 7 patients received PC and RBCs; and 6 patients received PC, RBCs, and FFP. Except for the 130 patients, it was unknown which blood product was responsible for the adverse reactions.

All sera were tested for antibody to alloantigen or serum protein. Antibodies to plasma protein were tested using enzyme-linked immunosorbent assay with several purified serum proteins, including IgA, IgG, IgM, and haptoglobin. Anti-human IgG was used as the second antibody in this assay. Therefore, only IgG was presumed to be detected. The remaining portions of these sera were used in this study. Sera derived from 54 patients who did not react adversely to transfusions were used as control (Group B). In addition, sera derived from 107 blood donors were used as another control (Group C). The patients in Group B were all under treatment of a hematologic disorder. Their sera were sent to a blood center for investigation of antibodies against platelets (PLTs) because they did not obtain a sufficient PLT increment after PLT transfusion. They were free from adverse reactions. All sera in Group B were negative for HLA and PLT antibody. No other information about them was available.

For Group B and Group C, sera were primarily taken to test for screening for antibody to PLT before transfusion and for routine blood screening, respectively. Again, the remaining portions of these sera were used. This study was reviewed and approved by the executive review board (functioning as an institutional review board) of our blood center.

Mast cell culture

Mast cells were cultured as described.¹⁰ CD34+ cells (5×10^4 - 20×10^4) derived from buffy coat were cocultured for 8 to 12 weeks with a human bone marrow stromal cell line (hTERT stromal cell line)¹¹ in serum-free conditioned medium, which consisted of fresh serum-free medium (QSBF60, Quality Biological, Inc., Gaithersburg, MD) and culture supernatant of hTERT stromal cells in the presence of rHu (rHu) stem cell factor (100 ng/mL) and rHuIL-6 (50 ng/mL). Every week, 80% of the conditioned medium was exchanged. Then the mast cells (3×10^5 - 10×10^5 /mL) were further cultured in fresh QSBF60 medium in the presence of rHuIL-4 (50 ng/mL, PeproTech EC Ltd., London, UK) for 3 days and used for the Ca^{2+} influx-inducing activity (CaIA) assay.

Ca²⁺ influx assay

To perform CaIA assay, 1 $\mu\text{mol/L}$ indo-1 acetoxymethyl-ester (Dojindo Laboratories, Kumamoto, Japan) was added to the culture (final concentration, 1 $\mu\text{mol/L}$) and cells were further incubated for 30 to 45 minutes in the dark. Subsequently, the cells were washed twice with Ca-free Hanks' balanced salt solution (HBSS; GIBCO, Rockville, MD) containing 0.1% human serum albumin (HSA). Finally, mast cells were suspended in RPMI 1640 medium (GIBCO) containing 0.1% HSA at a concentration of $3.33 \times 10^4/\text{mL}$ at 4°C in the dark until the assay.

First, 270 μL of the mast cell suspension ($3.33 \times 10^4/\text{mL}$) was put into a reaction tube and incubated at 37°C for 5 minutes. Then, 30 μL of the test serum was added to the sample tube and further incubated for 3 minutes. Next, cells into which Ca²⁺ had been mobilized were monitored using flow cytometry (LSR, BD Biosciences Immunocytometry System, San Diego, CA). The change of fluorescence intensity (Ca²⁺ mobilization) in each cell was fundamentally expressed as the ratio of fluorescence at FL5 (Ca²⁺-bound indo-1 acetoxymethyl-ester) to that at FL4 (Ca²⁺-free indo-1 acetoxymethyl-ester). The percentage of cells in which the FL5/FL4 ratio had been increased by the stimulant was taken as the CaIA of that stimulant. As a positive control, ionomycin (10 $\mu\text{g/mL}$) and substance P (10 $\mu\text{g/mL}$) were used. Data were analyzed using software (Cell Quest, Becton Dickinson). In addition, ethylene glycol tetraacetic acid (5 mmol/L, Sigma-Aldrich, Tokyo, Japan) was used as a negative control.

In general, when mast cells are stimulated, a rapid but transient release of Ca²⁺ from the endoplasmic reticulum stores occurs within 1 minute. This is called the first stage of Ca²⁺ mobilization. The second stage of Ca²⁺ mobilization is a sustained influx of extracellular calcium. Fluorescence intensity was monitored 3 minutes after the stimulation of mast cells. The changes of intensity were recorded and displayed. Thereby, the sustained influx of Ca²⁺ was confirmed. Furthermore, when we use calcium-free medium in the assay, only the initial elevation of intensity, which indicates calcium release from endoplasmic reticulum, was observed. It decreased soon. Therefore, what we observed must reflect an influx of extracellular calcium.

Usually, CaIA assays performed with flow cytometry are limited to 30 to 40 samples because of the number of mast cells available and time required to assay one sample. Furthermore, the mast cells used in each assay were derived from different donors. Consequently, the sensitivity of mast cells to stimuli varied among assays. Therefore, in determining the normal range of CaIA, as many as 107 serum samples from healthy donors (Group C) were assayed using mast cells from 11 subjects. More precisely, approximately 10 samples were assayed at one time: a total of 11 assays using mast cells from 11 donors were conducted. Individual values were then combined

and the mean value and standard deviation (SD) were determined. Similar to assays for Group C, assays for 145 serum samples of Group A were conducted eight times using cultures of mast cells from 8 donors. Assays for 54 serum samples of Group B were performed five times using cultures of mast cells from 5 donors.

In some experiments, Ptx (*Bordetella pertussi*, Wako Pure Chemical Industries, Ltd., Richmond, VA) was used to block stimulation via Gi-protein. Mast cells were suspended in RPMI 1640 containing 0.1% HSA at a concentration of $3.3 \times 10^4/\text{mL}$ in the presence of Ptx (0, 0.03, 0.1, 0.3 $\mu\text{g/mL}$) for 2 hours. Subsequently, cells were washed and resuspended in RPMI 1640 containing 0.1% HSA at a cell concentration of $3.33 \times 10^4/\text{mL}$. The assay for CaIA was then performed as described previously. In this experiment, a monoclonal anti-human Fc ϵ antibody (CRA1, Kyokuto Pharmaceutical Industrial Co. Ltd., Tokyo, Japan) was used (final concentration, 1 $\mu\text{g/mL}$) as a stimulus via the Fc ϵ receptor.¹²

Histamine release assay

Mast cells cultured for 8 weeks were further cultured in the presence of rHuIL-4 for 3 days to perform assay for HRA. After being washed, the cells were suspended at a density of 3.3×10^4 cells/mL in a HBSS (GIBCO) in the presence of 0.1% HSA. Then, 270 μL of the cell suspension was incubated for 10 minutes and subsequently challenged with 30 μL of serum and incubated for 2 hours. Next, the cells were centrifuged at $450 \times g$ for 10 minutes. The supernatant was collected, and then the amount of histamine was measured using a histamine assay kit (Immunotech, Marseille, France) according to the instruction manual. The cell suspension was boiled for 15 minutes and centrifuged at $450 \times g$ for 10 minutes to release all the histamine from the cells. The amount of histamine in the supernatant was then measured and designated as the total histamine content. The percentage of histamine release was calculated as (released histamine content)/(total histamine content) $\times 100$ and designated as the HRA.

Complement inactivation and immunoglobulin removal

Some sera were treated with 56°C for 30 minutes for complement inactivation or treated with protein L beads (immobilized protein L; Pierce Biotechnology, Inc., Rockford, IL) to remove immunoglobulin.

Statistical analysis

The significance of differences was assessed using a two-sided non-repeated-measures analysis of variance

(ANOVA), an unpaired t-test, and a paired t-test. p Values of less than 0.05 were inferred as significant.

RESULTS

No IgG antibody against plasma proteins such as IgA or haptoglobin was detected in any serum samples. Antibodies to HLA were detected in some samples from patients and donors. However, in no case did the donor (or patient) serum react with patient (or donor) cells by cross match testing, nor was the possibility shown of reacting with patient (or donor) cells, judging from its specificity.

The recorded symptoms included skin manifestations (wheals, rash, etc.), hypotension, fever, dyspnea, and others (nausea, vomiting, rigor, etc.). The definition of hypotension in this study was blood pressure less than 90 mmHg, or a greater than 30% decrease from the baseline, with a physician's judgment that it had resulted from transfusion. Many patients had more than one symptom. The reactions were classified with respect to type as follows: skin manifestations without hypotension (wheals or rash, $n = 63$), skin manifestations with hypotension (anaphylactic shock, $n = 25$), and hypotension without skin manifestations ($n = 34$). The reaction was also classified as fever ($n = 41$), dyspnea ($n = 37$), and others ($n = 13$; Table 1). The CaIA of serum samples from patients with adverse reactions (Group A), patients without adverse reactions (Group B), and healthy donors (Group C) are shown in Fig. 1A. No difference in CaIA between Group B and C existed, but a significant difference between Group A and Group B (or Group C; $p < 0.05$) was found. The CaIA of each category shown in Table 1 was higher than that of either Group B or Group C. However, no significant difference was found among categories. The adverse reactions were also divisible into two categories: with skin manifestations and without skin manifestations. No significant difference between the CaIA values of these two groups was found (Fig. 1B). However, when CaIA of 30 patients with pure urticaria (mean, 30.58 ± 21.3 [$n = 30$]; range, 70.43-4.1) and CaIA of 18 patients with only fever (mean, 18.61 ± 14.71 [$n = 18$]; range, 45.94-3.19) were compared, the former was higher than the latter ($p = 0.045$, unpaired t test; Fig. 2).

Setting the cutoff value for "CaIA positive" as the mean + 2SD of the CaIA of Group C, 21.43%, the percentages of sera positive for CaIA among patients with and without skin manifestations were 47.0 and 56.1% (Table 1), respectively. No difference was found between the two. In addition, the percentages of sera that turned out to be positive for CaIA among Groups A, B, and C were 51.0% (74/145), 5.6% (3/54), and 6.5% (7/107), respectively (Fig. 1 and Table 1). Furthermore, a substantial percentage of sera samples among each type of reaction were found to be positive for CaIA (Table 1).

TABLE 1. Percentage of CaIA-positive patients for each type of reaction

	Total	Type of reaction*					
		Skin manifestation (+)	Skin manifestation (-)	Skin manifestation without hypotension	Skin manifestation with hypotension	Hypotension	Others§
Number of patients	145	88	57	63	25	34	13
Number of positive cases (%)	74 (51.0)	41 (47.0)	32 (56.1)	34 (54.0)	7 (28.0)	14 (41.1)	8 (35.1)

* Many patients had two or more symptoms. CaIA positivity was calculated for each type of reaction written in the upper column.

† Only fever was observed in 18 patients.

‡ Five with skin rash, 8 with hypotension, and 7 with fever.

§ Nausea, vomiting, chilliness, lumbago, etc.

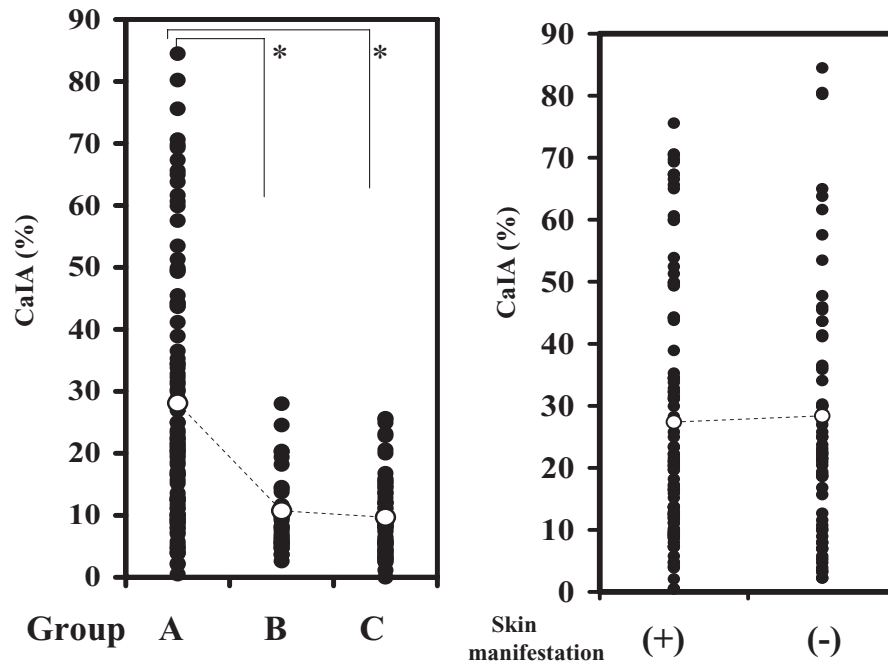


Fig. 1. (A) CaIA of patients with or without reactions and of normal donors. Group A = patients with reactions, $27.68 \pm 20.38\%$, $n = 145$, range 0.49-84.90. Group B = patients without reactions, $10.70 \pm 6.50\%$, $n = 54$, range 2.63-36.97. Group C = normal donors, $9.67 \pm 5.88\%$, $n = 107$, range 1.11-25.68. * $p < 0.05$, non-repeated-measures ANOVA. (B) CaIA of patients with or without skin manifestations. Group A patients were divided into two subgroups: with skin manifestations such as urticaria and rashes ($27.38 \pm 20.40\%$, $n = 88$) and without skin manifestations ($28.38 \pm 20.59\%$, $n = 57$).

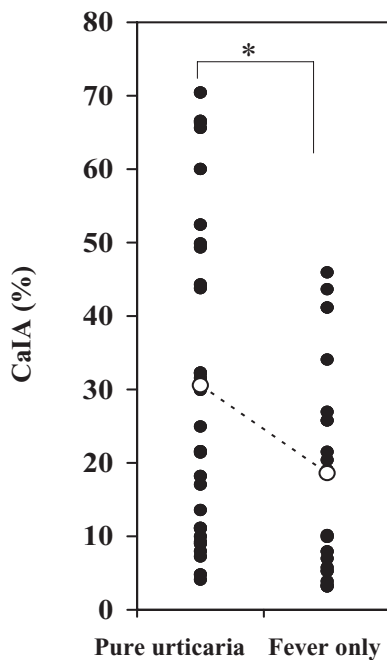


Fig. 2. CaIA of patients with pure urticaria and only fever. CaIA of 30 patients with pure urticaria (mean, 30.58 ± 21.3 [$n = 30$]; range, 70.43-4.1) was higher than those of 18 patients with fever alone (mean, 18.61 ± 14.71 [$n = 18$]; range, 45.94-3.19; $p < 0.05$, unpaired t test).

To elucidate whether the influx of Ca²⁺ was induced dose-dependently, five serum samples that had been shown previously to possess elevated CaIA were picked from Group A, serially diluted, and assayed for CaIA. In all samples tested, CaIA was observed dose-dependently (Fig. 3A).

To elucidate the mechanism by which the influx of Ca²⁺ was induced by the CaIA-positive sera, 10 samples that had been shown previously to possess elevated CaIA were selected randomly from Group A and the effect of Ptx, a Gi-protein blocker, on their CaIA was examined. As depicted in Fig. 3B, along with the influx of Ca²⁺ induced by substance P, the Ca²⁺ influx induced by 9 of 10 serum samples was clearly blocked dose-dependently by Ptx. In contrast, the Ca²⁺ influx induced by CRA1 was not blocked at all.

Ten CaIA-positive samples and 10 CaIA-negative samples from Group A, 10 samples from Group B, and 10 samples from Group C were selected randomly and tested for HRA. The CaIA-positive serum samples from Group A showed significantly higher levels of HRA than the others ($p < 0.001$; Fig. 4A). Furthermore, the release of histamine by these 10 samples was blocked in the presence of Ptx (Fig. 4B).

Six samples were treated with protein L beads to remove immunoglobulin. However, substantial activity of CaI remained (Table 2A). Additionally, heat treatment did

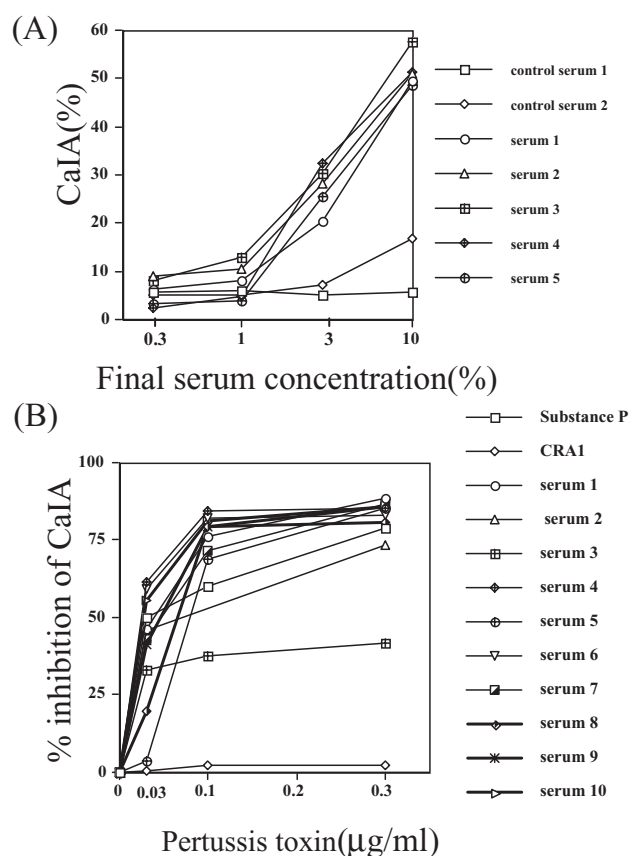


Fig. 3. (A) Dose-response curves of CaIA-positive sera from Group A. Five sera known to be positive for CaIA were diluted; 30 μL of original or diluted serum was added to 270 μL of a mast cell suspension. (B) Inhibitory effects of Ptx on Ca^{2+} influx induced by sera. Mast cells were stimulated with sera positive for CaIA from Group A in the presence of various concentrations of Ptx. The inhibitory effect of Ptx was expressed as percentage inhibition. Except for serum 3, the CaIA levels of 9 of 10 sera were inhibited by Ptx. CRA1, anti-human Fc ϵ R monoclonal antibody.

not affect the elevated activity of CaI of five samples with adverse reactions. Consequently, the factors in question appeared to be resistant to heat treatment (Table 2B).

DISCUSSION

Results of this study revealed that CaIA levels were higher in the sera from patients with adverse reactions (Group A) than in the sera from patients without adverse reactions (Group B) or from healthy donors (Group C; Fig. 1). Furthermore, approximately 50% of Group A had a CaIA level higher than the cutoff value. These findings suggest that the elevated level of CaIA in serum is related somehow with the occurrence of adverse reactions. It was of greater interest that when CaIA of 30 patients with pure urticaria and CaIA of 18 patients having only fever were compared,

the former was higher than the latter ($p < 0.05$), suggesting that elevated CaIA activity is attributed preferentially to the occurrence of urticaria-like reactions (Fig. 2).

The influx of Ca^{2+} was induced in a serum concentration-dependent manner (Fig. 3A) in five tested samples. Therefore, this response might result from the physiologic action of some factor in serum. Based on the observation that the CaIA of 9 of 10 samples tested was almost completely inhibited by Ptx, but that of CRA1 was not (Fig. 3B), the CaIA exerted by sera must be triggered through GPCRs.^{3,4} Therefore, the factor in question is likely to be a ligand of GPCRs. Inhibition was incomplete in serum 3, perhaps reflecting the involvement of Fc receptor-mediated stimulation in this case.

Because an influx of calcium is known to precede the release of histamine from mast cells,^{8,9} we speculated that CaIA-positive serum samples have the potential to induce histamine release, even if it does not cause maximum release. As speculated, the 10 CaIA-positive serum samples from Group A exhibited higher levels of HRA than the CaIA-negative samples (Fig. 4A). Furthermore, their activity was blocked by Ptx (Fig. 4B), suggesting a direct relation between CaIA and HRA. It is therefore concluded that the pretransfusion sera of some of the patients who developed adverse reactions do have CaIA and HRA. Sera from some patients with chronic idiopathic urticaria have been shown to possess HRA,^{6,7} which might support the idea described above: that elevated CaIA is preferentially attributable to the occurrence of a urticaria-like reaction. In other words, in patients with enhanced CaIA, mast cells might be preactivated to a certain degree beforehand and tend to be degranulated when an allogeneic blood product is transfused. However, it remains unknown what factor in the blood product triggers mast cell degranulation.

It is surprising that no significant difference was found in CaIA between patients with and without urticaria-like symptoms (Fig. 1B) and that a considerable number of serum samples in each adverse reaction type were shown to be positive for CaIA with the highest percentage (61%) among "others" (Table 1). The following explanation can be advanced: biologic active factors that differed from the factor in question coexist in the serum. Alternatively, the factor in question might induce Ca^{2+} influx on other cells, resulting in the occurrence of multiple manifestations of adverse reactions.

Whether the increase of serum CaIA was temporary or not, and the exact ligand and GPCR responsible for increase of CaIA, remain unknown at present. Nevertheless, it is unlikely that a component of the complement or immunoglobulin play a major role because substantial CaIA remained after heat treatment or immunoglobulin removal (Table 2). Involvement of IgE can also be excluded because Ptx eliminated CaIA completely (Fig. 3B). Moreover, substance P might not be involved

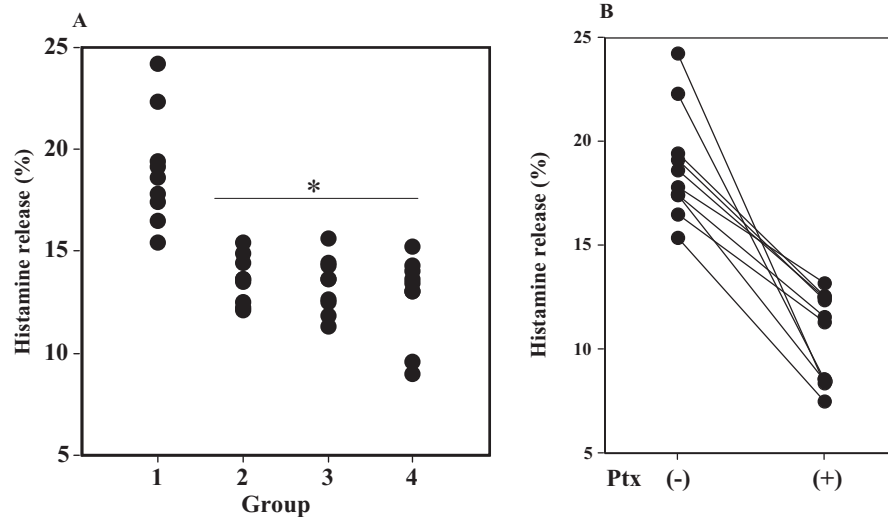


Fig. 4. (A) HRA. Group 1 = CaIA-positive sera from Group A; Group 2 = CaIA-negative sera from Group A; Group 3 = sera from Group B; Group 4 = sera from Group C. The HRAs of each group were $18.8 \pm 2.7\%$ ($n = 10$), $13.9 \pm 1.5\%$ ($n = 10$), $13.2 \pm 1.3\%$ ($n = 10$), and $13.0 \pm 2.2\%$ ($n = 10$), respectively. The HRA of Group 1 was significantly higher ($p < 0.001$, non-repeated-measures ANOVA) than that of Group 2, 3, or 4. (B) Blocking of HRA by Ptx. Simultaneously, 10 sera from Group 1 were mixed with mast cells pretreated with Ptx ($1 \mu\text{g/mL}$) to block the Gi-subunit; HRA was then assayed. The HRAs of all sera were clearly blocked by Ptx ($p < 0.001$, paired t test). All assays were performed simultaneously using the same mast cells.

TABLE 2. Effect of treatment on CaIA*

Sample	Before	After	Percentage reduction
A. Protein L beads			
1	42.9	32.4	24.4
2	11.6	16	0
3	53.9	42	22.0
4	55.8	44.5	20.0
5	57.2	38.4	32.9
6	53	36.5	31.0
B. Heat			
1	31.6	19.7	37.6
2	75.9	67.0	11.7
3	44.9	59.4	0
4	26.3	31.4	0
5	22.4	38.7	0

* Six samples were treated with protein L beads. On average, recoveries of IgG, IgA, and IgM were 12.1, 14.5, and 14.6%, respectively. Therefore, approximately 85% or more of each class of immunoglobulin was removed by protein L treatment. However, substantial activity of Cal was shown to remain. (B) Heat treatment (56°C , 30 minutes) did not affect CaIA of five samples with adverse reaction.

because we failed to see an apparent synergistic effect between substance P and CaIA positive sera (data not shown).

In conclusion, pretransfusion sera from some patients who reacted adversely to transfusions exhibited elevated levels of CaIA and HRA in mast cells. This activity might be one factor attributable to the manifestation of adverse reactions, especially urticaria-like reaction.

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CONFLICT OF INTEREST

The authors declare no competing financial interest.

REFERENCES

- Muyll L. The role of cytokines in blood transfusion reactions. *Blood Rev* 1995;9:77-83.
- Silliman CC, Ambruso DR, Boshkov LK. Transfusion-related acute lung injury. *Blood* 2005;105:2266-73.
- Segal DM, Taurog J, Metzger H. Dimeric immunoglobulin E serves as a unit signal for mast cell degranulation. *Proc Natl Acad Sci U S A* 1977;74:2993-7.
- Aridor M, Rajmilevich G, Beaven MA, Sagi-Eisenberg R. Activation of exocytosis by the heterotrimeric G protein Gi3. *Science* 1993;262:1569-72.
- Lagunoff D, Martin TW, Read G. Agents that release histamine from mast cells. *Annu Rev Pharmacol Toxicol* 1983; 23:331-51.

6. Asero R, Tedeschi A, Lorini M, Salimbeni R, Zanoletti T, Miadonna A. Chronic urticaria: novel clinical and serological aspects. *Clin Exp Allergy* 2001;31:1105-10.
7. Wedi B, Novacovic V, Koerner M, Kapp A. Chronic urticaria serum induces histamine release, leukotriene production, and basophil CD63 surface expression—inhibitory effects of anti-inflammatory drugs. *J Allergy Clin Immunol* 2000; 105:552-60.
8. Ozawa K, Szallasi Z, Kazanietz MG, Blumberg PM, Mischak H, Mushinski JF, Beaven MA. Ca^{2+} -dependent and Ca^{2+} -independent isozymes of protein kinase C mediate exocytosis in antigen-stimulated rat basophilic RBL-2H3 cells. Reconstitution of secretory responses with Ca^{2+} and purified isozymes in washed permeabilized cells. *J Biol Chem* 1993;268:1749-56.
9. Baba Y, Nishida K, Fujii Y, Hirano T, Hikida M, Kurosaki T. Essential function for the calcium sensor STIM1 in mast cell activation and anaphylactic responses. *Nat Immunol* 2008;9:81-8.
10. Yamaguchi M, Azuma H, Fujihara M, Hamada H, Ikeda H. Generation of a considerable number of functional mast cells with a high basal level of $\text{Fc}\epsilon\text{RI}$ expression from cord blood CD34+ cells by co-culturing them with bone marrow stromal cell line under serum-free conditions. *Scand J Immunol* 2007;65:581-8.
11. Kawano Y, Kobune M, Yamaguchi M, Nakamura K, Ito Y, Sasaki K, Takahashi S, Nakamura T, Chiba H, Sato T, Matsunaga T, Azuma H, Ikebuchi K, Ikeda H, Kato J, Niitsu Y, Hamada H. Ex vivo expansion of human umbilical cord blood hematopoietic progenitor cells using a coculture system with human telomerase catalytic subunit (hTERT)-transfected human stromal cells. *Blood* 2003;101:532-40.
12. Gounni AS, Lamkhioed B, Ochiai K, Tanaka Y, Delaporte E, Capron A, Kinet JP, Capron M. High-affinity IgE receptor on eosinophils is involved in defense against parasites. *Nature* 1994;367:183-6. 