

Immunoglobulin E oligomers identified in blood components activate mast cells: relevance to anaphylactic transfusion reaction

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BACKGROUND: In most cases of anaphylactic transfusion reaction, the mechanisms underlying its development are unclear. We found a donor whose transfused blood components were implicated in two cases of anaphylactic transfusion reaction, and we found that the donor plasma showed mast cell degranulation activity.

STUDY DESIGN AND METHODS: The donor plasma was examined to identify the mast cell-activating factors in it. Cultured mast cells prepared from cord blood were used for in vitro degranulation assay. Serum prepared from the donor plasma was fractionated by three-step chromatography using mast cell degranulation activity as a marker. The fractions selected from the third step of chromatography were analyzed by mass spectrometry after separation by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The characteristics of the identified proteins and other plasma samples that had been donated by the donor over several years were examined.

RESULTS: Two plasma proteins of high molecular weight were detected in the anion-exchange fractions and identified as human immunoglobulin (Ig)Es of 500 kDa and higher. The mast cell degranulation activity of the IgEs decreased in the presence of monomeric human IgE as well as an anti-human IgE antibody. Mast cell degranulation activity was detected in the donor plasma since January 4, 2002, when the first case was reported.

CONCLUSION: We identified high-molecular-weight IgEs as the mast cell-activating factors in the donor plasma. Results of analysis suggest that these IgEs were dimeric and trimeric and that they directly activated the transfusion recipient's mast cells by triggering the crosslinking of Fcε receptor I, thereby inducing an anaphylactic transfusion reaction.

Anaphylaxis is an acute, potentially life-threatening systemic reaction with various mechanisms, clinical presentations, and severity that result from the sudden systemic release of mediators from mast cells and basophils.¹ Severe anaphylaxis induced by transfusion, which is generally termed anaphylactic transfusion reaction, is one of the serious complication of therapeutic strategies for patients. The clinical features of anaphylactic transfusion reaction are cutaneous, respiratory, cardiovascular, and/or gastrointestinal reaction accompanied by intractable hypotension or shock, which appear during or immediately after transfusion. Many possible mechanisms of

ABBREVIATIONS: anti-hIgE pAb = anti-human immunoglobulin E polyclonal antibody; CBMC(s) = cord blood–derived mast cell(s); FcεRI = Fcε receptor I; HMW IgE(s) = high-molecular-weight immunoglobulin E(s); JRCs = Japanese Red Cross Society; PC(s) = platelet concentrate(s).

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anaphylactic transfusion reaction have been proposed; however, the cause of the reaction remains unclear in most cases.

The Japanese Red Cross Society (JRCS) has been collecting reports of transfusion reaction cases throughout Japan since 1992 to improve the safety of transfusion.² Anaphylactic transfusion reaction is one of the major transfusion reactions, and more than 200 cases of this reaction are reported each year in Japan, all of which were determined by a detailed symptom analysis.³ JRCS also asks hospitals to provide patient plasma or serum samples and the remaining blood components implicated in the transfusion reaction for analysis of their adverse effects. Several tests, including tests for deficiencies of haptoglobin and immunoglobulin (Ig)A and antibodies against them, are performed to identify the possible factors for transfusion reaction.^{2,4}

Tryptase concentration in patient samples collected before and after the onset of an anaphylactic transfusion reaction is also measured as a marker of systemic activation and degranulation of patient mast cells.⁵⁻⁷ A significant increase in tryptase concentration was observed after the onset of this transfusion reaction, indicating that the activation and degranulation of mast cells during anaphylactic transfusion reaction play an essential role.⁸ Hence, an *in vitro* assay of mast cell degranulation activity was considered to be useful for clarifying the causative factors for anaphylactic transfusion reaction in transfusion recipients or transfused blood components.

Using this assay, we preliminarily found that several plasma samples, which were prepared from the blood components transfused in the cases in which the tryptase concentration in patient plasma or serum increased after the transfusion reaction, activated mast cells, suggesting that there were some mast cell-activating factors in these blood components. One of these samples was obtained from a donor who has donated two bags of platelet concentrate (PC) associated with two cases of anaphylactic transfusion reaction. In this study, we analyzed this donor plasma to identify the mast cell-activating factors and examine the characteristics of the identified factors.

CASE REPORT

Two cases of anaphylactic transfusion reaction involving two bags of PC obtained from an index donor were reported to JRCS. All data recorded during transfusion were based on the medical records written by the doctors who treated each case.

Index donor

The index donor was a 24-year-old male with no subjective symptoms such as allergy. He had donated blood more than 30 times between 1997 and 2001. PC, red blood

cells (RBCs), and fresh-frozen plasma had been prepared from his blood and transfused into some recipients. No cases of transfusion reaction had been reported to JRCS before the two cases of anaphylactic transfusion reaction after transfusion with his blood were reported. After these cases, he donated only source plasma for fractionation.

Blood counts and results of blood chemistry tests of his blood collected in 2002 were essentially normal. The concentrations of IgA and IgG in his sera were, respectively, 77.6 and 773 mg/dL on January 4, 2002, and 86.5 and 631 mg/dL on February 7, 2002, which were slightly lower than the normal concentrations (110-410 and 870-1700 mg/dL, respectively). In contrast, the total IgE concentrations in his sera were 149,000 and 156,000 ng/mL on January 4 and February 7, 2002, respectively, which were higher than the normal concentration (lower than 415 ng/mL). Antibodies against 14 plasma proteins including haptoglobin and IgA were absent.

Case 1

The first case was reported on January 6, 2002. The recipient was a 71-year-old female with chronic disseminated intravascular coagulation and idiopathic hypothyroidism after undergoing surgery for thoracic aortic aneurysm. She received transfusion of 8600 mL of leukoreduced RBCs and 8400 mL of PC over 7 months. She experienced febrile reaction after PC transfusion once. She had no history of allergies. On January 6, 2002, two bags of gamma-irradiated PCs were transfused to her, one of which was donated by the index donor, because of uncontrolled hemorrhage after a fall. At the start of transfusion, her blood pressure was 144/68 mmHg. She complained of itchiness throughout her back at the end of transfusion, which was 2 hours after the start. She was administered a corticosteroid intravenously. Her blood pressure was 83/41 mmHg and decreased further to 56/32 mmHg 30 minutes later. She was administered dopamine. She was diagnosed as having anaphylactic transfusion reaction. Her clinical manifestations including itchiness and reduced blood pressure several hours after the start of PC transfusion satisfied the diagnostic criteria of anaphylaxis according to Lieberman and coworkers.¹

No antibodies against 15 plasma proteins were detected in her serum sample after the transfusion. Haptoglobin was detected in the sample by the sensitive enzyme-linked immunosorbent assay with a detection limit of 3 µg/dL.⁹ The IgA concentration in the sample was 250 mg/dL. She was neither haptoglobin- nor IgA-deficient. The total IgE concentration in the sample was 4100 ng/mL.

Case 2

The second case was reported on February 9, 2002. The recipient was a 49-year-old female with acute leukemia

transformed from myelodysplastic syndrome. She had a history of transfusion with PC twice for thrombocytopenia due to consolidation therapy. She had no history of allergies. A bag of gamma-irradiated PC was transfused, which was donated by the index donor, at a rate of 1 mL/min. Seven minutes after the start of transfusion, she started to feel itchy and her face turned red. The transfusion was stopped. After 2 minutes, hives appeared on her arms and legs and she felt chest tightness. Her systolic blood pressure decreased to less than 60 mmHg and saturation of peripheral oxygen was less than 90%. Her clinical manifestations including itchiness and reduced blood pressure several minutes after the start of PC transfusion also satisfied the diagnostic criteria of anaphylaxis.

Antibodies against 15 plasma proteins were not detected in her serum sample. The concentrations of haptoglobin and IgA in the sample were 41 and 316 mg/dL, respectively. The total IgE concentrations were less than 25 and 790 ng/mL before and after the transfusion, respectively. The tryptase concentrations were 2.22 and 18.4 µg/L before and after the transfusion, respectively.

MATERIALS AND METHODS

Plasma and serum

Plasma collected from the index donor on June 22, 2004, was analyzed to identify the responsible mast cell-activating factors. The other 15 plasma samples collected from the index donor between 1997 and 2008 were examined for mast cell degranulation activity and total IgE concentration. The index donor provided written informed consent to participate in the study. Control samples were collected from healthy donors. Ten serum samples with a high IgE concentration collected from atopic individuals were also examined. To prepare serum samples, plasma was incubated with 40 mmol/L CaCl₂ at 37°C for 2 hours and subsequently at 4°C overnight. Before degranulation assay, plasma and serum samples were dialyzed against saline overnight using dialysis cassettes (Slide-A-Lyzer 3.5K MWCO, Thermo Fisher Scientific, Kanagawa, Japan) at 4°C, and 8 mg/mL sodium heparin was added to the plasma.

Cord blood–derived mast cells

Cord blood–derived mast cells (CBMCs) are generally used for the *in vitro* study of mast cells.¹⁰ CBMCs were cultured from umbilical cord blood obtained from the Metro Tokyo Red Cross Cord Blood Bank and were prepared for degranulation assay using a modified method reported by Saito and colleagues.¹¹ Written informed consent was obtained from mothers for use of the cord

blood in transplantation- or transfusion-related studies in cases when the cell number is not sufficiently high for transplantation. A mononuclear cell layer isolated from a bag of cord blood was cultured at 2×10^6 to 2.5×10^6 cells/mL in RPMI 1640 (Life Technologies Japan, Tokyo, Japan) containing 10% (vol/vol) fetal bovine serum (SH30071.03, Hyclone Laboratories, Logan, UT), 50 ng/mL stem cell factor (300-07, PeproTech, Rocky Hill, NJ), and 50 ng/mL interleukin (IL)-6 (200-06, PeproTech) at 37°C in 5% CO₂ for 9 to 12 weeks. After more than 98% of cells were stained metachromatically with toluidine blue, CBMCs were cultured with 20 ng/mL IL-4 (CYT-211, ProSpec, Rehovot, Israel) for an additional week to induce Fcε receptor I (FcεRI) expression.¹² The cells were collected by centrifugation and washed with a degranulation buffer (20 mmol/L HEPES, 260 mmol/L NaCl, and 10 mmol/L KCl, pH 7.4) for degranulation assay or with the buffer diluted twice with ultrapure water for the assay of gel filtration fractions.

Assessment of mast cell degranulation activity

Mast cell degranulation activity was assessed using the percentage release of tryptase or β-hexosaminidase from activated CBMCs during degranulation assay. Sixty microliters of a sample solution containing 0.18% (wt/vol) dextrose, 2.6 mmol/L CaCl₂, 1.8 mmol/L MgCl₂, and 0.18% (wt/vol) bovine serum albumin was plated onto a 96-well plate, and 5×10^3 CBMCs suspended in 50 µL of the degranulation buffer were added to each well. After incubation at 37°C for 40 minutes, the supernatant of each well was collected and tryptase concentration or β-hexosaminidase activity was measured. Tryptase concentration in plasma and serum samples was measured using an automatic immunoanalyzer (UniCAP 100, Phadia, Tokyo, Japan). β-Hexosaminidase activity in purified protein fractions was measured following the method of Matsushima and coworkers.¹³ The percentage release of tryptase or β-hexosaminidase was calculated as $[(A - B)/(T - B) \times 100]$, where A is the concentration or activity of the enzyme in the supernatant in the presence of the test sample, B is that in the supernatant in the absence of the test sample, and T is the total concentration or activity of the enzymes from CBMCs lysed by three freeze-and-thaw cycles. When gel filtration fractions were assayed, A' was used instead of A ($A' = A - \text{the } \beta\text{-hexosaminidase activity in the fraction itself}$). To examine the effects of an anti-human IgE polyclonal antibody (anti-hIgE pAb; 01-10-04, KPL, Gaithersburg, MD), monomeric human IgE (hIgE; 16-16-090705, Athens & Technology, Athens, GA), and monomeric human IgG (hIgG; I4506, Sigma-Aldrich Japan, Tokyo, Japan) on mast cell degranulation activity, samples were incubated with each protein for 30 minutes at room temperature and then assayed.

Isolation of mast cell-activating factors by chromatography

The mast cell-activating factors in the index donor plasma were isolated by three-step chromatography using a liquid chromatography system (ÅKTApurifier GE Healthcare Japan, Tokyo, Japan) at 4°C. Two milliliters of the index donor serum was separated by gel filtration chromatography on a Superose 12 column (HR 16/50 column, GE Healthcare Japan) using 10 mmol/L HEPES (pH 7.4) containing 130 mmol/L NaCl and 5 mmol/L KCl. The gel filtration fractions that showed mast cell degranulation activity were pooled and diluted three times with ultra-pure water. The pooled fraction (5 mL) was separated by cation-exchange chromatography on a cation-exchange bead (MONO S 4.6/100 PE column, GE Healthcare Japan) using 20 mmol/L sodium phosphate, pH 6.0, with a 0 to 0.6 mol/L NaCl linear gradient. The fractions that showed degranulation activity were pooled and diluted similarly to the gel filtration fractions, and 5 mL of the pooled cation-exchange fraction was separated by anion-exchange chromatography on an anion-exchange column (MONO Q 4.6/100 PE, GE Healthcare Japan) using 20 mmol/L Tris/HCl, pH 7.0, with a 0 to 0.5 mol/L NaCl linear gradient. The anion-exchange fractions that showed the activity were selected for identification of mast cell-activating factors.

Identification of mast cell-activating factors

Nonreducing sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed using 7.5 µL of the selected anion-exchange fractions. For mass spectrometry, the electrophoresed gel was stained using a silver stain kit for mass spectrometry (24600, Thermo Fisher Scientific). The detected protein bands were excised from the gel and the corresponding proteins were identified by matrix-assisted laser desorption/ionization tandem mass spectrometry after in-gel tryptic digestion (Hitachi High-Technologies, Tokyo, Japan). The mass spectrum of the peptides derived from tryptic-digested monomeric hIgE from myeloma plasma (16-16-090705M, Athens & Technology) was also measured as the mass spectrum of standard monomeric hIgE. For Western blot analysis, the separated proteins on the gel were electrotransferred onto a transfer membrane (Immobilon-P, Nihon Millipore, Tokyo, Japan). After blocking, IgE was detected using a horseradish peroxidase–conjugated anti-hIgE (P0295, Dako Japan, Tokyo, Japan) and Western blotting detection reagents (ECL Plus, GE Healthcare Japan).

Visualization of mast cell-activating factors

To examine whether the mast cell-activating factors in the index donor were present, gel filtration chromatography

of three serum samples (200 µL) from the index donor collected on February 7, 2002, June 6, 2004, and August 28, 2008, was performed as described above. Gel filtration fractions A30 to A32 of each sample that presumably contained the mast cell-activating factors were selected, and IgE was detected by Western blot analysis.

Measurement of IgE concentration

IgE concentration in plasma, serum, and other samples was determined by peak-rate nephelometry using an automated immunochemistry system (Image 800, Beckman Coulter, Tokyo, Japan).

Statistical analysis

The mean percentage release of tryptase or β -hexosaminidase was compared using the two-tailed t test. A p value of less than 0.05 was considered significant.

RESULTS

Mast cell degranulation activity in index donor plasma and serum

Plasma collected from the index donor on June 22, 2004, showed mast cell degranulation activity (Fig. 1). The activation pattern of the plasma showed a bell-shaped curve with the maximum activity observed at a plasma volume of 2.5 µL in 110 µL of assay solution. A similar curve was observed for the index donor serum. On the other hand, the plasma and serum samples collected from five healthy donors did not activate CBMCs; the curves for one of the donors are shown in Fig. 1.

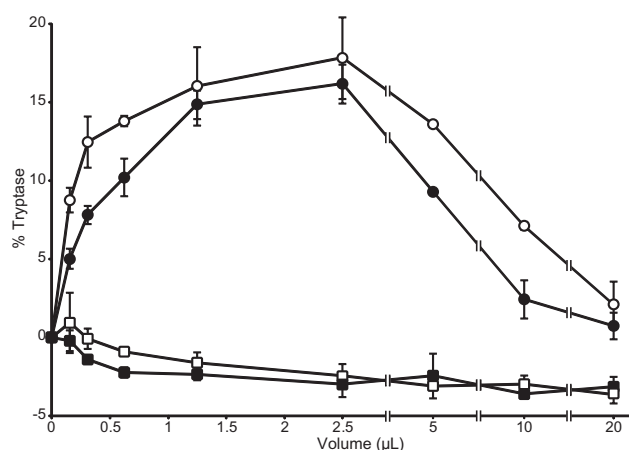


Fig. 1. Mast cell degranulation activity in index donor plasma and serum. CBMC degranulation assay was performed on the index donor plasma (●) collected on June 22, 2004, and serum (○) prepared from the plasma and on plasma (■) and serum (□) collected from a healthy donor. Mean $\pm$ SD of percentage of tryptase release is plotted. $n = 2$.

Isolation of mast cell-activating factors from index donor serum

As shown in Fig. 2A, mast cell degranulation activity in the index donor serum was detected in A29 to A34 fractions in

the gel filtration chromatogram, which were considered to contain plasma proteins of high molecular weight. Gel filtration fractions A30 to A32 were collected for the next step as a pooled fraction containing the mast cell-activating factors, separating other medium- and

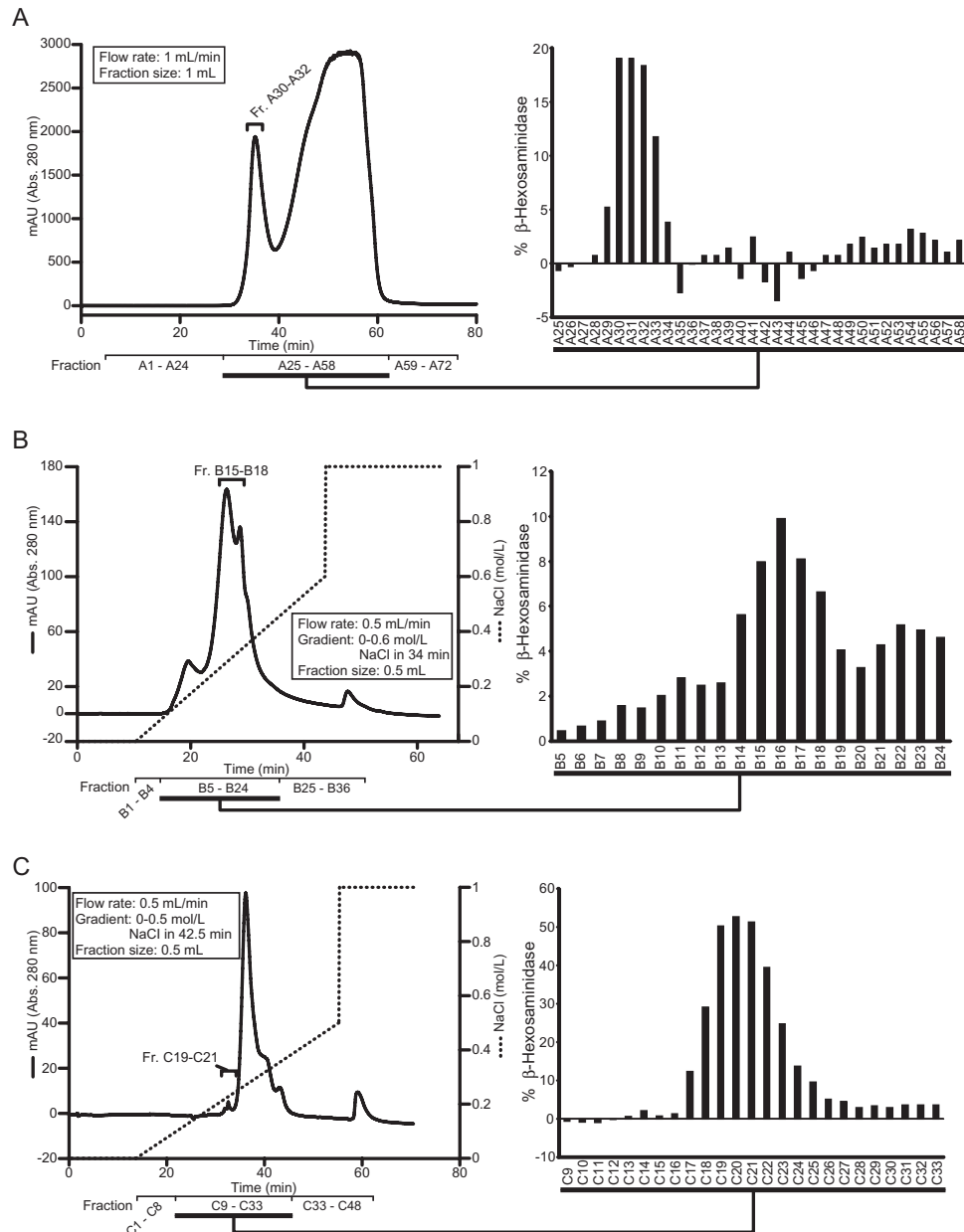


Fig. 2. Isolation of mast cell-activating factors from index donor serum by three-step chromatography. To isolate the mast cell-activating factors, gel filtration chromatography (A), cation-exchange chromatography (B), and anion-exchange chromatography (C) were performed sequentially using serum prepared from the index donor plasma collected on June 22, 2004, as a starting material. The chromatograms are shown in the graph on the left. The solid line represents OD 280. The dashed line represents the NaCl concentration in the elution buffer. The numbers of fractions are indicated at the bottom of each chromatogram, and 50 μ L of each of the underlined fractions was tested for mast cell degranulation activity. The fractions marked Fr. A30-A32 in A and Fr. B15-B18 in B were pooled and collected as the mast cell-activating factor-containing fractions from each step of chromatography. The histograms on the right show the mast cell degranulation activity in each fraction presented as the percentage of β -hexosaminidase release in degranulation assay.

low-molecular-weight plasma protein contaminants. In the cation-exchange chromatogram, a high β -hexosaminidase activity was detected at the top of the main protein peak, although a relatively low activity was also observed depending on the salt concentration of the elution buffer (Fig. 2B). Cation-exchange fractions B15 to B18 were collected for the next step. The third anion-exchange chromatography was effective in separating the factors from the other contaminants. Mast cell degranulation activity was detected in fractions C18 to C22, which were separated from the left shoulder of the main protein peak (Fig. 2C). Anion-exchange fractions C19 to C21 were further analyzed to identify the mast cell-activating factors.

Identification of mast cell-activating factors contained in anion-exchange fractions

Two bands corresponding to proteins of approximately 500 kDa (Band 1) and higher (Band 2) were detected in anion-exchange fractions C19 to C21 by nonreducing SDS-PAGE (Fig. 3A). These proteins were analyzed by mass spectrometry. In the comparison between the mass spectra of the tryptic peptides derived from the two bands (Fig. 3BI and 3BII) and standard monomeric hIgE (Fig. 3BIII), eight peptide peaks derived from IgE were detected in all mass spectra, showing that one of the proteins corresponding to the two bands was hIgE. The six other peptides derived from Band 1 were also detected in the mass spectrum of the peptides derived from Band 2 but not standard monomeric hIgE (Fig. 3B, Peptide Peaks #a to #f). Because of their identification as immunoglobulin chains, they were considered to be derived from IgE (Fig. 3BIV). Four of the six protein-matched peptides corresponded to a human Ig heavy-chain variable region or a human immunoglobulin κ chain variable region that contained a complementarity-determining region, indicating that both IgEs corresponding to the two bands might be produced by the same cell clone. The amino acid sequence of Peptide Peak #e was identified as a human immunoglobulin κ chain constant region, the first leucine of which was one of the amino acids constituting Km1 or Km1.2.¹⁴ The orthologous identification of Peptide Peak #f is probably due to the substitution of tryptophan by glycine in the peptide sequence because the sequence was otherwise identical to that of the Peptide Peak #g derived from standard monomeric hIgE. Other peptide peaks were assigned to trypsin or were not assigned to any proteins. By Western blot analysis, two bands corresponding to high-molecular-weight IgEs (HMW IgEs) were detected at the same position as the two bands visualized by silver staining (Fig. 3C). HMW IgEs were confirmed as the mast cell-activating factors because preincubation with anti-hIgE pAb decreased the mast cell degranulation activity in the anion-exchange fraction C20 (10 μ L) in a dose-dependent manner (Fig. 3D).

Effect of monomeric hIgE on mast cell degranulation activity in index donor

Mast cell degranulation activity in the anion-exchange fraction C21, which contained 11 ng of HMW IgEs per 10 μ L, in the presence of monomeric hIgE was measured. The activity was decreased by monomeric hIgE in a dose-dependent manner, whereas monomeric hIgG did not affect the activity (Fig. 4, circles). Incubation with monomeric hIgE also decreased the mast cell degranulation activity in the index donor plasma (Fig. 4, squares).

Correlation between mast cell degranulation activity and presence of HMW IgEs in index donor

Among 16 samples collected from the index donor between 1997 and 2008, mast cell degranulation activity was first observed on January 4, 2002, when the PC associated with anaphylactic transfusion reaction in the first case was donated (Fig. 5A). Since then, the activity was present in all 10 plasma samples and increased with time. HMW IgEs were detected by gel filtration followed by Western blot analysis in all of the three tested serum samples obtained between 2002 and 2008, including that prepared from the PC involved in the second case (Fig. 5B). The total IgE concentration was slightly higher than the normal concentration on June 4, 1997 (547 ng/mL). It increased markedly from February 4, 2000 (2500 ng/mL); on October 20, 2005, it was 712,000 ng/mL. To study the effect of total IgE concentration on mast cell degranulation activity, serum samples collected from 10 atopic individuals with high total IgE concentrations (18,000–1,180,000 ng/mL) were examined. The activity was not detected in any of these serum samples, although trace amounts of HMW IgEs were detected in a sample with a total IgE concentration of 1,180,000 ng/mL (data not shown). All of these data strongly suggest that mast cell degranulation activity occurs because of the high concentration of HMW IgEs but not total IgE. This was a unique feature of the index donor blood. We advised the index donor to undergo a medical check-up, although he was asymptomatic. He was diagnosed as having IgE κ -type multiple myeloma in an early stage on May 30, 2009.

DISCUSSION

The activation of mast cells plays a key role in anaphylactic reaction.^{15,16} We reported that mast cell activation is also involved in anaphylactic transfusion reaction, although the activation mechanism remains unclarified.⁸ Two bags of PC obtained from the same donor were reported as the causative blood component in two different cases of anaphylactic transfusion reaction involving two distinct recipients. The plasma prepared from one of these blood components showed direct mast cell

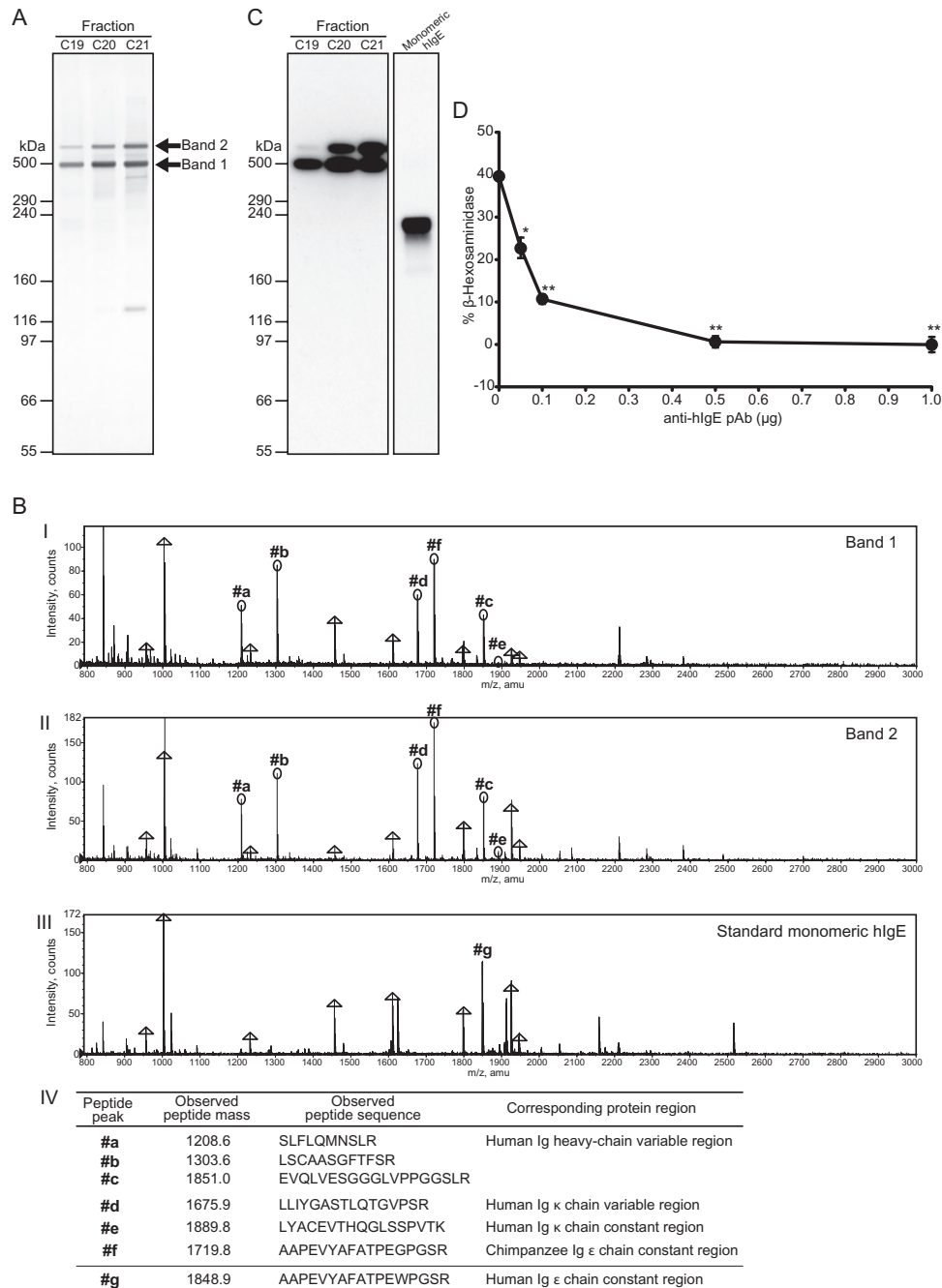


Fig. 3. Identification of mast cell-activating factors contained in anion-exchange fractions. (A) SDS-PAGE. Anion-exchange fractions C19 to C21 were analyzed by nonreducing SDS-PAGE followed by silver staining. (B) Mass spectrometry. The two protein bands, Band 1 and Band 2, indicated in A, were analyzed by tandem mass spectrometry. The mass spectra of peptides derived from these bands are shown in the panels (I and II), along with that of standard monomeric hIgE (III) for comparison. The peaks marked by triangles (Δ) are those of peptides that were derived from IgE and detected in all three mass spectra. Six peptide peaks marked by circles ($\circ$) are unique peaks detected only in Band 1 and Band 2. The amino acid sequences and the corresponding protein regions of these peptide peaks and Peptide Peak #g are listed in the table (IV). (C) Western blot analysis for IgE confirmation. Non-reducing SDS-PAGE and then Western blot analysis of anion-exchange fractions C19 to C21 and monomeric hIgE were carried out using a horseradish peroxidase-conjugated anti-hIgE. (D) Inhibitory effect of anti-hIgE pAb on mast cell degranulation activity. Mast cell degranulation activity in anion-exchange fraction C20 (10 μ L) in the presence of anti-hIgE pAb was determined. Mean $\pm$ SD of percentage of tryptase release is plotted. $n = 2$. Significant decreases compared with the case in the absence of anti-hIgE pAb are indicated by * $p < 0.05$ or ** $p < 0.01$.

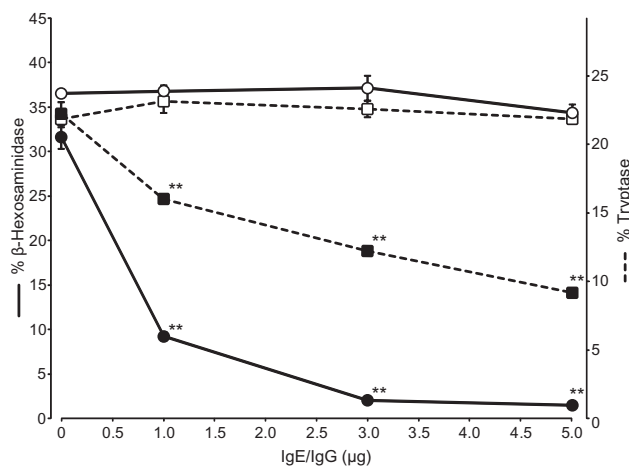


Fig. 4. Effect of monomeric hIgE on mast cell degranulation activity. Mast cell degranulation activity in samples was determined in the presence of monomeric hIgE (closed symbols) or hIgG (open symbols) as a control. Either the anion-exchange fraction C21 (10 µL: IgE, ●; IgG, ○) or the index donor plasma (1.25 µL: IgE, ■; IgG, □) collected on June 22, 2004, was tested. Mean $\pm$ SD of percentage of release of β -hexosaminidase and tryptase are plotted. $n = 2$. Significant decreases compared with the case in the absence of monomeric hIgE are indicated by ** $p < 0.01$.

degranulation activity, suggesting the presence of some mast cell-activating factors in the donor plasma. From the results, the factors were determined to be HMW IgEs.

The molecular weights of HMW IgEs seem to be two or three times higher than that of monomeric hIgE (approx. 230 kDa), as shown by nonreducing SDS-PAGE. The mass spectra of their tryptic peptides suggest that HMW IgEs are produced by the same cell clone. IgE of approximately 230 kDa presumably produced by the same cell clone as that producing HMW IgEs was also detected in the index donor (data not shown). HMW IgEs were shown by reducing SDS-PAGE to be reduced into 75-kDa immunoglobulin ϵ chains and 30-kDa immunoglobulin κ chains after incubation with β -mercaptoethanol similarly to monomeric hIgE (data not shown). These findings suggest that HMW IgEs are oligomeric, formed via disulfide bonds, and produced monoclonally in the index donor *in vivo*.

HMW IgEs activate mast cells directly without any antigens or stimulators. Under the IgE-mediated immunologic mechanism of anaphylaxis, mast cells and basophils are activated by the crosslinking of Fc ϵ RI-bound IgE monomers with multivalent antigens on their surface.^{15,16} Covalently oligomerized rat and human IgEs prepared using a chemical crosslinker reagent were also reported to bind to Fc ϵ RI on mast cells or basophils multivalently and activate these cells without antigens.^{17,18} Hence, it is suggested that HMW IgEs also act as a unit signal for mast cell degranulation via Fc ϵ RI. This proposed mechanism is also

supported by the finding that the mast cell degranulation activity of HMW IgEs decreased after incubation with monomeric hIgE in a dose-dependent manner, suggesting that monomeric hIgE acts as a competitor for HMW IgEs in binding to Fc ϵ RI. Thus, HMW IgEs are considered to have in one molecule at least two Fc regions that bind to at least two Fc ϵ RI molecules to activate mast cells competitively with monomeric hIgE.

The concentration of HMW IgEs in the index donor plasma seemed to increase with time between 2002 and 2008 accordingly with total IgE concentration and mast cell degranulation activity. It seems that HMW IgEs are produced by myeloma cells when considering the monoclonality of HMW IgEs and the index donor's disease of IgE-type multiple myeloma. Before the first case was reported, no mast cell degranulation activity was observed in the index donor plasma, showing no or only small amounts of HMW IgEs. Because of comparable mast cell degranulation activity, the PC transfused in the first case was considered to contain HMW IgEs as well as the PC transfused in the second case. These findings suggest the association between the occurrence of anaphylactic transfusion reactions and the presence of HMW IgEs with mast cell degranulation activity in the index donor blood. Thus, oligomeric myeloma IgE in blood components might induce anaphylactic transfusion reaction, as reported here. Because purified myeloma IgE is reported to contain an IgE dimer,¹⁹ which may activate mast cells, it is considered that some types of myeloma IgE tend to form oligomers. IgG aggregates in purified IgG fraction are reported to induce hypersensitive reactions after intravenous administration,²⁰ although its production mechanism through the fractionation processes of pooled human plasma and reaction mechanism via the activation of the complement system are thought to be different from those of HMW IgEs. Thus, polymerized immunoglobulins, including HMW IgE and IgG aggregates, may have to be focused on as the causative factors for adverse transfusion reaction.

In summary, HMW IgEs were identified as the mast cell-activating factors in the index donor plasma and may cause anaphylactic transfusion reaction via the activation and degranulation of mast cells, and/or probably basophils, in recipients by inducing Fc ϵ RI aggregation. It is commonly believed that anaphylactic transfusion reactions are mainly caused by the antibodies produced in recipients who have been transfused repeatedly. Our results raise another possible mechanism in which transfused donor plasma directly activates a recipient's mast cells. There are many cases of anaphylactic transfusion reaction in which the cause remains unresolved. Hence, it is valuable to analyze the mast cell-activating ability of a transfused blood component and to identify the mast cell-activating factors in it, although the case involving an IgE myeloma donor is presumably very rare.²¹ Degranula-

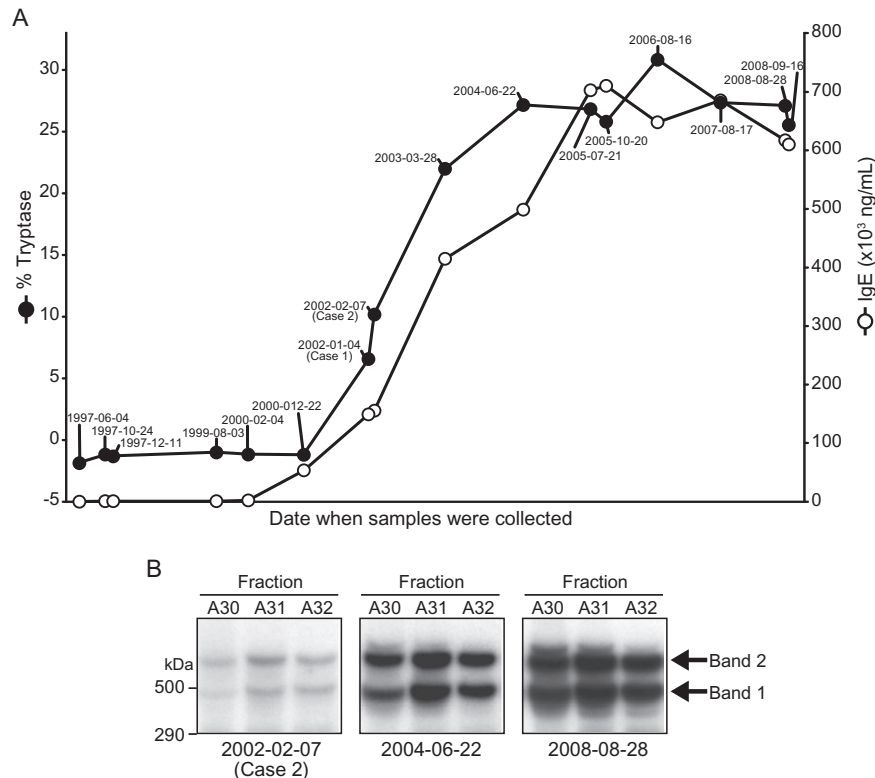


Fig. 5. Correlation between mast cell degranulation activity and presence of HMW IgE in index donor. Sixteen plasma samples collected between 1997 and 2008 were examined. (A) Changes in mast cell degranulation activity (●) and total IgE concentration (○) in index donor plasma. (B) HMW IgEs detected in index donor. Gel filtration chromatography of three serum samples (200 μ L) collected on the date indicated at the bottom of each panel was performed. The proteins contained in the obtained gel filtration fractions A30 to A32 were analyzed by nonreducing SDS-PAGE followed by Western blot analysis using the anti-hIgE antibody. When Western blot analysis was performed, the dilution rate of the antibody and the exposure time were the same between the samples.

tion assay using in vitro cultured mast cells in combination with plasma protein purification techniques is useful for identifying the factors.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest relevant to the manuscript submitted to **TRANSFUSION**.

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