Quantitation of HEV RNA

Quantitation of HEV RNA was performed following a previously reported method with minor modifications.[5] Briefly, RT-PCR was used to determine the number of copies of HEV RNA by targeting 75 nucleotides of a highly conserved sequence in the open reading frame (ORF) 2 region. The conditions for the RNA amplification stage were as follows: 25 μL of extracted RNA was incubated at 50°C for 30 minutes and at 95°C for 15 minutes, followed by 50 cycles of 94°C for 15 seconds and 60°C for 1 minute. Real-time RT-PCR was then performed using PCR and a Sequence Detection System (TaqMan and PRISM 7900, respectively, Applied Biosystems, Tokyo, Japan). The sensitivity of this system was determined to be 25 copies/mL, with a 95% confidence interval of 13 to 166 copies/mL by logistic analysis. The quantitation standard for HEV RNA was generated by transcribing the HEV complementary DNA (cDNA) of the HEV ORF2 region cloned into a plasmid (pCRII-TOPO, Invitrogen).

Full genome sequencing of HEV RNA

We referred to a previous report by Urayama and colleagues[12] for the method of full genome sequencing of HEV RNA. Total RNA was extracted either from 300 μL of HEV-positive blood specimens or from 200 μL of recovered cell culture supernatant. RT of HEV RNA was performed at 42°C for 1 hour. Consequently, nearly the full length of the cDNA was constructed. This cDNA was divided into two fragments: a 3′ fragment (3960 nucleotides) and a 5′ fragment (3160 nucleotides).

Phylogenetic analysis of HEV isolates

Complete or nearly complete sequences of HEV isolates were determined as previously described.[12] Sequences were aligned with the reported HEV strains using a computer program (ClustalW, Version 1.8, http://www.genome.jp/tools/clustalw/).

Photochemical inactivation of HEV in platelet samples

Preparation of platelets

Rejected platelets (PLTs), which are not clinically suitable to be used as blood derivatives because the alanine aminotransferase value exceeds the acceptance criteria, were used in this assay. All PLTs were collected by apheresis methods using equipment of the component collection system of Haemonetics (Braintree, MA), as well as TRIMA and TERUSYS of Terumo BCT (Lakewood, CO). All procedures were accomplished according to the manufacturers' protocols. The basic criteria for the volume and concentration of PLTs were 190 to 250 mL/bag and 1.9×10^{11} to 4.7×10^{11}/bag, respectively.

Treatment of PLTs with the Mirasol PRT system

Mirasol PRT treatment of PLTs was performed as previously described.[13] Briefly, 35 mL of riboflavin solution was added to a Mirasol PRT system kit to obtain a final concentration of 50 μmol/L, the unit was then exposed to a dose of UVB light (6.24 J/mL) and then left to rest for 30 minutes at an ambient temperature to prevent activation of the PLTs.

Sampling of HEV specimens from PLTs

Three milliliters of the pre-Mirasol-treated HEV PLT specimens was collected after the PLTs were thoroughly mixed with the virus in the dedicated bags, and the same volume of posttreated HEV specimens was likewise acquired. Titration samples for HEV infectivity were obtained from the supernatants of PLTs by centrifugation at 3000 rpm ($1750 \times g$) for 15 minutes under ambient atmosphere.

Confirmation of the noncytotoxicity against a cultured cell line caused by plasma from both pre- and post-Mirasol-treated PLTs

A cytotoxicity test against A549 cells was performed using virus-free control PLTs. This experimental condition was the same as the HEV infectivity assay, as described in the paragraph, "Cell culture and virus inoculation." Both pre- and post-Mirasol PRT-treated plasma obtained from the supernatant of PLTs were laid onto A549 cells for 2 hours. After the plasma was removed, the cells were cultured to confirm if any positive cell death or expression of a negative impact on cell growth were seen.

RESULTS

Establishment of the HEV culture systems

Selection of an HEV strain to establish a culture system using A549 cells

Fourteen G3- and G4-containing HEV specimens from plasma or serum (Table 1) were examined. All samples were diluted to $10^{4.8}$ copies/mL (total amount, $10^{5.2}$ copies in 2.5 mL) and then inoculated into A549 cells (Fig. 1). Consequently, only two HEV strains (JRC-HE3 for G3 and UA1 for G4) were found to be infectious. The HEV load of JRC-HE3 reached a plateau at an approximate concentration of 10^6 copies/mL (total amount, 10^7 copies in 10 mL of recovered medium) at around 42 days (6 weeks) postinoculation. On the other hand, UA1 reached a plateau at $10^{3.3}$ copies/mL at 60 days postinoculation.

HEV cultivation of JRC-HE3 using PLC/PRF/5 cells to procure a higher load of viral progeny

JRC-HE3 was diluted to $10^{5.5}$ copies/mL ($10^{5.9}$ copies in 2.5 mL) and then inoculated into alternative cells, namely, PLC/PRF/5 cells (Fig. 2). The viral concentration increased until 91 days (13 weeks) postinoculation and reached a plateau at an approximate concentration of 10^8 copies/mL (10^9 copies in 10 mL of recovered medium;

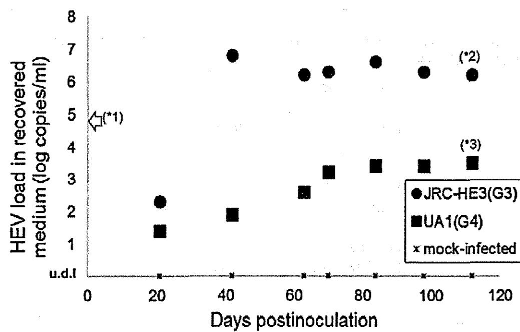


Fig. 1. Selection of HEV strains for the establishment of a culture system using A549 cells. The initial concentrations of the HEV strains used were all $10^{4.8}$ copies/mL ($10^{5.2}$ copies in 2.5 mL; *1). The concentrations of JRC-HE3 for G3 and UA1 for G4 in the recovered culture medium at 112 days postinoculation were $10^{6.2}$ copies/mL ($10^{7.2}$ copies in 10 mL) (*2) and $10^{3.3}$ copies/mL ($10^{4.3}$ copies in 10 mL; *3), respectively. A549 cells were utilized as the host for this assay. u.d.l. = under the detection limit.

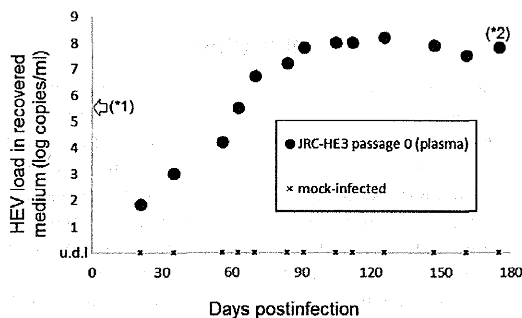


Fig. 2. Confirmation of a higher load of HEV progeny using PLC/PRF/5 cells. A high concentration of HEV progeny was obtained when PLC/PRF/5 cells were used. The HEV JRC-HE3 strain was inoculated at $10^{5.5}$ copies/mL ($10^{5.9}$ copies in 2.5 mL; *1). The load of JRC-HE3 in recovered culture medium at 175 days postinoculation was $10^{7.9}$ copies/mL ($10^{8.9}$ copies in 10 mL; *2). No viral progeny were detected in the mock-infected samples.

Fig. 2). It was possible to obtain a higher load of JRC-HE3 with PLC/PRF/5 cells than with A549 cells for virus proliferation.

Serial culture passage of JRC-HE3 using PLC/PRF/5 cells

HEV production resulting from different JRC-HE3 culture passages was examined using PLC/PRF/5 cells. For the first cultivation, an original HEV-positive-plasma specimen was used (Fig. 2, Passage 0). This means that the viral progeny referred to as Passage 1 was obtained from the result of Passage 0 infection. Each viral progeny from the specimens at 70 days (10 weeks) postinoculation was diluted to $10^{5.7}$ copies/mL before the next infection step (Fig. 3). The growth curves obtained for viral production were identical for all passages (Passages 1-4).

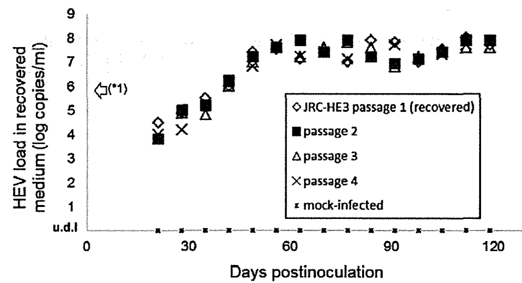


Fig. 3. JRC-HE3 serially passaged using PLC/PRF/5 cells. HEV samples in recovered culture media were diluted to $10^{5.7}$ copies/mL ($10^{6.1}$ copies in 2.5 mL; *1). Each viral sample from specimens at 70 days (10 weeks) postinoculation was inoculated into PLC/PRF/5 cells for each successive passage. There were no significant differences between the viral production curves from each passage. No viral progeny were detected in the mock-infected samples.

Genomic analysis of JRC-HE3 associated with culture passage and longer incubation of the virus

Full genome sequencing of JRC-HE3 was carried out on the original plasma (Passage 0) and progeny after long-term incubation (119 days [17 weeks], Passage 4), and genomic substitutions and amino acid alterations were investigated (Table 2). Consequently, only point substitutions at five bases in the ORF1 region and duplicate substitution at two bases in the ORF2 and ORF3 regions were found, respectively. These substitutions were demonstrated to result in alterations at four amino acid sites. Note that, due to triplet code degeneracy, some of the observed genomic base substitutions did not result in amino acid alteration.

Infectious titration of JRC-HE3 and UA1 using A549 cells and PLC/PRF/5 cells

Limited dilution assays were performed to determine the minimum HEV RNA load for infectivity against two cell lines. High loads of JRC-HE3 ($10^{9.4}$ copies/2.5 mL or $10^{8.5}$ copies/2.5 mL) were serially diluted 10-fold and inoculated into A549 cells and PLC/PRF/5 cells, respectively. Viral progeny were quantitatively investigated at 3, 6, and 9 weeks postinfection. Consequently, JRC-HE3 expressed its infectivity against A549 cells (Fig. 4A) and PLC/PRF/5 cells (Fig. 4B) even at 10^4- and 10^3-fold dilutions,

TABLE 2. HEV RNA and amino acid substitution associated with in vitro passaging and the longer incubation of HEV JRC-HE3 obtained during Passage 4 (Fig. 3)

ORF 1, five bases	Methyl transferase G137G [GGU(G) ⇒ GGC(G)] Papain-like cysteine protease D452D [GAU(D) ⇒ GAC(D)] Domain X W741P [UGG(W) ⇒ CGG(P)] Domain X E871K [GAG(E) ⇒ AAG(K)] Helicase F1124S [UUC(F) ⇒ UCC(S)]	
ORF 3 and ORF 2 (duplicative two bases)	ORF3 C20C [UGU(C) ⇒ UGC(C)] ORF3 P98P [CCG(P) ⇒ CCA(P)]	ORF2, Capsid L8L [UUG(L) ⇒ CUG(L)] ORF2, Capsid A119T [GCU(A) ⇒ ACU(T)]

respectively. This indicates that $10^{5.4}$ and $10^{5.5}$ copies of JRC-HE3 are necessary to achieve infectivity against A549 cells and PLC/PRF/5 cells (Table 3). Thus, for both cell lines, 1 TCID of JRC-HE3 was estimated to be approximately $10^{5.5}$ copies. A similar experiment was performed using UA1 ($10^{7.5}$ copies/2.5 mL) along with A549 cells (Fig. 4C). Consequently, 1 TCID of UA1 was found to be approximately $10^{5.5}$ copies, although infectivity against PLC/PRF/5 cells could not be confirmed (data not shown). Based on the above-mentioned results, A549 cells may have to be used as the hosts when titration of both JRC-HE3 and UA1 are performed.

Photochemical inactivation of HEV in PLTs

The assays of HEV inactivation of both G3 (JRC-HE3) and G4 (UA1) in PLTs were performed thrice. The mean concentration of each strain in PLTs was consequently $10^{9.8}$ and $10^{7.9}$ copies per total volume (approx. 200 mL) of PLTs, respectively. HEV specimens collected from PLTs were serially diluted 10^n-fold (n = 0-6). Consequently, JRC-HE3 was found to express infectivity even if the original specimen (10^0-fold) was diluted 10^3-fold (Fig. 5A). This was clearly demonstrated by the fact that the viral progeny was found in the cell culture supernatant when the 10^3-fold–diluted specimens were applied. On the other hand, UA1 showed infectivity to a level of 10^2-fold dilution (Fig. 5C). No infectivity was recognized in any specimens after treatment with the Mirasol PRT system at the ninth or 10th week postinfection (Figs. 5B and 5D). In some cases, HEV RNA was detected in the cell culture supernatant in posttreated samples (Figs. 5B and 5D, white arrows). However, it would be reasonable to understand that these HEV RNA samples are derived from originally challenged HEV, not from virus progeny. All the data described in Fig. 5 are representative data of the results of each assay that was performed thrice. There was little difference between the assays. Based on these results, it was concluded that the Mirasol PRT system lowers the infectivity of G3 and G4 of live HEV, by more than 3 log (99.9%) and more than 2 log (99%), respectively (Table 4). In this experiment, A549 cells were utilized for HEV titration. The effect of HEV-free plasma treated or not treated with the Mirasol PRT system on the cells was also investigated. Consequently, no positive factors for cell death nor a negative impact on cell growth was observed.

DISCUSSION

Since HEV was recently categorized as a transfusion-transmittable infectious disease, the risk of HEV infection via blood transfusion has become a major global concern in transfusion medicine.[5,6] Hence, there has been a demand to establish an HEV culture system, especially for G3 and G4, which are frequently detected in Japan, China, and southeastern Asia,[7,8] as well as for a method to assay the HEV infectious titer, TCID. We therefore attempted to establish a culture system for G3 and G4 HEV and have successfully obtained two positive systems: the JRC-HE3 strain for G3 and the UA1 strain for G4. The concentrations of both viral strains in plasma or serum originally exceeded 10^7 copies/mL; thus, it is plausible that the two strains may easily replicate and grow both in vivo and in vitro.

Although JRC-HE3 can infect both PLC/PRF/5 cells and A549 cells, the production efficiencies of the virions differed. A higher load of JRC-HE3 was inoculated into PLC/PRF/5 cells, and a higher concentration of approximately 10^8 copies/mL of virus progeny was confirmed. In contrast, A549 cells generated progeny at a concentration of approximately 10^6 copies/mL, with inoculations ranging from $10^{5.4}$ to $10^{9.4}$ copies in 2.5 mL. Since the PLC/PRF/5 cells are derived from human hepatoma, this cell line may be more suitable for HEV production than the A549 cells.

We examined the genomic alterations and amino acid substitutions of JRC-HE3 derived from PLC/PRF/5 cells after long-term culture. Full genome sequencing of JRC-

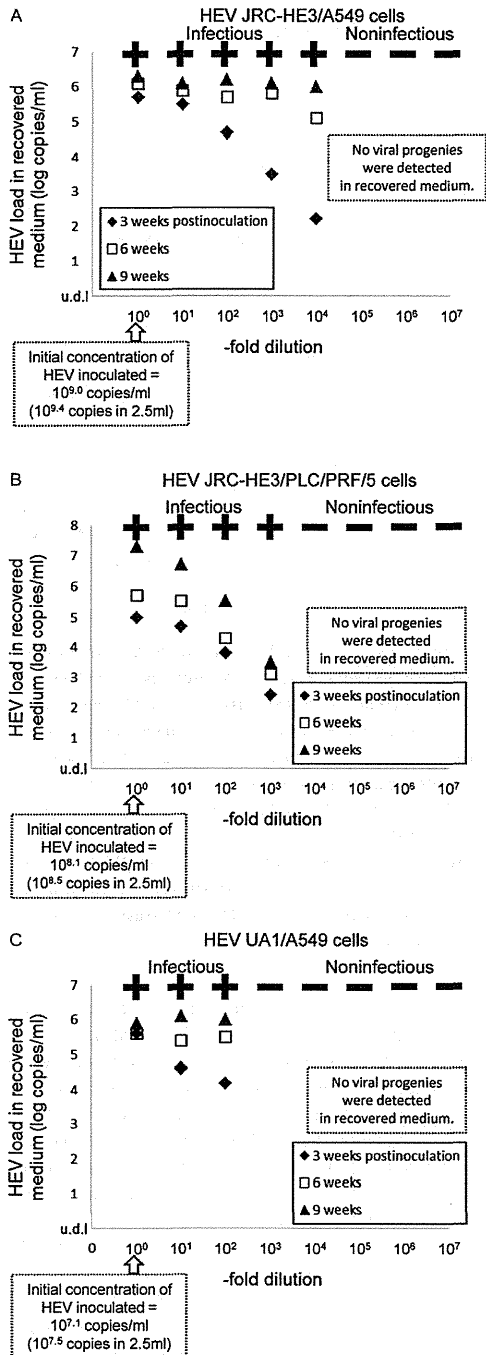


Fig. 4. (A) Titration of infectious HEV JRC-HE3 using A549 cells. A high HEV load of $10^{9.0}$ copies/mL was obtained by ultracentrifugation. The original HEV samples were serially diluted tenfold. Each diluted HEV sample was inoculated into A549 cells. The viral progeny in the recovered culture medium were examined every 3 weeks. Even the original HEV samples, diluted at 10^4, were found to be infectious. (B) Titration of infectious HEV JRC-HE3 using PLC/PRF/5 cells. A high HEV load of $10^{8.1}$ copies/mL was obtained by ultracentrifugation. Even the original HEV samples, diluted at 10^3, were found to be infectious. (C) Titration of infectious HEV UA1 using A549 cells. A high HEV load of $10^{7.1}$ copies/mL was obtained by ultracentrifugation. Even the original HEV samples, diluted at 10^2, were found to be infectious. u.d.l. = under the detection limit.

TABLE 3. Numerical relationship between two viral units: copies and TCID

Host cells	HEV strain	Copies per TCID
A549	HEV JRC-HE3 G3	$10^{5.4}$
PLC/PRF/5		$10^{5.5}$
A549	HEV UA1 G4	$10^{5.5}$
PLC/PRF/5		Could not be determined

HE3 revealed few variations. It may be concluded that JRC-HE3 has easily become habituated to in vitro cultivation. If the sequences of the viral genome would drastically change under long-term incubation, the efficacy of HEV production and viral infectiousness (infectious titer) may be negatively impacted. Based on these data, it could be concluded that HEV can stably and consistently be produced by this culture system, and its infectivity may remain almost the same in all situations. This consideration can be supported by the fact that the obtained growth curves demonstrating viral production were identical at a high level up to 120 days postinfection, regardless of the passage of the virus. Currently, a similar trial is being performed using UA1 produced by A549 cells.

In the first half of this article, we conclusively established an HEV culture system using HEV RNA–positive plasma or serum sample obtained from G3 or G4 HEV-infected individuals. Using the system established here, we attempted to undertake photochemical inactivation of HEV in PLTs using the Mirasol PRT system.

Log reduction values (LRVs) of more than 3 log for JRC-HE3 and more than 2 log for UA1 were achieved despite the fact that this agent is a nonenveloped virus, which are traditionally resistant to chemical inactivation. For the inactivation assay of JRC-HE3, a relatively higher concentration of the sample was obtained using PLC/PRF/5 cells as the virus producer. On the other hand, UA1 was not propagated in PLC/PRF/5 cells, but only in A549 cells. Therefore, more than 2-log reduction was marginally demonstrated due to the lower titer of the applied HEV.

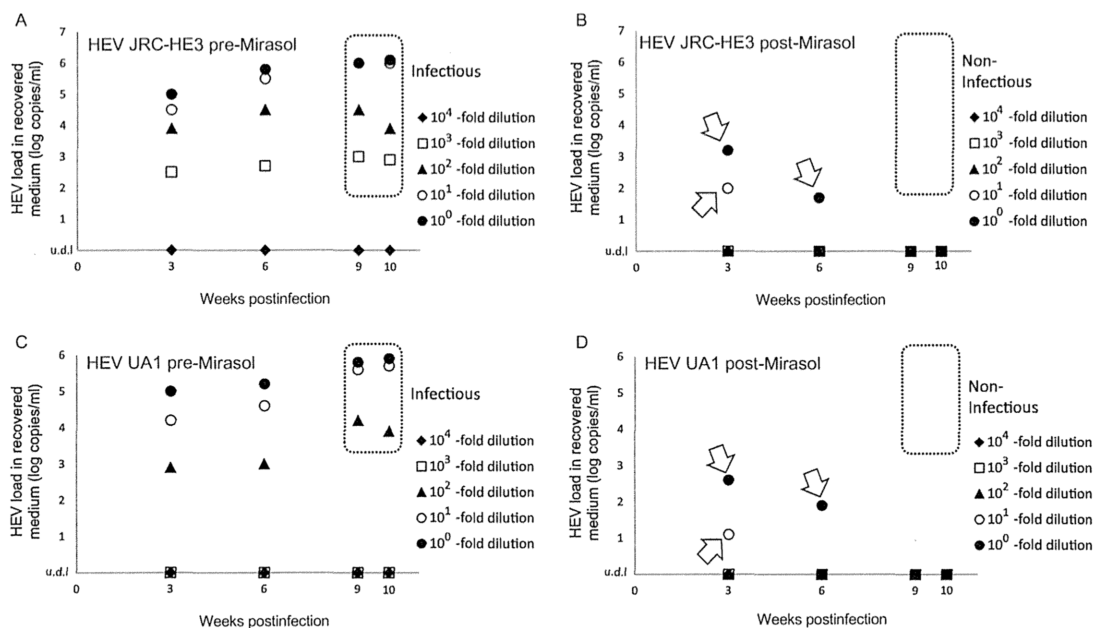


Fig. 5. (A and B) Inactivation of the live HEV JRC-HE3 in PLTs by the Mirasol PRT system. The change in the HEV load in the supernatant of A549 cell culture was investigated. The assayed HEVs were (A) Mirasol pretreated and (B) Mirasol posttreated HEV, respectively. The establishment of HEV infection was consequently confirmed at 9 and 10 weeks postinfection. The concentration of JRC-HE3 in PLTs was consequently $10^{9.8}$ copies per total volume of PLTs. JRC-HE3 expressed infectivity even when the original specimens were 10^3-fold diluted (A). No infectivity (viral progeny) was recognized in any Mirasol PRT system posttreated specimens of (B). HEV-RNAs detected in (B, white arrows) are derived from the originally challenged HEV, because the HEV loads in recovered medium were gradually decreasing. The Mirasol PRT system lowered the infectivity of JRC-HE3 more than 3 log (99.9%). These are the representative data of triplicate assays. (C and D) Inactivation of the live HEV UA1 in PLTs by the Mirasol PRT system. The HEVs assayed were (C) Mirasol pretreated and (D) Mirasol posttreated HEV, respectively. The concentration of UA1 in PLTs was consequently $10^{7.9}$ copies per total volume of PLTs. UA1 expressed infectivity even when the original specimens were 10^2-fold diluted (C). No infectivity (viral progeny) was recognized in any Mirasol PRT system posttreatment specimens (D). The Mirasol PRT system lowered the infectivity of UA1 more than 2 log (99%). These are the representative data of triplicate assays.

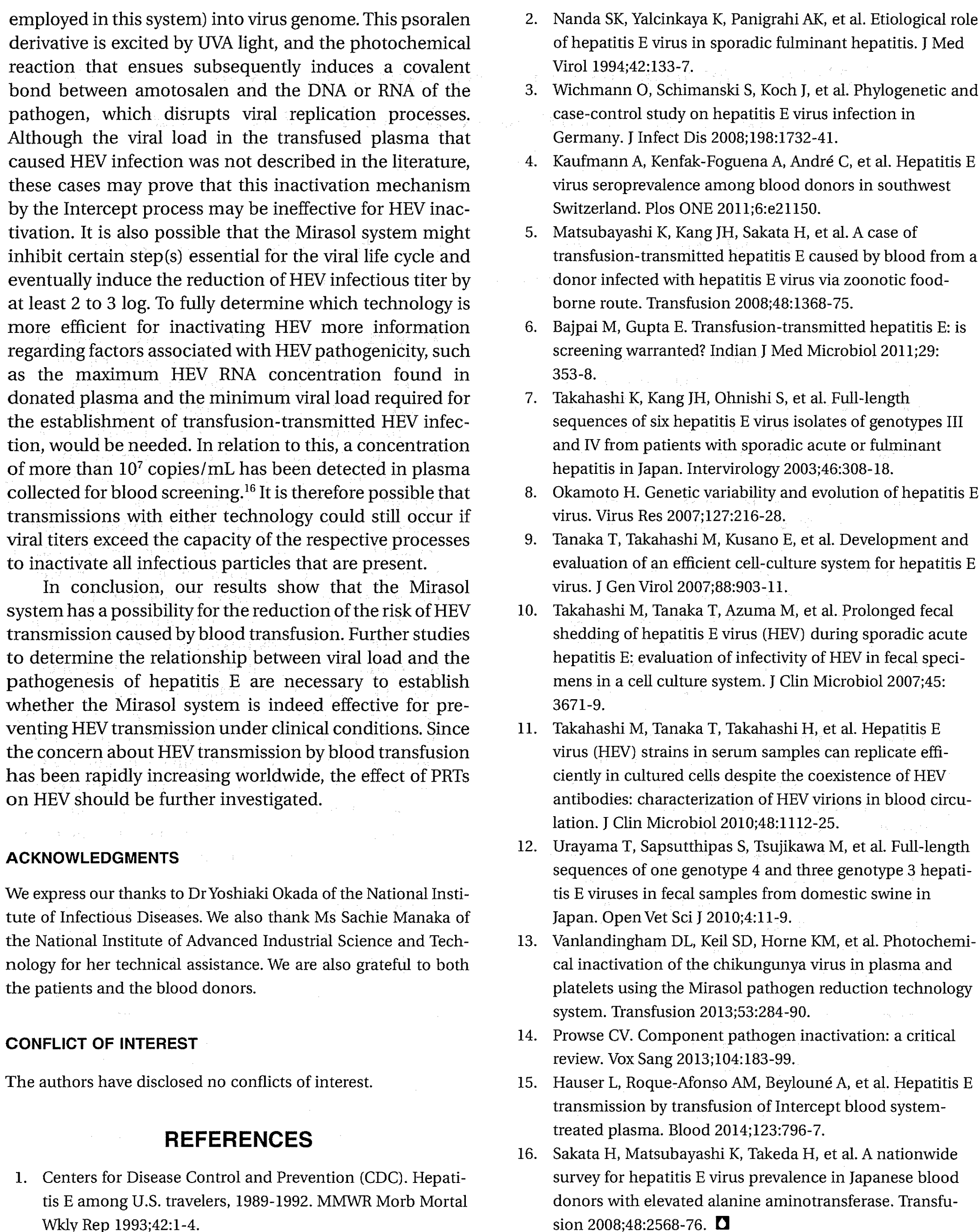
TABLE 4. Inactivation of the live HEV and HAV in PLTs by the Mirasol PRT system

HEV and HAV strain	Log reduction value (% of reduction value)
HEV JRC-HE3 G3	More than 3 log (more than 99.9%)
HEV UA1 G4	More than 2 log (more than 99%)
(Reference data)	
HAV VR-1402 GIB	1.8 log (98.4%)

The limited load of HEV obtained from in vitro cultivation precluded evaluation of the robustness of the measured LRV. If higher efficacy of viral infection against the hosts were attained, it could also be possible to acquire more robust data.

Of note, a 1.8-log (98.4%) reduction of LRV for the hepatitis A virus (HAV) strain of VR-1402 was previously obtained in our laboratory and an identical value was also reported by Prowse.[14] Thus, HEV inactivation is more efficient than that for HAV. VR-1402 has frequently been used for inactivation assays of HAV and is the usual model viruses for nonenveloped and relatively smaller-sized RNA viruses including HEV, because it is easy to titrate. We believe that the system we described here may be applied widely for infectivity studies of small, nonenveloped viruses, instead of VR-1402 as a model.

The Intercept blood system, similar to the Mirasol PRT system, is also well known as a photochemical method for PRT of blood products. Recently, two cases of HEV transmission caused by the transfusion of plasma treated with the Intercept were reported by Hauser and coworkers.[15] The primary mechanism of viral inactivation by the Intercept process has been described to involve an intercalation of amotosalen (the photochemical agent



employed in this system) into virus genome. This psoralen derivative is excited by UVA light, and the photochemical reaction that ensues subsequently induces a covalent bond between amotosalen and the DNA or RNA of the pathogen, which disrupts viral replication processes. Although the viral load in the transfused plasma that caused HEV infection was not described in the literature, these cases may prove that this inactivation mechanism by the Intercept process may be ineffective for HEV inactivation. It is also possible that the Mirasol system might inhibit certain step(s) essential for the viral life cycle and eventually induce the reduction of HEV infectious titer by at least 2 to 3 log. To fully determine which technology is more efficient for inactivating HEV more information regarding factors associated with HEV pathogenicity, such as the maximum HEV RNA concentration found in donated plasma and the minimum viral load required for the establishment of transfusion-transmitted HEV infection, would be needed. In relation to this, a concentration of more than 10^7 copies/mL has been detected in plasma collected for blood screening.[16] It is therefore possible that transmissions with either technology could still occur if viral titers exceed the capacity of the respective processes to inactivate all infectious particles that are present.

In conclusion, our results show that the Mirasol system has a possibility for the reduction of the risk of HEV transmission caused by blood transfusion. Further studies to determine the relationship between viral load and the pathogenesis of hepatitis E are necessary to establish whether the Mirasol system is indeed effective for preventing HEV transmission under clinical conditions. Since the concern about HEV transmission by blood transfusion has been rapidly increasing worldwide, the effect of PRTs on HEV should be further investigated.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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Antibody against immunoglobulin E contained in blood components as causative factor for anaphylactic transfusion reactions

Takaaki Abe,[1] Eiko Shimada,[1] Minoko Takanashi,[1] Takeshi Takamura,[2] Kyosuke Motoji,[3] Hitoshi Okazaki,[1] Masahiro Satake,[1] and Kenji Tadokoro[1]

BACKGROUND: Determining the mechanism underlying the development of transfusion reactions is important in transfusion therapy. Two bags of fresh-frozen plasma obtained from a donor (index donor) were implicated in two cases of anaphylactic transfusion reactions.

STUDY DESIGN AND METHODS: The serum prepared from the index donor plasma transfused into the second patient (Patient 2) was evaluated using cord blood–derived mast cells (CBMCs) incubated with Patient 2 plasma. The component in the serum required for the degranulation was determined and quantified by chromatography in combination with degranulation assay, Western blot analysis, and enzyme-linked immunosorbent assay. The component in the plasma required for CBMC sensitization was determined using human immunoglobulin (Ig)E or normal plasma in place of Patient 2 plasma in the assay. Sera collected from the index donor between 2001 and 2008 were examined for the CBMC degranulation factor.

RESULTS: The donor serum activated CBMCs incubated with Patient 2 plasma. The IgG fraction of the donor serum induced degranulation of CBMCs sensitized with IgE or plasma containing a normal IgE concentration. The IgG anti-IgE at a concentration higher than 2200 ng/mL, which showed CBMC degranulation activity, was detected in the donor sera for at least 7 years.

CONCLUSION: Transfusion of a high concentration of the anti-IgE in the donor plasma was suggested to induce mast cell degranulation in the patients leading to the development of anaphylactic transfusion reactions. Antibodies existing in not only the patient circulation but also the transfused blood might cause transfusion-induced anaphylaxis.

Transfusion reaction cases that occurred throughout Japan have been reported voluntarily to the Japanese Red Cross Society (JRCS) since 1993 to improve transfusion safety.[1] Among approximately 1500 cases of transfusion reactions reported each year, more than 200 cases have been classified as severe anaphylaxis, which is generally termed anaphylactic transfusion reactions.[2] JRCS has also been collecting patient serum or plasma samples as well as the remaining blood components implicated in transfusion reactions to analyze their adverse effects. However, the cause of the reactions remains unclear in most cases.

Mast cells are considered to play a key role in anaphylaxis.[3] That is, the cross-linking of Fcε receptor I (FcεRI) via the complex of an antigen and immunoglobulin (Ig)E molecules on the cell surface stimulates the release of several enzymes and chemical mediators such as tryptase and histamine from the cells. These phenomena are closely related to the development of anaphylaxis and increased concentrations of serum or plasma tryptase are considered as a marker of anaphylaxis.[4,5] The serum or plasma tryptase concentration in patients who developed anaphylactic transfusion reactions was observed to

ABBREVIATIONS: CBMC(s) = cord blood–derived mast cell(s); FcεRI = Fcε receptor I; hIgE = human immunoglobulin E from plasma; JRCS = Japanese Red Cross Society.

From the [1]Central Blood Institute, Blood Service Headquarters, Japanese Red Cross Society, Tokyo; the [2]Department of Cardiorenal Medicine, Yokohama City University School of Medicine, Kanagawa; and the [3]Motoji Medical Clinic, Tokyo, Japan.

Address reprint requests to: Takaaki Abe, Central Blood Institute, Blood Service Headquarters, Japanese Red Cross Society, 2-1-67 Tatsumi, Koto-ku, Tokyo 135-8521, Japan; e-mail: t-abe@jrc.or.jp.

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increase significantly after the onset of the reactions, suggesting that mast cell degranulation is triggered during these transfusion reactions.[6] We previously reported two cases of anaphylactic transfusion reactions associated with the transfusion of platelet (PLT) concentrates that contained oligomeric IgE capable of inducing the degranulation of cord blood–derived mast cells (CBMCs).[7]

JRCS was notified that another two bags of blood components obtained from a single donor were involved in two cases of anaphylactic transfusion reactions. In our preliminary analysis, the degranulation of CBMCs incubated with plasma from one of these patients upon stimulation with the serum sample prepared from one of the blood components was observed. In this study, we examined these samples by an in vitro degranulation assay using CBMCs and plasma protein purification techniques similar to those performed in a previous study.[7] We propose a new mechanism by which the adverse reactions were elicited in these cases.

CASE REPORTS

Two cases of anaphylactic transfusion reactions involving two bags of fresh-frozen plasma (FFP) obtained from a donor were reported to JRCS.

Index donor

The index donor was a 64-year-old female in 2004. She donated more than 100 times between 1994 and 2008 and at least 18 bags of blood components including FFP prepared from her blood were transfused into recipients. Two of them were implicated in anaphylactic transfusion reactions that developed in two different patients described below. She had no history of allergy. The concentrations of IgE and IgG in her serum prepared from the FFP used for the second patient (Patient 2) were determined to be 24.2 ng/mL and 10.5 mg/mL, respectively, which were normal concentrations (lower than 415 ng/mL and 8.70-17.0 mg/mL,[8,9] respectively). We examined retrospectively the hemovigilance data that had been accumulated in JRCS since 1993 by searching for reports concerning the blood component prepared from her blood. However, no cases were reported except these two cases of anaphylactic transfusion reactions. She had not donated since 2009 and currently she cannot donate owing to the age limitation for blood donation.

Patient 1

Patient 1 was a 53-year-old female suffering from pulmonary hypertension, renal failure, anemia, and thrombocytopenia. She had been diagnosed with thrombotic thrombocytopenic purpura and had been treated with plasma exchange four times without any transfusion reactions. She had no history of allergy. She received her fifth plasma exchange using five sequential bags of FFP in 2004. Within 10 minutes after the start of transfusion of the fifth bag of FFP, which was donated by the index donor on April 17, 2004, her systolic blood pressure decreased from 136 to 30 mmHg, and her pulse rate also decreased to 30 beats/min; she then stopped breathing. She was considered to have developed anaphylactic transfusion reaction from her clinical manifestation.[10,11] The IgE concentration in the pretransfusion plasma sample of the patient was 77.9 ng/mL. Her posttransfusion sample was not available for testing.

Patient 2

Patient 2 was a 62-year-old female with thrombotic thrombocytopenic purpura associated with systemic lupus erythematosus and underwent plasma exchange in 2005 using a bag of FFP obtained from the index donor on October 26, 2004. She had no history of allergy and she did not develop any adverse reactions during her transfusion therapies with four bags of FFP and a bag of PLT concentrate before the transfusion of FFP from the index donor. Ten minutes after the start of transfusion of FFP from the index donor, she started to feel throat pain and to wheeze, her forehead turned red, and her blood pressure dropped from 150/87 to 86/47 mmHg. Her oxygen saturation level measured by pulse oximetry was 86%. She was considered to have developed anaphylactic transfusion reaction from these clinical manifestations.[10,11] Her plasma IgE concentration decreased from 177 ng/mL before the transfusion to 90.7 ng/mL after the transfusion. The tryptase concentration in her plasma increased from 4.0 to 20.5 μg/L (normal range in healthy individuals, 2.1-9.0 μg/L),[12] indicating mast cell activation.

MATERIALS AND METHODS

Plasma and serum

Plasma samples were collected from Patient 1 before the transfusion and from Patient 2 before and after the transfusion. An aliquot of plasma from the bag of FFP transfused into Patient 2 was incubated with 40 mmol/L $CaCl_2$ at 37°C for 2 hours and then at 4°C overnight to prepare serum. Seven serum samples were also collected from the index donor when she donated her blood between 2001 and 2008. Control samples were collected from healthy donors. All the samples were dialyzed against or buffer exchanged with saline for the degranulation assay. IgE and IgG concentrations in the samples were determined by peak-rate nephelometry using an immunochemistry system (IMMAGE 800, Beckman Coulter, Tokyo, Japan).

CBMCs

CBMCs, which are generally used for the in vitro study of mast cells,[13] were prepared for degranulation assay.

Umbilical cord blood was obtained from the Japanese Red Cross Kanto-Koshinetsu Cord Blood Bank. Written informed consent was obtained from mothers for the use of the cord blood in transplantation- or transfusion-related studies in cases when the number of cord blood cells is not sufficient for transplantation. The differentiation of cord blood cells into mast cells was induced as previously described.[7]

CBMC degranulation assay

Mast cell degranulation activity in the test sample was determined from the percentage of tryptase or histamine release from activated CBMCs during the degranulation assay. CBMCs suspended in the culture medium were plated at 5×10^3 cells per well in a 96-well plate with 80 µg/mL sodium heparin and 20% (vol/vol) of the pretransfusion plasma from Patient 2, four plasma samples from healthy donors, or the index donor serum in a final volume of 50 µL. The cells were then incubated at 37°C in 5% CO_2 for 6 hours. Subsequently, 60 µL of a sample solution containing the index donor serum or a healthy donor serum in 0.18% (wt/vol) dextrose, 2.6 mmol/L $CaCl_2$, 1.8 mmol/L $MgCl_2$, and 0.18% (wt/vol) bovine serum albumin was added to each well. After incubation at 37°C for 40 minutes, the supernatant of each well was collected. The tryptase and histamine concentrations in the supernatant were measured using an allergy and autoimmunity analyzer (UniCAP 100, Phadia, Tokyo, Japan) and a histamine enzyme immunoassay kit (A05890, Bertin Pharma, Yvelines, France), respectively. The percentage of tryptase or histamine release was calculated as described previously.[7] To examine the effects of IgE and IgG on mast cell degranulation activity, CBMCs were incubated with 35.4 ng/mL human IgE from plasma (hIgE; 16-16-090705, Athens Research & Technology, Athens, GA) or human IgG from serum (hIgG; I4506, Sigma-Aldrich Japan, Tokyo, Japan). The CBMCs were then washed and incubated in a 96-well plate containing the sample solution described with or without 10,000 ng/mL hIgE or hIgG. The mast cell degranulation activity in the sample was then assessed.

Fractionation of index donor serum

To separate the components that induce CBMC degranulation from the other components of the serum, the index donor serum was fractionated by gel filtration and affinity chromatography. Gel filtration chromatography was carried out using 2 mL of the index donor serum on an column packed with prep grade (HR 16/50 and Superose 12, respectively, GE Healthcare Japan, Tokyo, Japan) at 4°C. The IgG fractions of the index donor serum and healthy donor serum were obtained using a protein G column (17-0404-01, GE Healthcare Japan) in accordance with the manufacturer's protocol. The unbound fraction was collected as the pass-through fraction. Chromatographic fractions were assessed to determine mast cell degranulation activity using CBMCs sensitized with Patient 2 plasma and IgG concentration.

Detection of IgG anti-human IgE

In the Western blot analysis to detect the IgG anti-IgE, hIgE was blotted onto a transfer membrane (Immobilon-P, Nihon Millipore, Tokyo, Japan). The membrane was incubated with the index donor serum, healthy donor serum, or the IgG fraction of these sera, and then the IgG anti-IgE was detected using a horseradish peroxidase–conjugated anti-human IgG antibody (HRP–anti-IgG; 054220, Life Technologies Japan, Tokyo, Japan) and Western blotting detection reagents (ECL Plus, GE Healthcare Japan). hIgE on the membrane was visualized using a gold staining kit (Protogold, British BioCell International, Cardiff, UK). The IgG anti-IgE was quantified by enzyme-linked immunosorbent assay (ELISA). The test serum samples from the index donor or healthy donor were incubated in a 96-well plate (Nunc Immuno Plate PolySorp, Thermo Fisher Scientific, Kanagawa, Japan) coated with hIgE. The IgG anti-IgE in each well was detected using HRP–anti-IgG with 3,3′,5,5′-tetramethylbenzidine (KPL, Gaithersburg, MD). A humanized monoclonal IgG anti-human IgE, omalizumab (Novartis Pharma, Tokyo, Japan), was used to generate a standard curve for quantification (detection range, 100-40,000 ng/mL).[14]

Statistical analysis

The percentage of tryptase release and the concentration of the IgG anti-IgE were determined in two and four independent experiments, respectively. The mean percentage of tryptase release was compared between samples by the two-tailed t test for independent samples using computer software (Excel 2010, Microsoft Corp., Redmond, WA). A p value of less than 0.05 was considered significant.

RESULTS

Index donor serum–induced degranulation of CBMCs sensitized with Patient 2 plasma

The index donor serum induced the degranulation of CBMCs incubated with Patient 2 plasma (Fig. 1). No significant difference was observed between the tryptase concentrations in the culture supernatants of CBMCs incubated with 20% (vol/vol) Patient 2 plasma only and in that incubated without it (data not shown). However, the CBMCs sensitized with Patient 2 plasma released tryptase when incubated with 2.5 to 40 µL of the index donor serum in 110 µL of assay solution in a dose-dependent manner; CBMCs incubated without Patient 2 plasma were

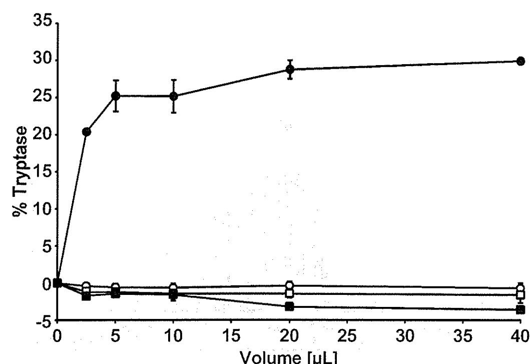

Fig. 1. Degranulation of CBMCs induced by index donor serum. CBMCs were incubated with (filled symbols) or without (open symbols) Patient 2 plasma followed by indicated volumes of index donor serum (●, ○) or healthy donor serum (■, □). The mean ± SD percentage of tryptase release is plotted.

not activated by the index donor serum. These results indicate that CBMCs were activated by the combination of Patient 2 plasma and the index donor serum.

CBMC degranulation induced by IgG fraction of index donor serum

Chromatographic fractionation was performed to separate the components of the index donor serum and identify those that activated CBMCs. Gel filtration fractions A37 to A49 induced the histamine release from CBMCs sensitized with Patient 2 plasma to a greater extent than the other fractions (Fig. 2A). These fractions contained a high concentration of IgG, suggesting that IgG is involved in the activation of CBMCs. The IgG fraction obtained from the index donor serum, as well as the index donor serum containing the same amount of IgG (3.56 μL), induced the degranulation of CBMCs sensitized with Patient 2 plasma (Fig. 2B). Thus, it is considered that the factors in the index donor serum that activated Patient 2 plasma–sensitized CBMCs were in the IgG fraction.

Requirement of human IgE for CBMC sensitization

Next, the components in Patient 2 plasma required for CBMC sensitization were determined. CBMCs washed after incubation with Patient 2 plasma were activated by the index donor serum (data not shown). Hence, IgE may be necessary for CBMC sensitization because IgE binds to FcεRI on the CBMC surface and is not removed by washing.[15] Indeed, CBMCs incubated with hIgE at the same concentration as that of IgE in Patient 2 plasma and then washed were activated by the index donor serum and the IgG fraction of the serum (Figs. 3A and 3B). Besides Patient 2 plasma, other plasma samples could also sensitize CBMCs to the index donor serum (Fig. 3C). CBMCs incubated with plasma with an IgE concentration similar to that in Patient 2 plasma were activated by the index donor serum, whereas CBMCs incubated with plasma containing a low IgE concentration or an extremely high IgE concentration were not activated by the serum. These results suggest that normal IgE generally contained in human plasma could contribute as a factor for CBMC sensitization at suitable concentrations in place of Patient 2 plasma.

IgG anti-IgE in index donor serum activating IgE-sensitized CBMCs

As shown in Figure 3C, CBMCs were not activated by the index donor serum in the presence of a high IgE concentration. Moreover, CBMC degranulation induced by the IgG fraction of the index donor serum was inhibited by the addition of an excess amount of hIgE (Fig. 4A), suggesting that the IgG fraction recognized IgE. Western blot analysis revealed that IgG in the index donor serum bound to hIgE (Fig. 4B). Hence, it is considered that the index donor serum contained the IgG antibody against human IgE and this antibody triggered CBMC degranulation by the cross-linking of IgE-bound FcεRI on the cells.

IgG anti-IgE in index donor serum detected over 7 years

The IgG anti-IgE in the index donor serum samples collected between 2001 and 2008 was examined to determine the relationship between the presence of the antibody and the occurrence of anaphylactic transfusion reactions (Table 1). The IgG anti-IgE was detected in all eight samples by Western blot analysis. The concentration of the antibody was calculated to be in the range between 2200 and 8900 ng/mL by ELISA. All the test samples activated hIgE-sensitized CBMCs. These results indicate that the index donor had continuously produced the IgG anti-IgE over a long period of at least 7 years. CBMCs incubated with Patient 1 plasma were also degranulated after incubation with the index donor serum obtained from FFP that was from the blood donated on April 17, 2004, and that was transfused to Patient 1 (data not shown).

DISCUSSION

Mast cells in transfusion recipients are activated and degranulate during anaphylactic transfusion reactions,[6] although the activation mechanism remains unclarified in most cases. In the cases presented in this study, the sera from the transfused FFP showed degranulation activity against CBMCs sensitized with the patient plasma and the

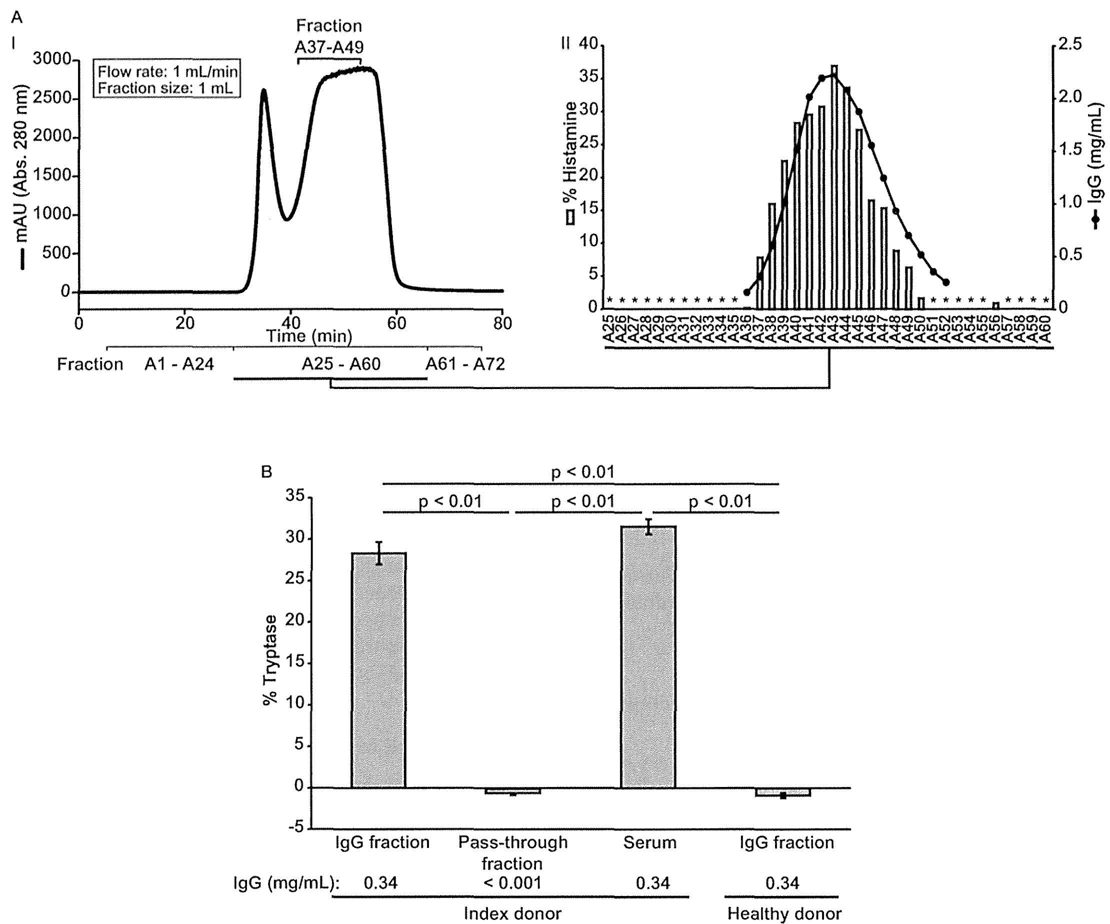

Fig. 2. Mast cell degranulation activity in fractions separated from index donor serum by chromatography. (A) The index donor serum was fractionated by gel filtration chromatography. (I) Chromatogram. (II) Elution patterns of mast cell degranulation activity and IgG. The histogram shows the percentage of histamine release during degranulation assay of 50 μL of each fraction. *The percentage of histamine release was not higher than 0%. The solid line represents the IgG concentration in each fraction. (B) CBMC degranulation assay was performed using the indicated samples. The concentrations of IgG from the test samples in the assay solutions are indicated at the bottom of the panel. p values indicating significant differences are shown. The mean ± SD percentage of tryptase release is plotted.

factor responsible for CBMC degranulation was determined to be the IgG anti-human IgE in the donor plasma. It was suggested that the antibody was the causative factor for the anaphylaxis.

Autoantibodies against human IgE are found in patients with various diseases such as allergic disorders,[14,16-19] systemic lupus erythematosus,[20] parasite infection,[21] systemic sclerosis,[22] Crohn's disease,[23] and visceral leishmaniasis.[24] The IgG anti-IgE was also detected in normal individuals in some studies.[14,23] Generally, no more than 300 ng/mL IgG anti-IgE was observed in patient serum samples.[17-22] However, another report showed that the IgG anti-IgE concentrations in two of 96 individuals were more than 4000 ng/mL.[14] The index donor was reported to have no history of allergic, autoimmune, or other diseases. Despite that, the IgG anti-IgE was detected in the index donor sera at relatively high concentrations ranging from 2200 to 8900 ng/mL. In our preliminary study to detect the IgG anti-IgE in approximately 250 serum samples prepared from the blood components, only one serum sample showed a high IgG anti-IgE concentration of 1200 ng/mL; however, that serum sample

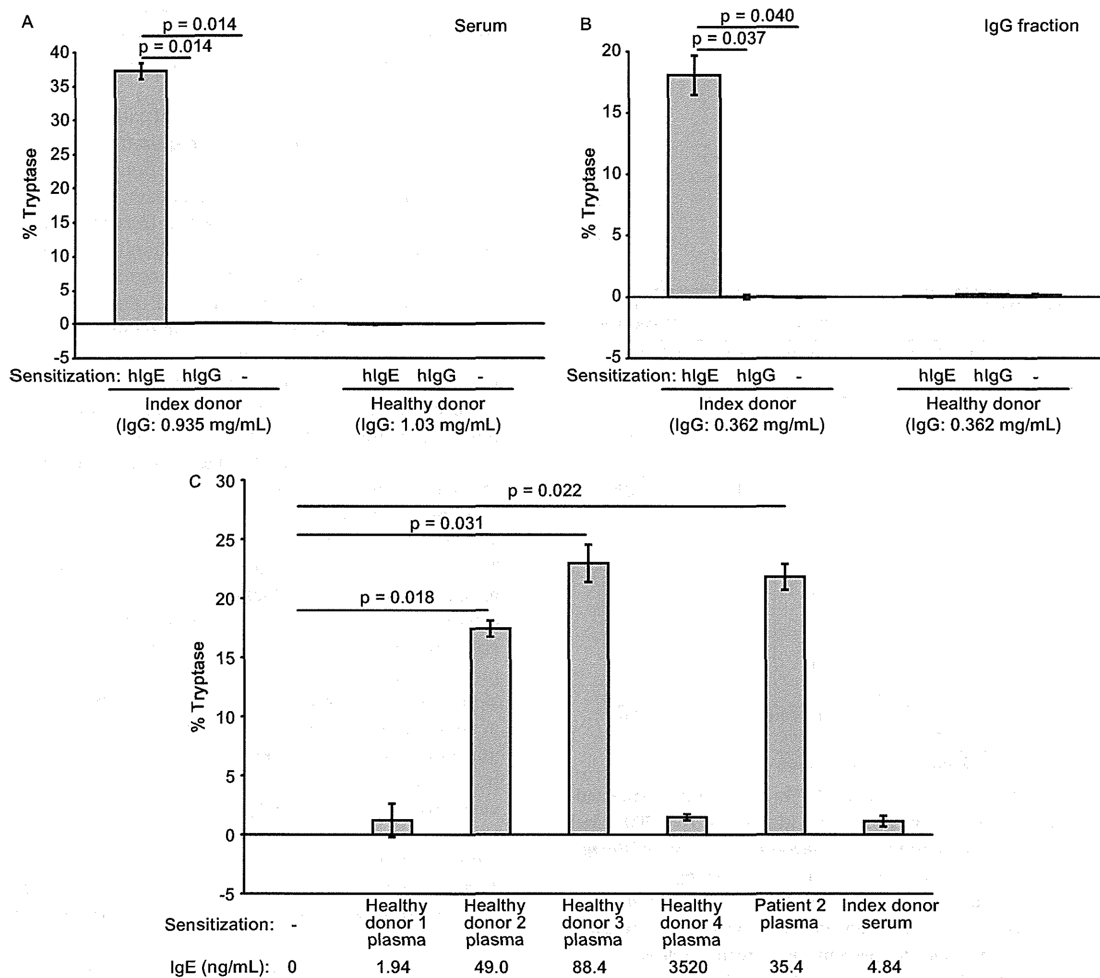

Fig. 3. Incubation of CBMCs for degranulation induced by index donor serum. CBMC degranulation assay was performed. (A and B) CBMCs incubated with 35.4 ng/mL hIgE or hIgG as the control were washed and then used for the degranulation assay of the indicated serum samples (A) and the IgG fractions of these samples (B). The IgG concentrations in the assay solutions are indicated at the bottom of the panel. (C) CBMCs were incubated with indicated plasma or serum, and then the degranulation assay was performed using 10 μL of the index donor serum. The IgE concentrations at which CBMCs were incubated are indicated at the bottom of the panel. p values indicating significant differences are shown. The mean ± SD percentage of tryptase release is plotted.

did not activate IgE-sensitized CBMCs. Thus, it is suggested that the blood component with a high concentration of the IgG anti-IgE possessing mast cell degranulation activity similarly to the index donor plasma is very rare.

The concentration of IgE that sensitizes the cells seems to regulate whether CBMCs are induced to degranulate by the index donor serum. CBMCs were activated by the index donor serum when incubated with plasma containing appropriate IgE concentrations, as shown under Results, and when incubated with hIgE at tens to a thousand of nanograms per milliliter but not at lower or higher than these concentrations (data not shown). The results suggest that at a low IgE concentration, the amount of IgE molecules on CBMCs was not sufficient to react with the IgG anti-IgE and that at an excessively high concentration of IgE, by forming an antigen–antibody complex, the IgE molecules in the assay solution inhibited the binding of the IgG anti-IgE to IgE on the CBMC surface to induce degranulation. Indeed, the IgE concentration in Patient 2 plasma decreased by half

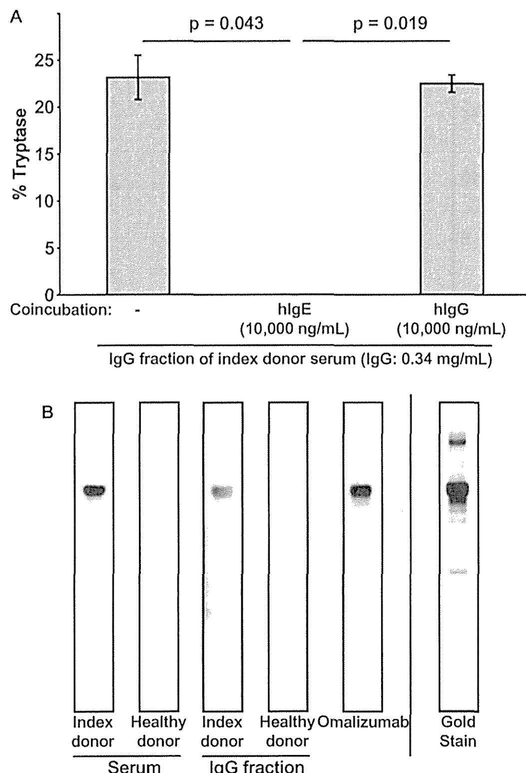

Fig. 4. Assessment of IgG anti-IgE in index donor serum activating IgE-sensitized CBMCs. (A) hIgE-sensitized CBMCs were washed and then used for the degranulation assay of the IgG fraction of the index donor serum (IgG: 0.34 mg/mL) coincubated with or without 10,000 ng/mL hIgE or with hIgG as the control. p values indicating significant differences are shown. The mean ± SD percentage of tryptase release is plotted. (B) The IgG antibody against IgE in the index donor serum was detected by Western blot analysis. The hIgE-blotted membrane was incubated with the samples indicated at the bottom of the panels.

after the occurrence of transfusion reactions. It might be suggested that the transfused IgG anti-IgE was consumed in part during its complexation with circulating IgE in the patient's blood.

In addition to the concentration of patient plasma IgE, a rapid transfusion rate might be required in the development of anaphylactic transfusion reactions. Both patients who developed anaphylactic transfusion reactions were treated by plasma exchange, in which the transfusion rate was considered to be much higher than that for supplementation of the blood coagulation factor. The rapid infusion of a high concentration of anti-IgE might facilitate the induction of the degranulation of the patients' mast cells. This may explain why no transfusion reaction cases other than the two cases presented in this study have been reported, despite the fact that many bags of blood components were prepared from the index donor blood.

In summary, the results suggest that the IgG anti-IgE in the transfused plasma caused anaphylactic transfusion reactions in these two patients. Thus, we propose a possible mechanism underlying these transfusion reactions. That is, the transferred antibody against a recipient's antigen causes anaphylactic transfusion reactions. An existing antibody in the patient blood that recognizes a substance in the transfused blood component has been proposed as a causative factor for anaphylactic transfusion reactions. However, concerning transfusion-related acute lung injury (TRALI), transferred antibodies, such as antibodies to human leukocyte antigens (HLA) and human neutrophil alloantigens (HNA) are considered as causative factors activating various cells in transfusion recipients.[25] The concentration of the transferred HLA antibody is suggested to be related to the development of TRALI.[26] Similarly, anaphylactic transfusion reactions could be induced in some patients in the presence of an adequate concentration of a transfused antibody against a specific antigen on the patient's cells, such as the IgG anti-IgE found in this study. Identification of causative antibodies in blood components would help reduce the risk of these transfusion reactions.

TABLE 1. IgG anti-IgE in index donor sera collected on various dates

	IgG anti-IgE			% Tryptase release†	
		Concentration*			
Collection date	Western blot analysis	Mean (ng/mL)	SEM	Mean (%)	SD
February 22, 2001	+	3200	210	30.7	2.0
August 23, 2002	+	3200	98	31.3	1.1
September 1, 2003	+	3000	230	30.8	2.4
April 17, 2004 (Case 1)	+	2300	130	23.3	2.1
September 17, 2004	+	2800	180	25.9	3.8
October 26, 2004 (Case 2)	+	2200	220	22.4	1.6
January 26, 2005	+	2500	250	21.1	3.0
July 11, 2008	+	8900	560	29.9	3.1

* The concentration of the IgG anti-IgE was measured by ELISA.

† For the degranulation assay, hIgE-sensitized CBMCs were activated with 10 μL of each of the index donor sera collected on the indicated dates.

CONFLICT OF INTEREST

The authors report no conflicts of interest or funding sources.

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第76回日本血液学会学術集会
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日本における輸血感染症のリスク

佐竹正博

Key words : Transfusion-transmitted infection, Emerging infectious disease, Blood screening

日本における輸血感染症の実態と残存リスク，これから有り得る輸血感染症について，その主なものを概説する。

1. 核酸増幅検査によるスクリーニング

B型肝炎ウイルス（HBV），C型肝炎ウイルス（HCV），ヒト免疫不全ウイルス（HIV）は，核酸増幅検査（nucleic acid amplification testing, NAT）によるスクリーニングの対象となっている。日本赤十字社血液センターでは，従来はドナー血清を20本プールした検体を用いて検査を行っていたが，平成26年8月より，プールをしない個別検体での検査が開始された。核酸の抽出効率・試薬の感度はほぼ究極の段階にあり，これ以上の感度の上昇は検体の容量に依存することになる。この態勢下でなおこれらのウイルスによる感染がおこるのは，いわゆるウィンドウ期にある献血者の献血血液によるものがほとんどである。すなわち，献血者が感染を受けてから，その末梢血液中のウイルス濃度がNATの感度に達するまでの期間に献血された血液である。ウイルス抗原やウイルスに対する抗体が検出されるのはそのあとになる。

① HBV

HBVは感染性が非常に高いうえに，国民の間の侵淫率も高かった[1]。さらに末梢血中でのあり方にも特有のものがあり，これまでの輸血感染対策は複雑なものであった[2]。HBVゲノムの末梢血中の倍加時間は約2.6日[3]と非常に長く，それだけウィンドウ期間も長い。したがってその間に献血される頻度も高くなる。試算では，個別NAT陰性のウィンドウ期の献血血液は1年に約90本はあるだろうと考えられる[4]。これは輸血用血液製剤1バッグにHBVが1個以上入っているものの数であり，そのうちのごく一部が実際に感染を起こすものと思われる[5]。実際に血液センターが把握する輸血感染例は年に2,3例である。もう一つの感染源は，HBV感染既往者の血液である。今日では，少年期以後の水平感染では治癒後HBVは体内から完全に駆逐されるという考えは間違いであり，肝細胞などにはHBVが残っていて，間歇的にあるいは持続的に末梢血中に低濃度のウイルスが放出されると考えられている[4,6,7]。近年では，極めて低濃度ながらこれらが原因となった輸血感染例はウィンドウ期血液による感染よりも多かった（Fig. 1）[4]。2012年，コア抗体陽性血液をすべて排除したことにより，このタイプの感染例はほとんどなくなったと考えられる。ただ，現行のコア抗体の感度以下の感染既往者の血液は存在するはずで，それらの血液による感染の可能性は残っている。

NATの高感度化により，出庫される血液製剤にHBVが混入したとしてもそれは極めて低濃度のものに限られる。このレベルになると，製剤の感染性は製剤1ユニット中の全ウイルス量に依存することが示されている[4]。したがって以後の感染は，赤血球製剤よりも，血漿量の多い新鮮凍結血漿や濃厚血小板に集中するであろうと推定される[4]。これまでは，HBVの高い感染性のために，プールNATの感度が感染性の有無を分けるレベルに達しておらず，そのほかの臨床的な要因が感染を規定していたと思われる。

血液製剤中に混入したHBVの量は非常に少なくなるため，輸血感染の後HBVマーカーが陽転したり実際に肝炎を発症するまでにはこれまでより時間がかかると思われる。遡及調査ガイドライン[8]で推奨されている輸血後3カ月では感染の有無をまだ断定できない可能性がある。したがって輸血時より遠く隔たった時期に肝炎を発症することが多くなり，フォローする医療機関の連携など，これまで以上に輸血後の患者のフォローに注意を必

日本赤十字社中央血液研究所

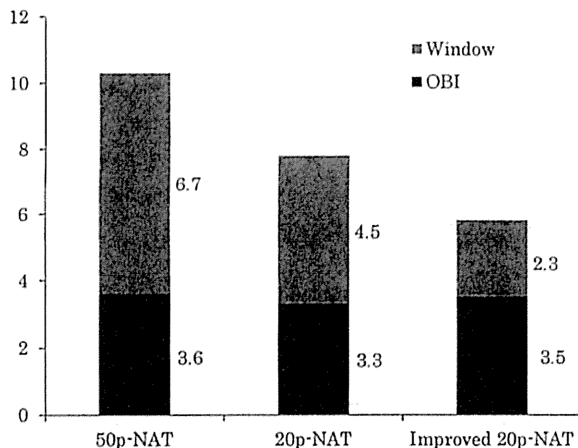

Fig. 1 Number of established Transfusion-Transmitted-HBV infections grouped according to pool-based NAT screening systems.
Window; Infection cases caused by window period-derived blood components.
OBI; Infection cases caused by occult HBV infection-derived components. OBI refers to anamnestic HBV infection stage typically represented by HBsAg-negative, HBcAb-positive, and HBV PCR-positive.
Intervals when indicated NAT systems were applied were Feb. 2000 - Jul. 2004 (4.5 y), Aug. 2004 - Jul. 2008 (4.0 y), and Aug. 2008 - Mar. 2010 (1.67 y), for 50 pool-NAT, 20 pool-NAT, and Improved 20 pool-NAT, respectively. Sensitivities of NAT systems used are 650, 260, and 76 copies/m*l*, for 50 pool-NAT, 20 pool-NAT, and Improved 20 pool-NAT, respectively. Vertical axis indicates the number of established transfusion-transmitted HBV infection cases per year.

要とする。

輸血後のB型肝炎と間違われやすいものに，HBVの再活性化がある。分子標的薬の投与や免疫抑制を行う患者はしばしば輸血も受けているので，時期的にどちらの肝炎であってもよい場合がある。再活性化による肝炎は重症で時に致命的であるので，どちらであるかを早急に鑑別し必要な治療を施さなければならない[9]。

② HCV

HCVの末梢血中での増殖速度は，倍加時間が約11時間と極めて短く[10]，たとえばHCVが1コピー/m*l*から個別NATの検出感度濃度に達するまで2日とかからない。たまたまこの短いウィンドウ期にあった個人が献血をするという確率は極めて低い。過去5年間の20プールNATの時期に，NATのみ陽性（抗体陰性）の感染初期の献血は37例であった。感染後のNAT単独陽性の時期の長さを90日とすると，2日間のウィンドウ期にある個人が献血する頻度は年間0.2回に満たない。プールNATの段階ですでにHCVの輸血感染はほとんど防がれていたと考えてよく，過去5年間に輸血によるHCV感染は1例のみであった。個別NAT下ではさらに少なくなることが期待される。しかも図に見るように，NATのみ陽性の献血（献血者が献血数カ月以内に感染の機会を有したことを示す）は年々減少してきている(Fig. 2)[11]。リスクを有することを自覚した人が献血をしないようになったか，あるいは民間医療を含めて観血的医療・施術に使用する器具や手技が，感染の伝播という点において改善されてきたことなどが考えられる（滅菌の徹底や使い捨て器具の使用など）。

③ HIV

HIVの末梢血中の倍加時間は20.5時間とされる[12]。20プールNAT下でのウィンドウ期は約2週間であったが，個別NAT下では約11日となる。日本では海外に比べて，献血者中のHIV抗体陽性者の割合が一般人口でのそれに比べて高い状態が続いてきた[13]。感染してから間もない抗体陰性・NAT陽性の献血者はここ14年間に27人であった。これらから計算すると，個別NAT陰性のHIV感染者の献血は1年に2例近くあることが推定される。実際2013年暮れに起きた輸血によるHIV感染事例は，HIVの輸血感染リスクが依然残っていることを示している[14]。この例においては，献血者がHIV感染後1, 2週で献血をし，その血液に由来するFFPの輸血によってひとりの患者に感染が起きた。献血者の保管検体の検査では3つのNATシステムのうち一つのみで検出することができた（ただしそのうちの一つは定量系）。世界的に，個別NAT陰性の血液によるHIV感染の報告はまだないが，今回の例は個別NATでも検出できない，感染性のある献血があり得ることを示している。同じ血液に由来する赤血球製剤では感染は起きなかった。ここでも感染性が輸注ウイルス量に依存することが示唆される。なおこの事例は，当該献血者が2度目の献血を行った際，HIV抗体が陽転しており，血液センターで前回の献血血液の保管検体を個別NATで調べた結果，HIV RNAがわずかに陽性であり，該当する血液を輸血された患者を至急遡及調査して判明したものである。

以上，NATでスクリーニングしている3つのウイルスについては，蓄積された検査データから残存リスクを推定することができる[15,16]。それはウイルスを含む献血の頻度としてあらわされ，上記のようにHBVやHIVに関しては依然として大きな数である。しかし実際の感染性はまた別の話であり，製剤の形態（PC，凍結品であるFFP，赤血球製剤の違い，血漿量の違いなど），献血者の感染ステージ，患者の免疫状態・全身状態などが複

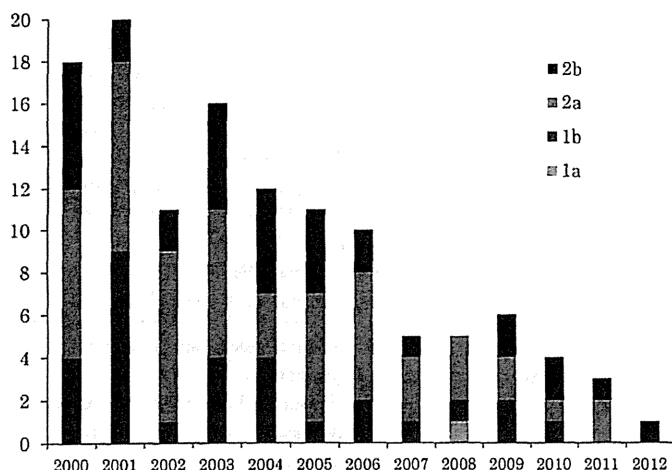

Fig. 2 Number of blood donors positive for NAT but negative for HCV-Ab in relation to year. Number of donors is shown separately with each HCV genotype indicated with different color. Vertical axis shows the number of donors by year.

雑に関与してくる。その結果例えば，HBV ウィンドウ期の血液の感染率は 50%，感染既往の HBV NAT 陽性血液の感染率は 3%などと計算された[5]。いっぽう臨床の現場では，輸血を受けた患者の半数は 1 年以内に死亡しており，それらの患者が輸血感染を受けたかどうかはほとんど不明である。さらに，輸血感染症をフォローできているのは全国で 30～40%と言われており[17]，合わせると，厚労省と血液センターが把握している感染数の 5, 6 倍の感染が実際には起きているのではないかと推定される。

2. ヒトT細胞白血病ウイルスI型（human T-cell leukemia virus type 1, HTLV-1）

成人 T 細胞白血病（Adult T-cell Leukemia, ATL）や HTLV-1 関連脊髄症（HTLV-1-Associated Myelopathy, HAM/TSP）の原因となるレトロウイルスである。成人後に輸血を含めた水平感染によって感染した人から ATL が発症することは極めてまれとされている[18]が，HAM/TSP は輸血後比較的短い期間で発症しうる[19,20]。献血血液に対しては現在日本では CLEIA 法による抗体スクリーニングが行われている。HTLV-1 はプロウイルスとして宿主 CD4＋陽性 T リンパ球のゲノムに組み込まれ，輸血に際してはリンパ球と共に患者血中に入ることになるが，2007 年 2 月以降はすべての輸血用血液製剤は保存前白血球除去がされており，その点からも輸血による感染の可能性は低い。妊婦検診などでの検査では，抗体スクリーニングは陽性であるが，蛍光抗体法やウェスタンブロット法などの確認検査が陰性の場合があり，患者への説明が問題となっている[21]。献血血液のスクリーニングでも同様であるが，スクリーニング陽性の血液はすべて不合格となっている。

3. ヒトパルボウイルス B19（PB19）

PB19 は主に小児の感染症である伝染性紅斑の病原ウイルスである。妊婦が感染すると胎児水腫を起こす危険がある。非常に感染力が強く，約 4 年に 1 回大流行をきたす。最も小型のウイルスに属し，エンベロープはなくカプシドが頑丈で，物理化学的に厳しい環境でも破壊されにくい。したがって，血漿分画製剤の製造工程でも最後まで不活化されずに残り，最終製品に濃縮されることになる。このため，血漿分画製剤の投与による PB19 感染例が少なからず報告されている[22]。血漿分画製剤の原料血漿を NAT スクリーニングするか，またはナノフィルトレーションすることにより効果的に PB19 の混入を防ぐことができる[23,24]。

健常人における PB19 感染後のウイルスの血中の動態はきわめて特徴的である[25]。気道からの感染後約 10 日で血中濃度は極めて高くなり，10^{12}コピー/m*l* にも達する[26]。その直後から IgM/IgG 特異抗体が出現して急激に血中濃度は下がる。しかし 10^{4}コピー/m*l* 付近からはなかなか下がりきらない例が多く，年余にわたって血中にウイルスゲノムが見つかることが多い[27]。しかしながら特異的 IgG 出現後は，ウイルスゲノムが検出されても感染性はほとんどないと考えられる。ウイルス血症の極期にあっても症状を自覚しないことも多く，したがってその時期に献血するリスクも高い。実際ウイルスコピー数が $10^{6.7}$コピー/m*l* 以上を示す献血は日本全国で 1 年に 2,000 件以上ある（日赤データ）。日赤ではこの感度を有する抗原検査を施行しており，効果的に高ウイルス血症の献血を排除しているが[28]，これよりも低い濃度でも輸血感染を起こすことがある[29]。

PB19 のレセプターは赤血球グロボシドであり，赤芽球系の細胞に強い親和性を持つ。健康人に感染した場合

は網状赤血球が一時的に低下する程度で済むが，盛んな造血によって末梢の Hb レベルをようやく維持しているような患者では，感染によって赤血球造血が損なわれ重症の貧血，あるいは一時的な骨髄無形成クリーゼとなる[25]。溶血性貧血の患者や，化学療法からの回復期の患者などでリスクが高い。重度の免疫抑制下にある患者が感染すると，特異抗体が産生されない為に感染が持続し，赤芽球ろうとなる。汎血球減少に至る場合もある[30]。

輸血用血液製剤の投与によってこれらの病態を引き起こす可能性は十分に高いが，症例の報告は世界でも 20 例に満たない。その半分以上は日本からのものである (Table 1)[29]。日本の臨床医の患者の観察が極めて行き届いているのではないかと思われる。なお一層注意すれば症例が隠れている可能性もある。なお，輸血用血液について PB19 をルーチーンでスクリーニングしているのは日赤とドイツ・オーストリアの一部の血液センターだけである[31]。

4. ヒトサイトメガロウイルス (CMV)

CMV はその感染が引き起こす病態の重篤さから，特に免疫抑制を受ける患者が増加している現代の医療において大きな問題を提起する。CMV は白血球との親和性が強く，輸血の場合は白血球と共に患者血中に入るので，白血球を除去した血液が特異抗体陰性血の代用として使用できるのではないかという議論がなされてきた[32]。これは，国民の間での CMV の感染率が高い為に，CMV 陰性血を見出すことがしばしば困難であることから起きている問題である。日本では衛生環境の改善と共に CMV の感染率が下がってきているのではないかとの推察もあるが[33]，最新の首都圏の献血者の調査では 20 年前の感染率とほとんど同じで，20 歳代は 58%，30 歳代は 73%，60 歳代で 98%（平均 77%）の感染率であった (Fig. 3)[34]。さらに，血液型その他の条件で患者に適合する血液を選択する候補血の範囲は小さくなり，血液センターに大きな負担がかかる。世界中で，臨床の困難な状況下で前方視的あるいは後方視的スタディが施行されてきたが，抗体スクリーニングをしていない白血球除去血による CMV 感染頻度は抗体陰性血の場合よりわずかに高いか，あるいは有意差はない，というのが全体的な結論である[35,36]。

現実には輸血感染はほとんど防がれているのではないかと思われるが，それぞれの方策には理論的な欠点がある。抗体陰性血が最も安全のように思われるが，CMV のウィンドウ期というものがある。上記のように CMV は青壮年の年代で急激に感染率が上昇する。献血者の中でも盛んに新規感染が起こっているはずである。CMV が感染してから抗体が陽性となるまでには約 6 週間を要するとされており[37]，その間の献血は抗体陰性でも高濃度のウイルス血症を示す可能性がある。これらのことから抗体陰性血よりも白血球除去血の方が安全であるとす

Table 1 Clinical course of parvovirus B19 infection caused by the transfusion of parvovirus-contaminated blood products. Day 0; implicated transfusion.

Patient	Clinical course
61y Male, AML	Day 22; high fever Day 24; Systemic erythema, Low reticulocyte Pure red cell aplasia on bone marrow puncture Day 25; PB19 DNA+ 7 weeks; Anemia recovered
57y Male, AML	Recovered leukocyte and platelet but low erythropoiesis Day 21; pure red cell aplasia and complete remission of AML on bone marrow puncture Day 24; PB19 DNA+, αPB19-IgM+, αPB19-IgG+ Day 35; αPB19-IgM−, erythropoiesis recovered
41y Male, Hairy cell leukemia	Day 10; ret. 0.3% Day 11; PB19 DNA 1×10^{12} copies/*ml* Red cell aplasia sustained 3 months; Complete recovery from anemia
30y Female, Caesarean section	Day 6; fever (39℃) Day 9; WBC 1530, CRP 0.6 mg, high fever sustained Day 19; αPB19-IgM+, αPB19-IgG+, ret. 0.2% Day 22; ret. 8.9% Day 31; PB19 DNA 4.8×10^{4} copies/*ml*
35y Female, Placenta previa	Day 7; high fever (38℃) Day 11; systemic eruption Day 12; αPB19-IgM+ 1 month; all recovered
59y Male, Rectal cancer	Day 6-11; high fever (38-40℃) Day 22; PB19 DNA+, αPB19-IgM+, αPB19-IgG+
50s Female, Paroxysmal nocturnal hematuria	High fever, general malaise, pancytopenia (1 M), sustained anemia
20s Female, Hemolytic anemia	Reticulocytopenia (1 m), sustained severe anemia
70s Male, Pelvis tumor	Reticulocytopenia (1 w), asymptomatic

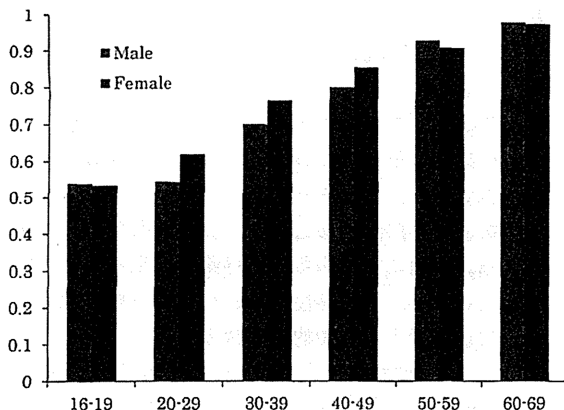

Fig. 3 Yearly change of CMV seroprevalence. Horizontal axis indicates age range of blood donors examined. For each group of donors categorized by age and sex, 200 randomly selected blood samples were investigated for IgG CMV-specific antibody. A total of 2,400 samples were collected in Tokyo metropolitan area. Vertical axis indicates ratio of CMV seroprevalence.

る論調もある[38]。しかしながら我々の検討でも，抗体陰性で high viremia の献血というものは極めてまれである[34,39]。いっぽう白血球除去血は，白血球数やウイルス濃度が白血球除去フィルターの除去能力を超えた場合にオーバーフローしてしまう危険と，白血球に結合していないフリーの CMV が濾過されないリスクがある。ただしそのようなフリーの CMV が感染性をもつか否かは不明である。また血液センターのろ過工程での不備の可能性もあるが，日赤では全国の施設の採血から常時サンプリングして白血球数をカウントしており，基準達成率は常に 99.9％前後である[40]。

いっぽう，CMV 抗体陰性血の適応については，造血細胞移植や固形臓器移植の分野では CMV 感染のモニタリングと先制治療が臨床的に非常に有効であるため[41]，厳密に CMV 抗体陰性血が必要とされることが少なくなった。日本の造血細胞移植ガイドラインの CMV 感染症に関する記述でも，移植後の CMV 感染症の多くは患者に潜伏感染している CMV の再活性化によるものであるとし，患者とドナーがともに CMV 抗体陰性の場合にのみ，CMV 陰性血液製剤を使用することが望ましいとしている[42,43]。そして CMV 抗体陽性血液製剤を用いても，CMV 初感染のリスクは低いとしている。最も尖鋭なのはイギリスで，2012 年の血液・組織及び臓器の安全性に関する諮問委員会（SaBTO）の報告では，CMV 抗体陰性血の適応を子宮内輸血，新生児・妊婦への輸血のみとし，その他の場合は，患者・ドナー共に CMV 陰性の場合も含めて，白血球除去血で十分であると結論付けている[44]。シアトルの移植センターではすでに抗体陰性血の使用は全面的に中止されている。

日本での CMV の問題は主に新生児医療の分野にある。日赤の血液センターに，輸血の副作用報告として早産児・極超低出生体重児への CMV 感染疑い例が多く寄せられるようになった。使用されたのはほとんどの場合白血球除去血ではあるが CMV 抗体陰性血ではない。これまで，該当する輸血製剤の保管検体の精査で CMV-DNA や IgM 型抗 CMV 抗体が検出された例はない。白血球除去血以上の安全を期す場合には，血液センターに CMV 抗体陰性血をあらかじめ依頼することが望まれる。また，可能であれば輸血前の患者検体，母親の血液または母乳などの検体を同時に提出すれば輸血との因果関係を明らかにする手助けとなる。

5. E 型肝炎ウイルス（HEV）

HEV が日本を含めた先進国の間で土着のウイルスであることはすでに確立された。日本で認められる遺伝子型は 3 と 4 である。日本ではブタ，イノシシ，シカなどが主な自然宿主で人獣共通感染症である[45,46]。それらの内臓肉などを十分に加熱調理しないまま摂取することで感染する。市販されている食用豚肉にもまれに混入していることがある[47]。まれに劇症肝炎を起こすことがあるが通常は不顕性感染を起こし[48]，国民の 3.4％が抗体陽性である（東日本で 5.6％，西日本で 1.8％）[49]。これら無症状の急性感染期の感染者が献血した場合に，その血液が感染性を持つ可能性がある。2005 年より北海道血液センターで NAT によるスクリーニングを試行的に実施しているが[50]，そのデータから推定すると，日本全国で 1 年間に 300 人から 400 人がウイルス血症のまま献血をしていると思われる。北海道での試行的スクリーニングや血漿分画製剤メーカーからの情報[51]などから HEV 陽性の献血が判明することがある。それをもとに行った遡及調査によれば，HEV 陽性の血液製剤を輸血した場合の感染性は 30％と推定される（未発表データ）。日赤が把握している輸血による HEV 感染例は過去 15 年間に 14 例である。また惹起される肝炎については，ALT の最高値は，全身状態の悪い患者において 2,000 に近いレベルであり，多くは 100 から 1,000 の間にある。この中で臨床医からの自発的な感染報告はここ 12 年間でわずか 3 例であり[52]，その他はすべて血液センターからの情報に基づく調査で判明したものである。臨床医の間で HEV の輸血感染，あるいは HEV 肝炎そのものについての認識がまだ低いと思われる。E 型肝炎は国への届け出が必要な 4 類感染症であり，輸血後の肝機能の定期的なチェックや，非 A，B，C 型肝炎の診断などでは HEV

を考慮に入れる必要がある。現在国に報告されているE型肝炎の発症例数は年間100例を少し超える程度である。HEVの特異的IgA検査は2012年に保険収載されている。

6. 新興・再興感染症

① ウェストナイルウイルス（WNV）感染は1999年にアメリカ合衆国で初めて報告され[53]，その後2002，2003年に大流行を見た[54]。以後しだいに感染数は減る傾向にあったが，2012年に再び感染報告数が大幅に増えた（報告数5,674）。その原因はよくわかっていない[55]。2013年にはほぼ半減した。それでも死亡例は114例を数えており依然猛威を振るっている。WNVは哺乳類の血中では高いウイルス濃度に達せず[56]，そのためヒト—蚊—ヒトの感染環は成立しない[57]。高いウイルス濃度を示す鳥類を吸血した蚊からヒトは感染を受ける。したがって鳥類のモニターが非常に重要である。

全米の血液センターではNATによるWNVのスクリーニングを行っており[58]，2013年には420名のWNVウイルス血症のドナーを検出している。適切なNATスクリーニングの施行により，ここ数年は輸血感染は一例も報告されていない。日本ではこれまで1例も国内での感染が報告されていない。感染した渡り鳥が飛来しても，その時期には媒介蚊がいないためと思われるが，日本人が持っている日本脳炎ウイルス抗体が防御的にWNVに交差反応している可能性もある[59, 60]。日赤の血液センターでは国立感染症研究所と協力して，限定された地域での献血血液のNATスクリーニングを施行できる体制を整えている。

② デングウイルスは赤道をはさむ地域を中心に，毎年全世界で5千万人から1億人が感染するといわれている。ヒト血中の濃度が10^9コピー/m*l*レベルにまで達するので[61]，ヒト—蚊—ヒトの感染環が成立する[57]。熱帯特有のウイルスと思われがちであるが，日本でも第二次世界大戦中に西日本で約20万人が感染したことがある[62]。それ以後は国内での感染報告はなく，毎年200例を超える感染報告はすべて海外からの持ち込みによるものとされている。しかしながら，隣国台湾では毎年のように千例を超えるデングウイルス感染が報告され[63]，日本にも媒介蚊（ヒトスジシマカ）がいるので，国内でも感染がいつ起こっても不思議ではない。アメリカのフロリダでは輸入感染症ではない国内での感染例の報告が相次いでいる[64]。

これまでデング熱の輸血感染の文献報告は極めて少ないが，その理由の一つに，輸血を受ける集団もすでに既感染であるため発症しないということが挙げられている[65]。この点からみると，日本のようにこれまで流行していなかった地域で輸血感染が顕在化する可能性がある。プエルトリコではアメリカ赤十字の援助を受けてNATによる献血血域の試験的なスクリーニングが行われている[66]。

③ チクングニヤウイルス感染例[67]も，これまでは海外からの持ち込み例であるが，デング熱に匹敵する高いウイルス血症[68]，ベクターの存在（ヒトスジシマカ），海外での感染の広がりなどから，日本でも蔓延する可能性がある。輸血による明らかな感染例は世界でもまだ報告されていない。急性熱性疾患としての一般的な症状に加え，関節痛などが特徴的な症状である。

バベシア症は，マダニによって媒介される原虫疾患で，マラリアとよく似た症状，検査所見を呈する。発熱，溶血，腎不全，ARDSにまで至る多彩な臨床像を呈し，予後も不良である。赤血球に感染し輸血によって伝播しうる[69]。アメリカではこれまで150例以上の輸血によるバベシア感染があり，少なくとも12人の死亡例が報告されている[70]。日本では2000年に赤血球製剤による感染例が報告されている[71]。バベシア原虫（*Babesia microti*）は4℃に保存された赤血球製剤中で35日間生存しうる[72]。

日本でのシャーガス病の輸血感染リスクを調査するため，約4,000人の南米出身の献血者の血液を調べたところ，1検体において*Tripanosoma cruzi*特異抗体が陽性，PCRも陽性となった。同献血者の過去の献血9本のうち6本が同様にPCR陽性であった。このように，日本においてもシャーガス病の輸血感染のリスクは存在する[73]。幸いこの例では，確認できた5人の受血者はすべて感染していなかった。現在血液センターでは問診にて，南米で生まれたかあるいはその母親が南米で生まれた献血者からの血液は，血漿のみを血漿分画製剤の原料として用いている。

そのほか，新興再興感染症としては，マラリアや重症熱性血小板減少症候群（SFTS，SFTSブニヤウイルスによる）[74]などが重要である。

7. 細菌感染症

献血の際，採血バッグの中に1個の細菌が混入しても保存中に膨大な数にまで増殖しうる。皮膚上の細菌だけではなく，献血者の血中の細菌も製剤の汚染の大きな原因となる。健常者の日常生活においても一時的な菌血症が頻繁に起こることは周知のとおりである[75, 76]。通常献血の際，細菌が混入するとすれば，1バッグあたり1～20個レベルといわれている[77]。その後保存中に増殖を開始するまでの時間や増殖する速度は細菌の種類によってまちまちである[78]。一般に病原性の強い細菌の増殖は速い（*Bacillus cereus*，*Staphylococcus aureus*など）。