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Case Report

Fatal delayed hemolytic transfusion reaction associated with anti-Di[b] and anti-E

Yuichiro Hatano [a,*], Setsuko Otsuka [c], Mitsuhiro Chousa [c], Shunnichi Saito [d], Kenneth E. Nollet [d], Hitoshi Ohto [d], Katsuya Shimabukuro [b], Hirofumi Takemura [b], Akira Hara [a]

[a] Department of Tumor Pathology, Gifu University Graduate School of Medicine, Gifu, Japan
[b] Department of General and Cardiothoracic Surgery, Gifu University Graduate School of Medicine, Gifu, Japan
[c] Department of Transfusion Medicine and Cell Therapy, Gifu University Hospital, Gifu, Japan
[d] Department of Blood Transfusion and Transplantation Immunology, Fukushima Medical University, Fukushima, Japan

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ABSTRACT

We report the death of a 61-year-old Japanese man massively transfused during and after emergency aortic surgery. Postoperative on day 8, he died after cardiac arrest associated with hyperkalemia. Indirect antiglobulin testing demonstrated both anti-Di[b] and anti-E antibodies pre-transfusion, and elevation of their titers as the delayed hemolytic transfusion reaction evolved. Monocyte monolayer assay (induction of reactive monocytes) and flow cytometry (increase of IgG1 and/or IgG3) gave evidence of the clinical significance of both antibodies. Anti-Di[b] must be considered when an antibody to a high incidence antigen is found in Japanese and other Mongoloid populations.

1. Introduction

Delayed hemolytic transfusion reaction (DHTR) is widely recognized as a complication that occurs 2–14 days after transfusion of incompatible red blood cells (RBCs) [1–9]. DHTR is an immune response characterized by erythrocyte opsonization and phagocytosis by macrophages in the spleen and liver. The opsonizing antibody is generally IgG, typically arising in response to previous transfusion or pregnancy. This extravascular hemolytic response is provoked, on average, by one in every 2424 RBC units transfused [9], or one in 804 recipients [10], thus making DHTR among the most common serious adverse reactions of transfusion. The Serious Hazards of Transfusion (SHOT) study in the United Kingdom reported that 4% of 1464 adverse events reviewed in 2010 were hemolytic transfusion reactions (58 cases total, 6 acute and 52 delayed) [11]. Jaundice, fever, bilirubinuria and anemia are typical manifestations of DHTR, which may in turn provoke respiratory insufficiency, renal failure, and even death.

Here we report a fatal case of DHTR after massive transfusion of Di(b+) and E(+) incompatible RBCs during and after emergency surgery.

2. Case report

A 61-year-old Japanese man, admitted to hospital with acute chest pain, was transferred to Gifu University Hospital (his clinical course is summarized in Fig. 1). He was diagnosed with Stanford type A acute aortic dissection by contrast-enhanced CT, and underwent emergency surgery.

Pre-transfusion testing revealed his blood group as B, Rh(CCDee). The direct antiglobulin test (DAT) was negative, while the indirect antiglobulin test (IAT) was strongly positive (3+): his serum reacted with all panel cells but not with his own RBCs. History revealed that he had surgery after a head injury approximately 40 years ago, but details of prior transfusions were unavailable.

* Corresponding author. Address: Department of Tumor Pathology, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu 501-1194, Japan. Tel.: +81 58 230 6225; fax: +81 58 230 6226.
E-mail address: yuha@gifu-u.ac.jp (Y. Hatano).

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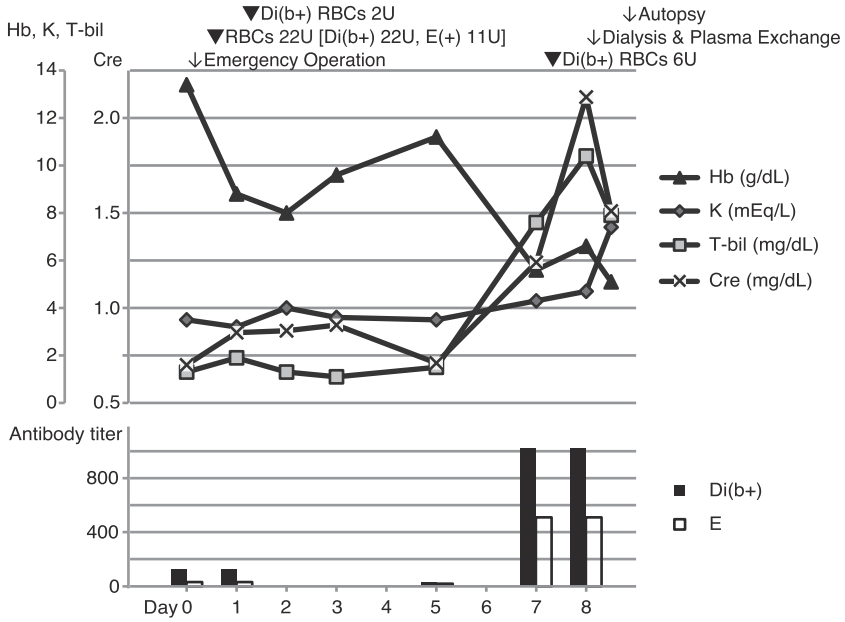

Fig. 1. Clinical course of the present case, a 61 year-old male. Day 0 is the day of surgery. The patient received red blood cell (RBC) transfusion 3 times: 22 units on Day 0, 2 units on Day 1, and 6 units on Day 7. Eleven units of RBCs contained E antigen. All RBCs transfused were Di(b+) positive. On Day 7, blood tests revealed a sharp decrease of hemoglobin and increase of total-bilirubin, suggesting hemolysis. On the follow day (Day 8), dialysis and plasma exchange were performed for acute renal failure. The rapid potassium elevation associated with hemolysis was considered to cause cardiac arrest. U, units; Hb, hemoglobin; K, potassium; T-bil, total bilirubin; Cre, creatinine.

During emergent repair of the dissecting aorta, he was given 22 RBC units (1 "unit" in Japan corresponds to what can be derived from 200 mL of whole blood), 20 fresh frozen plasma (FFP) units and 40 platelet units to compensate for 5214 mL of blood loss. Postoperative lab work revealed that the patient had anti-E by the Bromelin test in addition to an unidentified broadly reactive antibody. Of the 22 RBC units, 11 were E(+) and the other 11 were E(−), and the surgeons informed the patient and his family of potential complications. At the time, Di(b+) incompatibility was not appreciated because the panel study was unable to identify the patient's anti-Di^b.

Two more E(−) RBCs were transfused the day following the operation and the DAT turned positive. Although otherwise uneventful, the postoperative course was marked by abrupt onset of anemia and jaundice after 1 week (Fig. 1). Elevation of the AST (27–163 IU/L, Day 5 to Day 7), LDH (357–1524 IU/L) and urea nitrogen (15.6–48.7 mg/dL) was also observed, consistent with hemolysis. Another 6 E(−) RBCs did little to correct the anemia; instead there was a further progression of jaundice. Simultaneously, he suffered from oliguria, prompting dialysis and plasma exchange. Two hours after initiation of this treatment, the patient went into cardiac arrest. He died an hour later despite aggressive cardiopulmonary resuscitation.

This death followed symptoms of a delayed hemolytic transfusion reaction for which an antibody to a high frequency antigen (other than E) was suspected. Therefore, samples were sent to a regional reference lab at the Japanese Red Cross Aichi Blood Center. One week after his death, Di(b+) incompatibility was determined. Indeed, the patient's phenotype was Di(a + b−) and all the RBCs transfused turned out to be Di(b+).

In accordance with Japanese law, this transfusion-associated death was deemed an adverse drug reaction eligible for compensation by the Pharmaceutical and Medical Devices Agency of Japan's Ministry of Health, Labour and Welfare.

3. Materials and methods

3.1. Blood phenotyping and antibody screening, identification and titration

Blood grouping and screening for irregular antibodies were performed with an ORTHO AutoVue Innova (Ortho Clinical Diagnostics, Raritan, NJ). A low ionic strength saline-indirect antiglobulin test (LISS-IAT) for antibody identification and titration was performed with a Gamma LO-ION (IMMUCOR, Norcross, GA). Reagent RBCs used for antibody screening, identification and titration were Resolve Panel C (Ortho Clinical Diagnostics) and Di(a + b−) Rh(E+) RBCs obtained from the Japanese Red Cross Aichi Blood Center.

3.2. Monocyte monolayer assay

The monocyte monolayer assay (MMA) was performed using a modification of the method described by Arndt and Garratty [12,13]. Mononuclear cells (MNCs) from a normal donor were separated by centrifugation using Lymphocepal-I (Immuno-Biological Laboratories, Gunma, Japan). After washing with phosphate buffered saline (PBS), the MNCs were suspended in a culture medium (RPMI 1640, Life Technologies, Carlsbad, CA) containing

Table 1
MMA.

Specificity of reaction	Sera	Target RBC	Reaction[c]	Phagocytosis[d]	Adherence[e]
E	Day 7[a]	ccDEE, Di(a + b−)	37.2	19	75.3
Di(b+)	Day 7[a]	ccDee, Di(a − b+)	11.2	13.3	1.3
Positive control	Anti-E[b]	ccDEE, Di(a + b−)	7	6.8	3.5
Background	Day 7[a]	ccDee, Di(a + b−)	4.2	0.3	3.8

Each value was derived from randomly counting at least 600 monocytes. Values >5 (either% or RBCs/100 monocytes) are underlined.
[a] Day 7, the post-transfusion serum of the patient.
[b] Anti-E, another serum containing anti-E antibody.
[c] Reaction, the percentage of monocytes either ingesting or adhering to the erythrocytes (%).
[d] Phagocytosis, the number of ingested erythrocytes per 100 monocytes.
[e] Adherence, the number of adherent erythrocytes per 100 monocytes.

5% fetal bovine serum (Life Technologies) and incubated for an hour at 37 °C in 5% CO_2 on the cover glass slides. After the non-adherent lymphocytes were removed using a pipette, sensitized RBCs [postoperative Day 7 serum plus group O, Rh(ccDEE), Di(a + b−) group O, Rh(CCDee), Di(a − b+) or group O, Rh(CCDee), Di(a + b−) RBCs] were added and incubated for 2 h at 37 °C in 5% CO_2. As a positive control, serum containing anti-E was used. After washing with PBS three times, the slides were stained with May-Giemsa. Six hundred monocytes were counted and the percentage of reactive monocytes showing phagocytosis and/or adherence was calculated. Values >5 (i.e., percent or RBCs/100 monocytes) are underlined in Table 1.

3.3. IgG subclass

IgG subclass was analyzed using standard methods with minor modification, as described previously [13]. The patient's serum samples from Day 0 (pre-transfusion) and Day 7 (post-transfusion) were incubated with group O, Rh(ccDEE), Di(a + b−) and group O, Rh(CCDee), Di(a − b+) RBCs for an hour at 37 °C in a Fisher tube, respectively. The human monoclonal anti-Jr^a (Hokkaido Red Cross Blood Center) was used as a positive control. After washing with 5% bovine serum albumin (BSA) in PBS two times, 10 μL of a 1-in-100 diluted mouse monoclonal anti-human IgG1, IgG2, IgG3 and IgG4 (The Binding Site Limited, Birmingham, UK) was added to 90 μL of 0.2% sensitized RBCs and incubated for 30 min at 37 °C. After washing with 5% BSA in PBS two times, 20 μL of 1-in-100 diluted Phycoerythrin-labeled antibody (Goat anti-Mouse IgG-R-Phycoerythrin, Jackson ImmunoResearch Laboratories, West Grove, PA) was added to each sample and incubated for an hour at room temperature. Fluorescence was measured by flow cytometry using a Gallios (Beckman Coulter, Brea, CA). The values were considered significant if more than twofold higher than that of the non-specific background reaction.

4. Results

4.1. Autopsy findings

Autopsy was performed 2 h after death. We confirmed properly sutured incisions and repairs consistent with the aortic surgery. There was no finding of an anastomotic leak or infection. In addition, we found not only a left temporal operation scar consistent with a previous head injury but also an abdominal operation scar indicative of prior surgery. However, information about this abdominal surgery was not found in his medical records.

Two histological findings of significance were systemic fibrin thrombi and erythrophagocytosis, consistent with disseminated intravascular coagulation (DIC) and extravascular hemolysis, respectively (Fig. 2). In addition to multiorgan failure related to DIC, hemolysis-related hyperkalemia was determined as a reason for the patient's cardiac arrest (Fig. 1).

4.2. Titration of anti-Di^b and anti-E antibodies

We retrospectively measured the antibody titer of anti-Di^b and anti-E by LISS-IAT in every available patient serum sample (Fig. 1). Anti-Di^b (titer of 128) and anti-E (titer of 32) were present before the initial transfusion. Although both remained low until Day 5, their elevation (titers of 1024 and 512, anti-Di^b and anti-E, respectively) was observed on Day 7, in parallel with the degree of hemolysis.

4.3. MMA

To evaluate the antibody potential for erythrophagocytosis, we performed MMA. The reactivity indices of the post-transfusion serum against group O, Rh(ccDEE), Di(a + b−), group O, Rh(CCDee), Di(a − b+) and group O, Rh(CCDee), Di(a + b−) RBCs were 37.2%, 11.2% and 4.2%, respectively (Table 1). These results suggest that both anti-E and anti-Di^b, but especially anti-E, induced monocytes to ingest and/or strongly adhere to RBCs with the corresponding antigens.

4.4. IgG subclass

To clarify the relation between antibody IgG subclass and macrophage activation, we performed flow cytometry using RBCs containing either Di(b+) or E antigens. Previous reports indicate that either IgG1 or especially IgG3 bind to Fcγ receptors on monocytes to activate erythrophagocytosis [8,14,15]. Anti-Di^b IgG1 was significant before transfusion and increased 3.3 fold by Day 7 (Table 2). No subclass of anti-E was higher than two times the non-specific binding result pre-transfusion, but anti-E IgG1

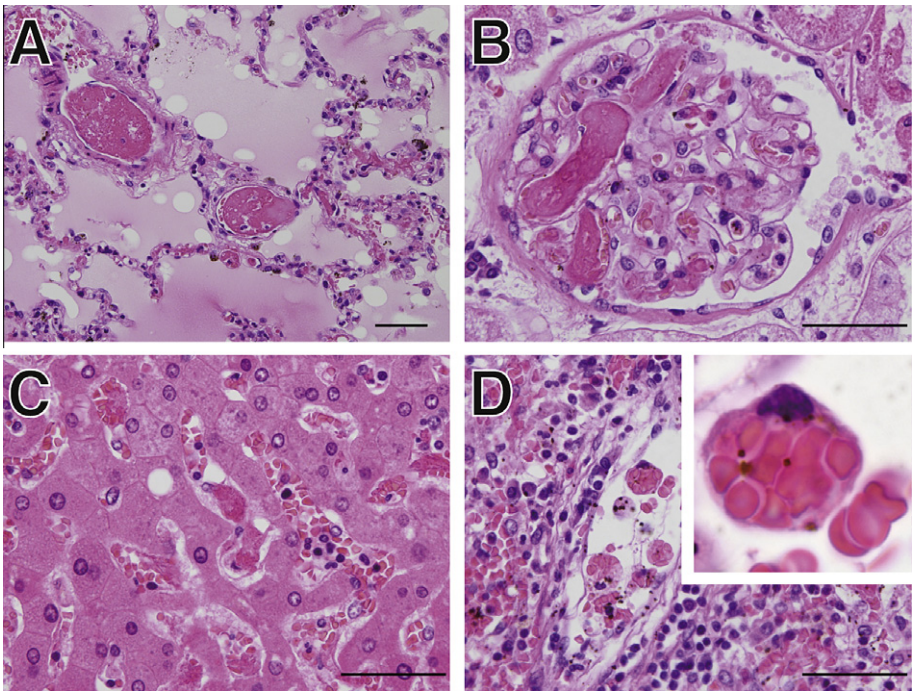

Fig. 2. Autopsy findings of the patient. A–C: Systemic fibrin thrombi. The fibrin thrombi were observed in the lung capillaries and small arteries (A), the renal glomeruli (B), the hepatic sinusoids (C) and the small arteries of other organs. D: Erythrophagocytosis. The process by which macrophages engulf and digest erythrocytes predominated in the spleen, which weighed 190 g, twice as heavy as the average. Inset: an erythrophage and erythrocytes. Hematoxylin and eosin, scale bars = 50 μm.

Table 2
IgG subclass.

Specificity of reaction	Sera	Target RBC	IgG1	IgG2	IgG3	IgG4
E(+)	Day 0[a]	ccDEE, Di(a + b−)	1.6	1.2	1.6	1.0
E(+)	Day 7[b]	ccDEE, Di(a + b−)	4.8	1.9	40.8	1.8
Di(b+)	Day 0[a]	ccDee, Di(a − b+)	3.0	1.1	1.3	0.9
Di(b+)	Day 7[b]	ccDee, Di(a − b+)	9.9	1.6	1.9	1.3
Positive control	Anti-Jr[ac]	ccDee, Di(a − b+)	1.0	1.0	7.3	1.0
Background	Day 7[b]	ccDee, Di(a + b−)	1.0	1.0	1.0	1.0

Each value of signal intensity of IgG subclass was normalized to the corresponding value of a non-specific reaction. The values underlined are those more than twofold higher than that of the non-specific background reaction.
[a] Day 0, pre-transfusion serum of the patient.
[b] Day 7, post-transfusion serum of the patient.
[c] Anti-Jr[a], human monoclonal anti-Jr[a] antibody.

and IgG3 significantly increased by Day 7 (Table 2, 4.8 and 40.8 times background, respectively).

5. Discussion

Although great strides have been made in blood safety, serious hazards persist, especially in regard to incompatible transfusion [16]. The United States Food and Drug Administration (US FDA) concluded that of 69 transfusion recipient fatalities from October 2010 through September 2011, 30 (43%) were transfusion-related, including 9 cases of hemolytic transfusion reaction (3 ABO and 6 non-ABO) [17]. Donor RBCs are routinely selected according to ABO and RhD compatibility [18]. ABO typing is chiefly to avoid immediate intravascular RBC hemolysis via the classical complement pathway, and Rh D typing is made to avoid anti-D alloimmunization.

Compatibility is also assessed by screening for non-ABO RBC alloantibodies (also known as irregular antibodies) in the recipient's serum using antigen-antibody reactions, e.g. column agglutination and IATs. Takeshita et al. estimated that the frequency of irregular RBC antibodies was 1.43% (3554 of 248,785 patients tested) from Japanese data from 2008 to 2009 [19]. Alloantibodies, detected or not, have the potential to hemolyse RBCs through intravascular or extravascular pathways. As a countermeasure, detection of RBC alloantibodies is routinely performed, but difficulties still occur in emergency cases when compatible RBCs

are scarce or unavailable. The paucity of rare units may be exacerbated by ever-expanding donor exclusion criteria.

Clinically significant antibodies are defined by the British Committee for Standards in Haematology (BCSH) as those that are capable of causing morbidity or mortality due to accelerated destruction of a significant proportion of transfused RBCs [18]. In the present case, the rise in antibody titers of both anti-Di^b and anti-E corresponded to the abrupt onset of hemolysis. Flow cytometry showed that these IgG1 or IgG3 subclasses reacted with corresponding antigen-positive RBCs, and MMA indicated that sensitized RBCs were engulfed by monocytes. Consistent with the serological findings, autopsy revealed that extravascular hemolysis occurred predominantly in the spleen. These findings demonstrated a causal relationship between alloantibodies and DHTR, suggesting that either antibody is clinically significant.

The Diego blood group system consists of antithetical antigens Di^a and Di^b [20]. Accordingly, there are three principal Diego phenotypes: Di(a − b+), Di(a + b+) and Di(a + b−). Fewer than 0.01% of Caucasians have Di^a, whereas the frequency of Di^a is 5–15% in South American Indians and in most Mongoloid populations [21]. In Japanese population, the frequencies of Diego phenotype are 0.1% for Di(a + b−), 8.3% for Di(a + b+) and 91.6% for Di(a − b+) [13]. These data suggest that Diego phenotyping may be dispensable in Caucasians, but beneficial for Mongoloid or Native American patients when an antibody to a high incidence antigen is suspected. However, Di(a + b−) panel cells for identifying irregular antibodies are not currently available in Japan, despite the relatively high frequency.

The Diego phenotype Di(a + b−) allows the production of anti-Di^b, whose clinical significance is reported in a limited number of DHTR cases [20,22–25] and around 30 cases of hemolytic disease of the newborn [13,26–33]. In contrast, DHTRs due to anti-E and other Rh system antibodies have been reported with some frequency [1–5,7,9,34]. The present case may be the first fatal DHTR associated with clinically significant anti-Di^b and massive Di(b+) incompatible transfusion reported in the English literature, although coexistence of E incompatibility, whose reactivity was stronger than that of Di(b+) in MMA, must be acknowledged.

Transfusion of Di(a + b−) RBCs for patients with an anti-Di^b is a challenge because of its rarity, which limits the availability of diagnostic panel cell reagents and compatible blood. The US FDA mandates that commercial cell panels include RBCs with C, D, E, c, e, M, N, S, s, P_1, Le^a, Le^b, K, k, Fy^a, Fy^b, Jk^a and Jk^b antigens [34], but there is no requirement for Di(a + b−) RBCs. This omission is regrettable because there are several hundred Di(b−) blood donors listed on the rare donor panel in Japan and therefore Di(b−) RBCs might have been provided if the patient's Di(a + b−) phenotype had been known. Anti-Di^b must be considered as a possibility when an antibody to a high incidence antigen is found in a Japanese patient, and, therefore, the spread of Di(a + b−) panel cells is advisable for appropriate management of Japanese and other non-Caucasian populations.

DNA-based genotyping, including rare antigens like Di(b+), is gaining favor as an alternative to conventional serological studies [22,35–37]. However, comprehensive blood group genotyping remains elusive because the 328 RBC antigens currently recognized by the International Society of Blood Transfusion (ISBT) are widely distributed among the human chromosomes [38]. It is premature to suggest that emerging methods will be applicable to every situation, but in the future, genetic characterization of a patient's RBC antigen profile may be a means to optimize transfusion product selection and minimize both short and long term adverse events. In the meantime, this fatal case should serve as a somber reminder that the presence of broad-reactive antibodies should be investigated as thoroughly as possible in critically bleeding patients. Panel cells capable of identifying rare but clinical significant antibodies, like anti-Di^b, should be widely available.

Acknowledgement

A preliminary report of this case was made at the XXth Regional Congress of the ISBT, Asia, November 2009, Nagoya [39].

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