

Anaphylactic transfusion reactions in haptoglobin-deficient patients with IgE and IgG haptoglobin antibodies

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BACKGROUND: Patients with haptoglobin deficiency associated with haptoglobin IgG antibodies, who experienced severe nonhemolytic transfusion reactions (NHTRs), have been identified in Japan. Haptoglobin deficiency therefore might be a risk factor for NHTRs.

STUDY DESIGN AND METHODS: A total of 4138 cases of voluntarily reported NHTRs in Japan, including 367 cases of immediate-onset anaphylactic NHTRs, were examined to identify haptoglobin deficiency. Serum haptoglobin IgG and IgE antibodies were determined in haptoglobin-deficient patients to elucidate the mechanism underlying the transfusion reactions.

RESULTS: Seven patients with haptoglobin deficiency were identified. Six of them experienced severe and acute NHTRs. Six of them were identified to be homozygous for the *Hp^{del}* allele of the haptoglobin gene. Both haptoglobin IgG and IgE antibodies were detected in serum samples of all the patients. The stimulative effects of blood transfusion on the production of haptoglobin antibodies in the patients and the relationship between the presence of the antibodies and the occurrence of the transfusion reactions were observed.

CONCLUSION: Anaphylactic NHTRs in these patients with haptoglobin deficiency associated with serum haptoglobin antibodies were suggested to be prevalent in Japan. In addition to IgG antibodies, IgE haptoglobin antibodies detected in the sera of such patients were suggested to play a role in the occurrence of the reactions.

Life-threatening anaphylactic nonhemolytic transfusion reactions (NHTRs) are rare but certainly occur, and a number of cases of anaphylactic NHTRs have not been analyzed in clinical practice. Although existing alloantibodies against several plasma proteins in patients with deficiency of the respective plasma proteins were thought to be responsible for anaphylactic NHTRs, a limited number of antibodies against plasma proteins, such as anti-IgA,¹⁻³ anti-C4,^{4,5} and coagulation factor antibodies,^{6,7} are recognized as the causative antibodies of anaphylactic NHTRs. Thus, it is possible that there is a type of plasma protein deficiency that is not pathogenic, similar to selective IgA deficiency, but in which antibodies are produced due to alloimmunization, which leads to the development of anaphylactic NHTRs.

Haptoglobin is a plasma glycoprotein that is capable of binding free Hb within the normal range (152 ± 83 mg/dL, mean $\pm$ 1 SD). It consists of two subunits of α - and β -chains, and there are three major phenotypes: type 1-1, type 2-1, and type 2-2. These phenotypes are related to the α -chains, α_1 - and α_2 -chains, encoded by a pair of codominant alleles, *HP1* and *HP2*.⁸ A 30-year-old man with asymptomatic haptoglobin deficiency was previously identified among 9711 healthy Japanese blood donors,⁹ and a Japanese patient suffering from anaphylactic transfusion reactions was judged to have congenital haptoglobin deficiency associated with serum haptoglobin

ABBREVIATIONS: NHTR(s) = nonhemolytic transfusion reaction(s); PC(s) = platelet concentrate(s).

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antibodies.¹⁰ These findings suggest that haptoglobin deficiency may be a risk factor for the occurrence of NHTRs in Japan.

The Japanese Red Cross has been conducting a nationwide search for the occurrence of transfusion reactions since 1992 to improve the safety of transfusion practices.¹¹ Information on adverse reactions associated with transfusion was reported voluntarily by hospitals to the Japanese Red Cross Central Blood Center (Tokyo, Japan) through the medical information network. If possible, patients' serum samples and any remaining transfused blood components were simultaneously sent to the blood center to determine the cause of the reactions. Several tests, selected for each case depending on the severity and the resultant symptoms of the reactions, were performed, and the results were reported to the hospitals. Serum haptoglobin concentrations and haptoglobin antibodies were also analyzed in patients exhibiting NHTRs. During the course of the study, two patients having haptoglobin deficiency associated with haptoglobin IgG antibodies, who suffered from anaphylactic transfusion reactions, were identified.^{12,13} They were identified to be homozygous for a deleted allele of the haptoglobin gene (*Hp*), the *Hp^{del}* allele, in which a large 28-kb deletion from the upstream of the promoter region of *Hp* to intron 4 of the haptoglobin-related (*Hpr*) gene was identified.¹⁴ Additional cases of haptoglobin deficiency were also found among the cases of transfusion reactions reported to the Japanese Red Cross.

In this study, we report on haptoglobin deficiency identified in Japanese patients with NHTRs during the nationwide search and their production of haptoglobin antibodies, not only IgG but also IgE antibodies, that may play a role in the occurrence of the anaphylactic reactions via high-affinity IgE receptors on mast cells and basophils leading to development of type I hypersensitivity reactions.

MATERIALS AND METHODS

Patients and blood samples

Blood samples from 4138 patients with NHTRs who were referred to the Japanese Red Cross between May 1993 and December 2000 were analyzed. The male to female ratio was 1:0.8, with age ranging from 0 to 98 years (average age, 53.1 years). Forty-two percent of the patients suffered from hematologic diseases, 23 percent from cancer, and 35 percent from other diseases. Sixty-five percent had a history of transfusion, and 33 percent had previously experienced transfusion side-effects. Forty-one percent of the female patients had a history of pregnancy. Seventeen percent had neither a history of transfusion nor of pregnancy. The transfused blood components used were platelet concentrates (PCs) (48.2%), RBCs (27.9%), FFP (16.0%), and others (7.9%) including

plasma fractionation components and washed blood cells. Seventy-one percent of the patients suffered from allergic NHTRs, including 367 cases of immediate-onset (within 20 min of the start of transfusion), anaphylactic NHTRs.

Patients' blood samples, collected in hospitals, were sent to the blood center. Sera were separated and stored at -80°C until assays were performed. Genomic DNA was extracted from PBMNCs.

Serum haptoglobin concentrations

Serum haptoglobin concentrations were measured by means of peak-rate nephelometry (Array 360 System, Beckman Coulter, Tokyo, Japan).

PCR analysis for identification of the *Hp^{del}* allele

PCR analysis was carried out according to the method of Koda et al.¹⁴

Haptoglobin IgG antibody ELISA

One hundred μ L of the purified mixed-type haptoglobin solution (#16-16-08116, Athens Research and Technology, Athens, GA, 1 μ g/mL in 0.02 M sodium carbonate-bicarbonate, pH 9.6) was added to each well, and the plate was incubated overnight at 4°C. After blocking the plate with 1-percent (wt/vol) BSA in PBS, serum (diluted with 1% [wt/vol] BSA in PBS) was added at 100 μ L per well, and the plate was further incubated overnight at 4°C. Haptoglobin IgG antibody was detected by using horseradish peroxidase-conjugated mouse monoclonal antihuman IgG (Fc) (#05-4220, Zymed Laboratories, South San Francisco, CA) and 3,3',5,5'-tetramethylbenzidine microwell peroxidase substrate (#50-76-00, Kirkegaard & Perry Laboratories, Gaithersburg, MD) by determining the increment in the differential OD at 450 nm minus that at 650 nm and comparing the result with that from normal healthy donors as controls. Serum samples that gave values higher than the average plus 3 SD for normal healthy donors were considered to be positive for haptoglobin IgG antibody. Antihaptoglobin IgG titer was represented by the maximum number of dilutions after which the sample showed a positive reaction.

Haptoglobin IgE antibody ELISA

In the methods to detect allergen-specific IgE antibodies such as radioallergosorbent tests, a large amount of antigen is designed to coat the materials in order to trap antigen-specific IgE antibodies effectively in the presence of an excess amount of allergen-specific IgG antibody. Because the amount of haptoglobin coated on microtiter plates is thought to be small, sera were subjected to IgG depletion to eliminate the effects of haptoglobin IgG antibodies on haptoglobin IgE antibody analysis. Serum (30 μ L) was added to each well of a 96-well membrane titer plate (Multiscreen #MAGV N22 10, Millipore, Tokyo, Ja-

pan) filled with 100 μ L of protein-G Sepharose 4FF (#17-0618-02, Amersham Pharmacia Biochemical, Tokyo, Japan), and this was incubated overnight at 4°C. The Sepharose gels were washed three times by adding 100 μ L of saline to each well, followed by centrifugation, and the washed filtrates were pooled as the "protein-G pass fraction." A haptoglobin-coated plate was prepared according to the method of haptoglobin IgG antibody ELISA except that the concentration of haptoglobin (10 μ g/mL) and the type of microtiter plate (Fluoro Nunc plate #43958, Nalge Nunc International, Tokyo, Japan) were different. The protein-G pass fraction was added to the haptoglobin-coated plate (100 μ L/well), and this was incubated overnight at 4°C. Haptoglobin IgE antibody was detected using antihuman IgE- β -D-galactosidase-conjugated antibody and the 4-methylumbelliferyl- β -D-galactoside substrate from the CAP RAST FEIA enzyme-conjugated reagent kit (CAPRAST FEIA #10-9184-10, Pharmacia & Upjohn, Tokyo, Japan), as the change in fluorescence intensity at an excitation wavelength of 360 nm and an emission wavelength of 450 nm with a multilabel counter (1420 ARVosx Multilabel Counter, Wallac Berthold Japan, Tokyo, Japan), which was compared with the fluorescence of samples from normal healthy donors as controls. Serum samples that gave values higher than the average plus 3 SD for normal healthy donors were considered to be positive for haptoglobin IgE antibody.

Western blot analysis

Human haptoglobins (1.7 μ g), mixed type, type 1-1, type 2-1, and type 2-2 (H-0138, H-9887, and H-9762, respectively, Sigma-Aldrich Japan, Tokyo, Japan), incubated overnight with 0.0625 M Tris containing 2.4-percent (wt/vol) SDS and 10-percent (vol/vol) glycerol, pH 7.4, in the presence or absence of 2-percent (vol/vol) 2-ME at room temperature, were electrophoresed on a polyacrylamide slab gel (84 $\times$ 90 $\times$ 1 mm³, Multigel 2/15, Daiichi Pure Chemicals, Tokyo, Japan) with 0.04 M Tris-acetate (pH 8.5) containing 0.2-percent SDS and 0.002 M EDTA and then electrophoretically transferred onto a polyvinylidene difluoride-based membrane (Immobilon-PTM, #IPVH 151 50, Millipore) by using 0.025 M Tris, 0.192 M glycine, and 20-percent (vol/vol) methanol (pH 8.3). After blocking with 5-percent (wt/vol) BSA in PBS, the membrane was incubated for 18 hours with the patients' serum diluted 1 in 150 with 1-percent BSA in PBS. Haptoglobin IgG antibody was detected with the horseradish peroxidase-conjugated mouse monoclonal antihuman IgG (Fc) and an enhanced chemiluminescent reagent (ECL Western Blotting Detection Reagents, #RPN2134, Amersham Pharmacia). For the detection of haptoglobin IgE antibody, the membrane was incubated with the protein-G pass fraction, and haptoglobin IgE antibody was detected with the affinity chromatography-isolated biotin-conjugated goat antihuman IgE (ϵ) (catalog num-

ber 2594, Bio Source International, Camarillo, CA), streptavidin-biotinylated horseradish peroxidase complex (#RPN1051, Amersham Pharmacia), and an enhanced chemiluminescent reagent.

Ouchterlony double immunodiffusion

Serum (20 μ L) and haptoglobin (0.5 μ g in 20 μ L) were added to each well of a 1.7-percent (wt/vol) agarose plate in PBS containing 3-percent (wt/vol) PEG (molecular weight 4000), and the plate was incubated for 3 days at 25°C. After removing free serum proteins from the agarose gel by washing with an excess volume of saline at 4°C, the gel was stained by Coomassie Brilliant Blue.

Measurement of total IgG and IgE

Total serum IgE and IgG were determined with a conventional sandwich ELISA.

RESULTS

Patients with haptoglobin deficiency and transfusion reactions

Seven patients were suggested to be haptoglobin deficient because their serum haptoglobin concentrations were lower than the detection limit (<3 mg/dL), and the low levels did not change during the observation period that averaged 3.3 months. PCR analysis of the haptoglobin gene showed that six of the patients were homozygous for a deleted allele of the haptoglobin gene, the *Hp^{del}* allele. The observed prevalence of haptoglobin deficiency among the patients with NHTRs was 7 of 4138 and that among patients with anaphylactic NHTRs was 6 of 367. The characteristics, backgrounds, and transfusion reactions of the patients are summarized in Table 1.

The patients' age ranged from 33 to 94 years, with an average of 66. The male to female ratio was 1:0.75, and the patients had suffered from various diseases. Transfusion reactions observed in the patients with haptoglobin deficiency were so severe that six of the seven patients went into shock immediately after the start of transfusion. The symptoms resembled those of typical hypersensitive type I anaphylactic shock characterized by a sudden drop in blood pressure, unconsciousness, respiratory distress or arrest, weakened or no heartbeat, and/or hives and skin rash immediately after the start of transfusion. Six of the patients showing these symptoms were considered to have gone into anaphylactic shock. One patient who showed relatively mild reactions of urticaria, discomfort, vomiting, and dyspnea was considered to have an anaphylactoid reaction. Although the transfusion reactions were very severe, the patients were successfully resuscitated by using conventional methods and corticosteroid medication. The patients had received various types of blood components at the time of the adverse transfusion, and five of them had a history of transfusion

TABLE 1. Patients identified as haptoglobin deficient and reported transfusion reactions*

Patient number	Age	Sex	Disease	Serum haptoglobin/Genotype	Transfusion reaction onset (min)	Transfused blood component	History		
							Transfusion	NHTRs	Pregnancy
1	94	F	Myelodysplastic syndrome	ND†/Hp ^{del}	2	PC	Yes	No	Yes
2	33	F	Hydramnion, fetal malformation	ND/Hp ^{del}	2	RBCs, plasma protein fraction, HAS‡	No		Yes§
3	66	M	Myelodysplastic syndrome	ND/Hp ^{del}	2	PC	Yes	No	
4	71	M	Myelodysplastic syndrome and others	ND/Hp ^{del}	20	PC	Yes	No	
5	71	M	Arteriosclerosis obliterans	ND/Hp ^{del}	5	FFP, plasma protein fraction	Yes	No	
6	33	M	Chronic renal failure	ND/Hp ^{del}	5	RBCs	Not clear		
7	50	F	Bladder cancer	ND/NT	5	PC	Yes	No	Yes

* Patients 1, 2, 3, 5, 6, and 7 experienced anaphylactic transfusion reactions. Patient 4 had an anaphylactoid reaction.

† Not detected.

‡ Human serum albumin.

§ Patient was in Week 31 of pregnancy.

but had not previously experienced any adverse transfusion reactions.

Haptoglobin IgG antibody detected in patients' sera

Haptoglobin IgG antibody was detected in the serum samples of all seven patients by ELISA (Fig. 1A), and the haptoglobin antibody was neutralized by incubation with purified human haptoglobin at 37°C for 30 minutes (data not shown). Haptoglobin antibodies were also confirmed by Western blotting. Typical results obtained for Patient 2 are presented in Figure 1B. Antibody bands corresponding to the electrophoretic patterns of the complex polymeric structure of haptoglobins and indicating antibodies against three major phenotypes of human haptoglobin—type 1-1, type 2-1, and type 2-2—were observed under nonreducing conditions. Antibodies against α_1 -, α_2 -, and β -chains derived from these phenotypes were detected under reducing conditions. The haptoglobin antibody was also detected by Ouchterlony double immunodiffusion. Serum samples from six of the seven patients, except Patient 7, were positive in the assay. Figure 1C shows the typical positive results obtained for Patient 3. Antibodies against the three phenotypes of haptoglobin were detected.

Haptoglobin IgE antibody detected in patients' sera

The recoveries of total serum IgE and IgG in the protein-G pass fractions prepared from the sera were 85.8 percent and less than 0.01 percent, respectively. In addition, when a protein-G pass fraction was prepared from the serum of a haptoglobin-deficient patient, haptoglobin IgG antibody was effectively eliminated from the sera

by the treatment (data not shown). These results suggest that both total IgG and specific IgG were effectively eliminated from the sera by the treatment. Haptoglobin IgE antibody was detected in all seven cases by ELISA by using protein-G pass fractions (Fig. 2A) and that was neutralized by purified human haptoglobin (data not shown). Haptoglobin IgE antibody was also detected by Western blotting. Figure 2B shows typical results obtained for Patient 5. Antibodies against the three major phenotypes of human haptoglobin and against the β -chain were detected under nonreducing and reducing conditions, respectively.

Production of haptoglobin antibodies in patients and development of NHTRs

The production of haptoglobin antibodies in the serum of patients during the clinical course of their illness could be observed in two patients. In Patient 1, a 94-year-old woman suffering from myelodysplastic syndrome and having a history of pregnancy had been admitted to the hospital and had received blood transfusions four times to improve her anemia without any observed transfusion reactions during the following 6 months. Haptoglobin IgG antibody was detected in high titer after receiving the first PC transfusion. Simultaneously, haptoglobin IgE antibody became detectable in her serum. A severe transfusion reaction occurred at the second PC transfusion. Approximately 2 minutes after the transfusion, the patient suddenly went into shock, became unconscious with unmeasurable low blood pressure, stopped breathing, and had cardiac arrest. Although the transfusion was stopped immediately and the volume of the transfused blood component was very low, the haptoglobin IgG antibody titer was slightly lower, and the haptoglobin IgE

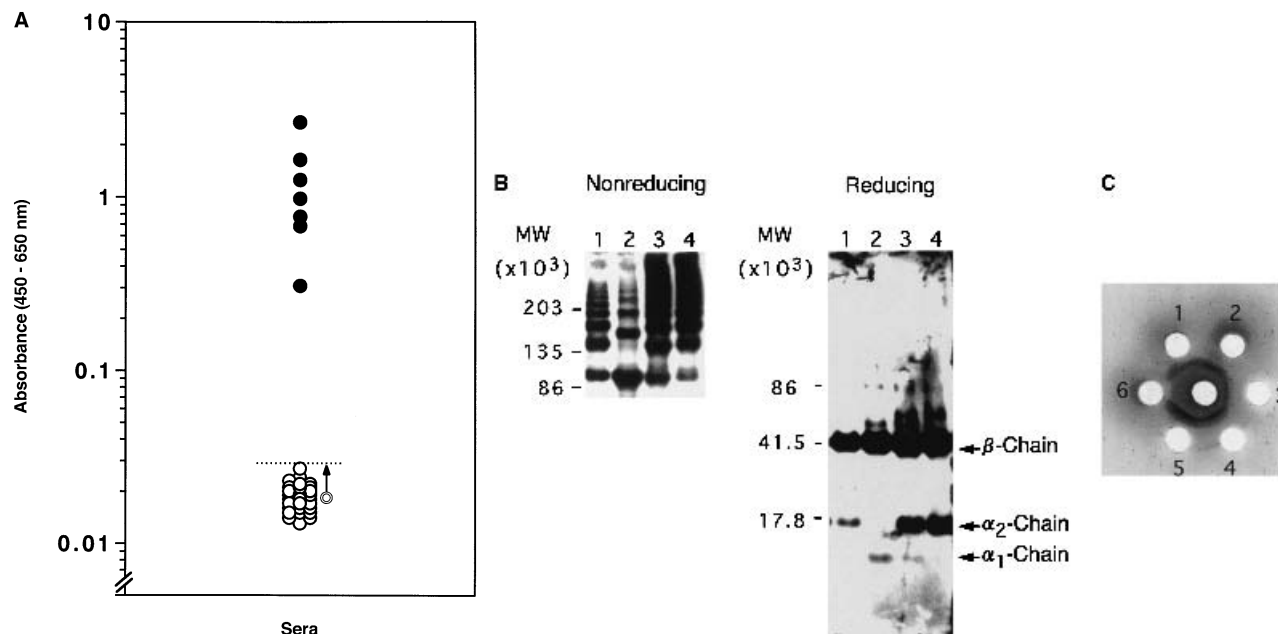


Fig. 1. Haptoglobin IgG antibody detected in the sera obtained from haptoglobin-deficient patients with severe NHTRs. (A) Haptoglobin IgG antibody detected by ELISA. Sera obtained from haptoglobin-deficient patients (●) and normal healthy donors (○) diluted 500 times were assayed. ○→, mean + 3 SD shown by normal healthy donors ($n = 96$). (B) Western blot analysis. Serum from patient in Patient 2 diluted 150 times was assayed for haptoglobin IgG antibody. Human haptoglobins were electrophoresed under nonreducing and reducing conditions. Lane 1, haptoglobin mixed type; Lane 2, type 1-1; Lane 3, type 2-1; and Lane 4, type 2-2. (C) Ouchterlony double immunodiffusion. Center well, serum from Patient 3; Side well 1, serum prepared from the transfused blood component (diluted 10 times); 2, human haptoglobin mixed phenotype; 3, type 1-1; 4, type 2-1; 5, type 2-2; and 6, serum from haptoglobin-deficient patients without haptoglobin antibody. Serum samples that presented precipitating lines against haptoglobin were considered as positive for anti-haptoglobin.

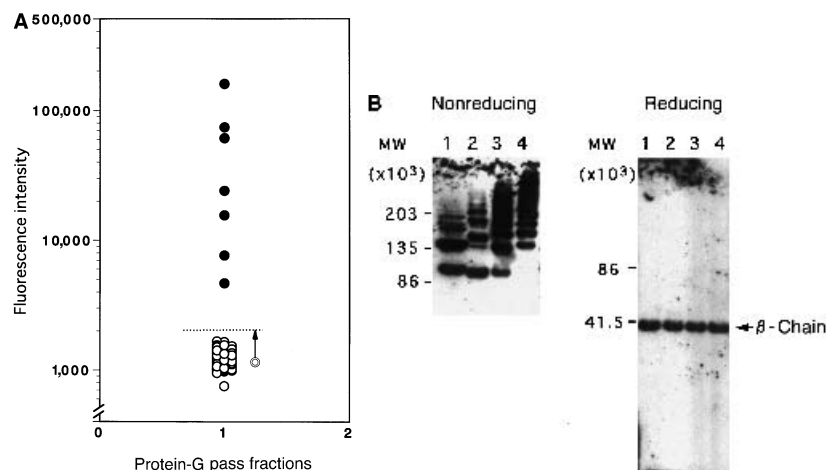


Fig. 2. Haptoglobin IgE antibody detected in the sera of haptoglobin-deficient patients with severe NHTRs. (A) Haptoglobin IgE antibody detected in the protein G-pass fractions prepared from the patients' sera by ELISA. Protein-G pass fractions prepared from the sera of the haptoglobin-deficient patients (●) and normal healthy donors (○) were assayed. ○→, mean + 3 SD shown by normal healthy donors ($n = 96$). (B) Western blot analysis. Protein-G pass fraction from Patient 5 diluted 150 times were assayed for haptoglobin IgE antibody. Human haptoglobins were electrophoresed under nonreducing and reducing conditions. Lane 1, haptoglobin mixed type; Lane 2, type 1-1; Lane 3, type 2-1; and Lane 4, type 2-2.

antibody could not be detected after the transfusion. The haptoglobin IgG antibody titer recovered to the same level as that immediately before the transfusion, and haptoglobin IgE antibody could be detected within 2 weeks after the transfusion. Saline-washed RBCs were transfused successfully to the patient without the occurrence of any transfusion reactions in the presence of haptoglobin antibodies (Table 2).

Patient 6 was a 33-year-old man with chronic renal failure whose history of transfusion was unclear. He had received one unit of RBCs to improve anemia after hemodialysis and suffered from severe NHTR. Immediately after the start of transfusion, he complained of an uncomfortable feeling and suddenly went into shock, became unconscious with unmeasurably low blood pressure, and stopped breathing. Both haptoglobin IgG and IgE antibodies were detected in the serum that was

TABLE 2. Development of haptoglobin antibodies and occurrence of anaphylactic NHTRs

	Time (day)	Blood transfusion	Transfusion reaction	Antihaptoglobin (titer)§	
				IgG	IgE
Patient 1	-212	RBCs	ND*	NT	NT
	-148	RBCs	ND	NT	NT
	-22	RBCs	ND	Positive (100)†	Negative†
	-18	PC	ND	NT	NT
	0	PC	Anaphylactic	Positive (3200)†	Positive†
	1			Positive (800)	Negative
	2	Saline-washed RBCs	ND	NT	NT
	19	Saline-washed RBCs	ND	Positive (6400)†	Positive†
				Positive (200)	Positive
Patient 6	-1			Negative‡	Negative‡
	0	RBCs	Anaphylactic		
	25			Positive (102,400)	Positive

* Not detected.

† Results determined before transfusion.

‡ Results determined after transfusion.

§ Antibody titers were measured by ELISA.

collected before the start of transfusion, although the haptoglobin IgG antibody titer was low. Both classes of antibodies could not be detected immediately after the transfusion but could be detected within 3 weeks after the transfusion, during which the haptoglobin IgG antibody titer had increased markedly.

DISCUSSION

Seven patients with haptoglobin deficiency, six of whom experienced severe anaphylactic NHTRs, were identified among 4138 patients with NHTRs, including 367 patients with anaphylactic NHTRs, reported voluntarily by hospitals to the Japanese Red Cross between May 1993 and December 2000. These seven haptoglobin-deficient patients were asymptomatic. They were found to be haptoglobin deficient after they experienced severe NHTRs in the course of treatment of their illness since middle age. The symptoms of NHTRs in the six patients with haptoglobin deficiency were very severe and acute, similar to anaphylactic NHTRs reported for selective IgA deficiency associated with IgA antibodies,¹⁻³ and other plasma protein deficiencies associated with antibodies against the respective plasma proteins.⁴⁻⁷ Strongly precipitating haptoglobin IgG and IgE antibodies against three major phenotypes of human haptoglobin—type 1-1, type 2-1, and type 2-2—were detected in the sera of the haptoglobin-deficient patients, and a relationship between the presence in the serum of haptoglobin antibodies and the occurrence of anaphylactic NHTRs was observed (Table 2). These data indicate that these antibodies may play a role in the occurrence of anaphylactic NHTRs.

Most of the seven patients experienced alloimmunization when they suffered from NHTRs. Five had histories of transfusion, and one was in her 31st week of pregnancy. Six of the seven patients were congenitally

haptoglobin deficient, being homozygous for *Hp^{del}*.¹⁴ The haptoglobin antigen was absent in the patients because of a large, almost complete deletion of the haptoglobin gene and the lack of immune tolerance caused by the absence of the haptoglobin antigen might contribute to the development of haptoglobin antibodies by exposure of patients to exogenous haptoglobin. Anti-haptoglobin IgG in the transfused blood components for the seven haptoglobin-deficient patients was analyzed to be negative (data not shown), suggesting the haptoglobin antibodies in the patients were obtained by alloimmunization rather than by passive immunizations. Enhancement of the pro-

duction of haptoglobin antibodies by blood transfusion was observed in Patients 1 and 6 (Table 2). The other four patients, who had a history of transfusion but had not experienced any transfusion reactions, were postulated to have been immunized by earlier transfusions resulting in severe NHTRs at subsequent transfusions.

It would be interesting to determine whether IgG class or IgE class haptoglobin antibodies mediate such anaphylactic NHTRs. It is known that IgG antibodies to IgA are associated with the occurrence of anaphylactic transfusion reactions in selective IgA deficiency.¹⁻³ Precipitating C4 antibodies were reported to be associated with the occurrence of anaphylactic reactions in C4 allotype deficiency.^{4,5} The formation of a complement-fixing immunocomplex of antigen-IgG alloantibodies and the activation of the complement system associated with the occurrence of anaphylactic NHTRs were reported in a patient with severe vWD.⁶ These data support the idea that the formation of circulating immune complexes leads to the activation of the complement system to release anaphylatoxins (C4a, C3a, C5a) that activate effector cells to release various mediators that cause anaphylactic reactions. On the other hand, several studies reported on IgE antibodies against plasma proteins thought to cause type I hypersensitivity reactions.¹⁵⁻¹⁷ Burks et al.¹⁵ identified IgE antibodies to IgA in a patient with hypogammaglobulinemia who also suffered from anaphylactic reactions after administration of gammaglobulin and suggested the importance of these antibodies. Dioun et al.¹⁷ reported that anaphylactic reactions in hemophilia B patients, which were associated with F IX antibodies after infusion of F IX concentrate, were IgE-mediated reactions because after desensitization, the disappearance of the allergic reactions was accompanied by the disappearance of serum F IX-specific IgE antibodies. In the seven cases of haptoglobin deficiency presented in this article, both haptoglobin IgG and IgE antibodies were

detected in the patients' sera. Thus, both mechanisms, activation of complement by immune complexes of infused haptoglobin and haptoglobin IgG antibodies and haptoglobin IgE-mediated type I hypersensitivity reactions, are possible. Patient 1 exhibited anaphylactic NHTRs after the production of high titers of haptoglobin IgG and IgE antibodies. On the other hand, in Patient 6, the patient suffered from anaphylactic NHTRs when he was found to have haptoglobin IgE antibodies and only low levels of haptoglobin IgG antibodies. High titers of haptoglobin IgG antibodies were observed in the patients after anaphylactic NHTRs. IgE-mediated type I hypersensitivity reactions were needed to play a major role in the generation of such NHTRs at least in Case 6. Only a small amount of haptoglobin was suggested to generate NHTRs because NHTRs occurred immediately after the start of transfusion when a very small volume of the blood component had been transfused. Highly elevated levels of serum tryptase (193 IU/mL) compared with the usual level of 3.3 IU per mL were observed in Patient 2 during the transfusion reactions (data not shown), suggesting that mast cell activation and degranulation occurred by means of the high-affinity IgE receptors (FcεRI) as a result of the transfusions.¹⁸ These data suggest the importance of haptoglobin IgE antibodies in the generation of anaphylactic NHTRs in haptoglobin-deficient patients.

The prevalence of haptoglobin deficiency among the patients with immediate-onset anaphylactic NHTRs reported voluntarily to the Japanese Red Cross was 6 to 367. That is approximately six times higher than the prevalence of selective IgA deficiency among the same patients with anaphylactic NHTRs determined simultaneously (data not shown). The increased prevalence among the patients implies that haptoglobin deficiency is an important risk factor for anaphylactic NHTRs in Japan. Six of the seven patients were identified to have a congenital haptoglobin deficiency, being homozygous for *Hp^{del}*. We previously reported that the incidence of haptoglobin deficiency associated with homozygosity for *Hp^{del}* in Japanese patients was expected to be higher than that of IgA deficiency¹⁹ from the calculated allele frequency of the *Hp^{del}* allele among healthy Japanese individuals.¹⁴ The results presented here support this expectation and suggest that anaphylactic NHTRs in patients with haptoglobin deficiency associated with serum haptoglobin antibodies are prevalent in Japan.

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