

Hemagglutination Assays for the Diagnosis and Prevention of IgA Anaphylactic Transfusion Reactions

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Passive hemagglutination assays (PHA) may be used to detect IgA antibodies to confirm clinical diagnoses of suspected IgA anaphylactic transfusion reactions. Passive hemagglutination inhibition assays (PHIA) may be used to identify IgA-deficient blood donors whose plasma-containing components are transfused to prevent anaphylactic transfusion reactions in prospective recipients at risk because of the presence of IgA antibodies. Using a standard PHA, we detected class-specific anti-IgA in 76.3% of 80 IgA-deficient patients with a history of an anaphylactic transfusion reaction, and in 21.7% of 97 asymptomatic IgA-deficient blood donors or their IgA-deficient family members. Using PHIA, we confirmed IgA deficiency (<0.05 mg/dL) for the donors of 525 plasma-containing blood components that were transfused without acute clinical reactions to 48 IgA-

deficient recipients with anti-IgA and/or a history of an anaphylactic transfusion reaction. The frequency of IgA-deficiency with class-specific anti-IgA among 32,376 random blood donors was 0.08% (1/1,200). The combined use of PHA for detecting anti-IgA and PHIA for measuring IgA concentration provides an effective and safe strategy for the diagnosis and prevention of IgA anaphylactic transfusion reactions. However, PHA for anti-IgA lacks specificity for identifying persons who are truly at risk for significant anaphylactic transfusion reactions. The consequence is an overdiagnosis of IgA anaphylactic transfusion reactions and an overestimation of the number of persons at risk for IgA anaphylactic transfusion reactions because of the detection of an IgA antibody in their serum.

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THE OCCURRENCE OF AN anaphylactic reaction during a blood transfusion requires the exclusion of IgA deficiency and/or the presence of anti-IgA before proceeding with additional transfusions of standard blood components. Suspected IgA anaphylactic transfusion reactions are confirmed when class-specific anti-IgA is detected in the serum of an IgA-deficient recipient.¹⁻¹³ Clinically less severe, anaphylactoid transfusion reactions are attributed to subclass- (anti-IgA1 or -IgA2) or allotype- [anti-IgA2m(1) or -IgA2m(2)] specific IgA antibodies in persons with a normal concentration of total IgA.^{1,3,9,14} Most laboratories test for IgA antibodies by passive hemagglutination assay (PHA) using group O red blood cells (RBCs) coated with serologically defined IgA multiple myeloma proteins as indicator cells.¹⁻¹⁵

In patients who have class-specific anti-IgA in their serum and a history of an anaphylactic transfusion reaction, subsequent transfusions of plasma-containing components (fresh-frozen plasma, cryoprecipitated antihemophilic factor [AHF], platelets) require blood components collected from IgA-deficient donors. Typically, IgA-deficient donors are identified by screening random blood donors using immunodiffusion methods and by confirming true IgA deficiency (<0.05 mg/dL) by highly sensitive passive hemagglutination inhibition assays (PHIA).^{3,15-18} In the PHIA, the presence of IgA in the test sample results in inhibition of agglutination of IgA myeloma-coated group O red blood cells by standardized reagent anti-IgA.^{15,19}

In this report, we describe the results of testing 328 IgA-deficient patients, healthy blood donors, or their family members for IgA antibodies by PHA. We also describe the outcome of transfusing 48 patients known to have anti-IgA and/or to have a history of an anaphylactic reaction with plasma-containing blood components that had been determined to be IgA-deficient by PHIA.

MATERIALS AND METHODS

Patients with anaphylactic reactions. Sera were obtained from 359 patients with a recent history of an anaphylactic or anaphylactoid transfusion reaction. These sera were collected in hospitals or blood centers from patients who had experienced acute clinically severe

reactions that were suspected to be IgA anaphylactic transfusion reactions. None of these 359 reactions were fatal.

Radial immunodiffusion (RID) for IgA. All serum samples from patients with suspected IgA anaphylactic reactions were screened for IgA concentration by RID according to the method of Fahey and McKelvey²⁰ (Radial Immunodiffusion Test; Kent Laboratories, Redmond, WA). If IgA was not detected by RID (<0.5 mg/dL), the samples were retested by PHIA.

PHIA for IgA. Assays were performed in V-bottom microplates using the method of Vyas et al,¹ as modified by Fudenberg and Koistinen,¹⁹ and as previously described.¹⁵ Six aliquots of RBCs were coated with two examples each of IgA1, IgA2m(1), and IgA2m(2) myeloma proteins. Twenty-five microliters of serum to be tested, 25 μ L of diluted commercial rabbit anti-human IgA (Axell, Accurate Chemical & Scientific, Westbury, NY), and 25 μ L of a 0.2% suspension of indicator RBCs were incubated in V-bottom microplates at room temperature for 25 minutes. After centrifugation, the presence of IgA in the sample was demonstrated by the lack of agglutination of indicator RBCs. Conversely, the absence of IgA was demonstrated by the agglutination of indicator RBCs. All samples were tested initially at a 1:10 dilution, and those that appeared to be IgA-deficient were confirmed so by retesting at a 1:2 dilution. The sensitivity of the PHIA assay was 0.05 mg/dL (0.005 g/L), which was determined by dilutions of the United States National Reference Preparation (Lot 12-0575C, US Public Health Service, Atlanta, GA). IgA deficiency was defined as a serum concentration of less than 0.05 mg/dL (0.0005 g/L), which is the limit of sensitivity of PHIA.

PHA for IgA antibodies. All serum samples from patients with suspected IgA anaphylactic reactions were tested for IgA antibodies by PHA in V-bottom microplates, according to the method of Vyas

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et al¹ as modified by Fudenberg and Koistinen,¹⁹ and as previously described.¹⁵ Twenty-five microliters of samples to be tested and 25 μ L of a 0.2% suspension of the same indicator RBCs as used in the PHA1 assay were incubated in V-bottom microplates at room temperature for 25 minutes. After centrifugation, the presence of anti-IgA was demonstrated by the agglutination of indicator RBCs; conversely, the absence of anti-IgA was demonstrated by the lack of agglutination of indicator RBCs. We determined the specificity of all antibodies by neutralization by diluted myeloma proteins and/or serum of known IgA subclass and allotype. An antibody was categorized as class-specific if it reacted with all 6 myeloma proteins of both IgA1 and IgA2 subclasses, as subclass-specific if it reacted only with IgA1 or IgA2 myeloma proteins, and as allotype-specific if it reacted only with IgA2m(1) or IgA2m(2) myeloma proteins.

Blood donors. Sera were obtained from 32,376 random volunteer blood donors in American Red Cross blood services in Detroit, MI, Farmington, CT, and St Louis, MO. Screening for IgA concentration was performed at the blood centers using the Ochterlony double-diffusion (ODD) method.²¹ Samples with no detectable IgA by ODD were shipped frozen from the regional blood center for retesting for IgA by PHIA and for IgA antibodies by PHA at the American Red Cross National Reference Laboratories, Rockville, MD.

Additional IgA-deficient samples. An additional 161 serum samples were obtained from other IgA-deficient blood donors, patients with known immunodeficiency syndromes, or their IgA-deficient family members. All serum samples in this category were retested by PHIA to confirm IgA deficiency and were screened for IgA antibodies by PHA.

Transfusion in IgA-deficient recipients. Between 1985 and 1992, the American Red Cross Rare Donor Registry coordinated the supply of plasma-containing blood components from IgA-deficient blood donors for 48 different IgA-deficient transfusion recipients who had anti-IgA and/or a history of an anaphylactic transfusion reaction. Requirements for transfusion of RBCs for these patients were met by autologous blood collections, by automated washing of standard allogeneic RBCs, or by washed deglycerolized RBCs.²² Typically, RBCs were not supplied from IgA-deficient blood donors.

RESULTS

Patients with anaphylactic reactions. Of 359 serum samples from patients with suspected IgA transfusion reactions, 59 (16.4%) had an abnormally low concentration of IgA (0.05 to 75 mg/dL) and 80 (22.3%) were IgA-deficient (<0.05 mg/dL) by PHIA (Table 1). Class-specific anti-IgA was detected in 61 (76.3%) of the 80 sera from IgA-deficient

patients. Subclass-specific anti-IgA2 was detected in two patients with low concentrations of IgA. Subclass-specific anti-IgA2 and allotype-specific anti-IgA2m(1) were detected in one IgA-deficient patient, and allotype-specific anti-IgA2m(2) was detected in another (Table 1). The median titer of class-specific anti-IgA in the serum of 63 IgA-deficient recipients with anaphylactic transfusion reactions was 640 (range, <10 to >10,240) (Fig 1). The clinical severity of the reactions did not necessarily correlate with the serologic specificity(ies). Some patients with severe clinical reactions had only low-titer or undetectable anti-IgA.

IgA deficiency in blood donors. IgA was not detectable by RID in 111 (0.34%) of 32,376 random blood donors. When these 111 sera were retested by PHIA, 87 (0.26%) were confirmed to be IgA-deficient. Of these 87 IgA-deficient sera, 27 (31.0%) contained class-specific anti-IgA. None of these 27 donors had a known history of blood transfusion. The frequency of IgA deficiency among the 32,376 blood donors was one in 372. One in 1,200 (0.08%) blood donors was IgA-deficient and also had class-specific anti-IgA detected by PHA.

Anti-IgA in asymptomatic IgA deficiency. In addition to the 87 sera from IgA-deficient blood donors, we tested 261 serum samples from other blood donors known to be IgA-deficient or from their IgA-deficient family members (Table 2); of the total 358 samples from IgA-deficient donors or their family members, class specific anti-IgA (alone or together with a subclass or allotype-specific antibody) was detected in 97 (27.1%) (Table 2). The median titer of class-specific anti-IgA in IgA-deficient blood donors was 120 (range, <10 to >1,024) (Fig 2). Thus, there was considerable overlap in titers of anti-IgA among asymptomatic blood donors (Fig 2) compared with patients with anti-IgA and a history of an anaphylactic transfusion reaction (Fig 1).

Transfusion of IgA-deficient blood components. Five hundred twenty-five plasma-containing blood components from IgA-deficient blood donors were transfused without acute reactions to 48 patients with known anti-IgA and/or a history of an anaphylactic transfusion reaction. These blood components were fresh-frozen plasma, or single-donor plasma (506 U), cryoprecipitated AHF (15 U), and platelets (pheresis; 4 U). Of 48 recipients, 38 underwent surgery, including six liver and two heart transplants. These statistics reflect most, but not all, IgA-deficient plasma-containing blood components supplied by American Red Cross Blood Services nationwide between 1985 and 1992 for patients with suspected IgA anaphylactic reactions. Not included are blood components supplied locally by IgA-deficient donors, but not reported to the Red Cross Rare Donor Registry, or IgA-deficient blood products supplied by non-Red Cross Blood Services.

Three patients with high-titer, class-specific anti-IgA had recurrent anaphylactic reactions when subsequently transfused with fresh-frozen plasma that was presumed to be IgA-deficient. On retesting these units of fresh-frozen plasma by PHIA, measurable, although low, concentrations of IgA were detected. A review of records showed that the donors' IgA concentrations had been determined to be IgA-deficient by a method less sensitive than PHIA.

Table 1. IgA and Anti-IgA in Patients With Suspected IgA Anaphylactic Transfusion Reactions (N = 359)

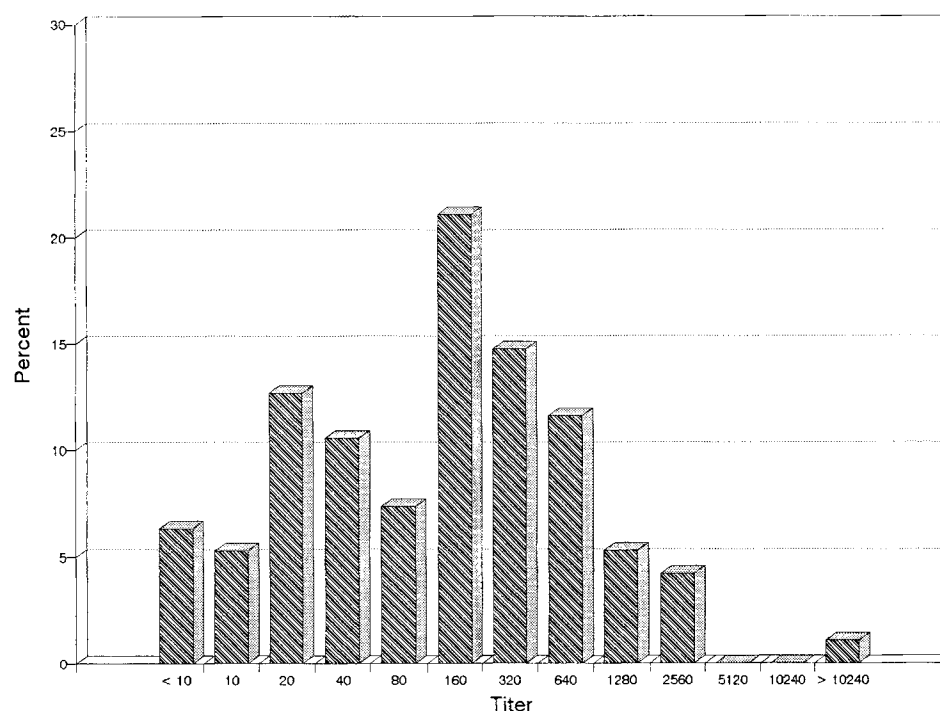
Anti-IgA Specificity	Serum IgA Concentration		
	Normal, >75 mg/dL (n = 220)	Low, 0.05-75 mg/dL (n = 59)	Deficient, <0.05 mg/dL (n = 80)
None detected	220	57	17
Class-specific	0	0	61
Subclass-specific	0	2*	0
Allotype-specific	0	0	1†
Subclass- and allotype-specific	0	0	1‡

* Anti-IgA2.

† Anti-IgA2m(2).

‡ Anti-IgA2 and anti-IgA2m(1).

Fig 1. PHA titers of class-specific anti-IgA for 63 IgA-deficient patients with a history of an anaphylactic transfusion reaction. The titer represents the reciprocal of the highest dilution of patient's serum that agglutinated all of 6 IgA myeloma-coated RBCs.



DISCUSSION

Our findings confirm other reports that PHIA for IgA is sensitive and effective for identifying safe IgA-deficient plasma-containing blood components and preventing IgA anaphylactic transfusion reactions in recipients with IgA antibodies and/or a history of an anaphylactoid transfusion reaction. This conclusion is based on the absence of acute clinical reactions when 525 plasma-containing blood components from donors determined to be IgA-deficient by PHIA were transfused in 48 patients at risk for IgA anaphylactic reactions.

Nevertheless, we cannot attribute unequivocally the absence of acute reactions in these 48 recipients to the fact that all subsequent transfusions were limited to IgA-deficient blood components. The relatively high frequency (one in 1,200) of class-specific anti-IgA detected by standard PHA in IgA-deficient donors, compared with the rarity of anaphylactic reactions in transfusion patients, strongly suggests that

using the standard PHA for anti-IgA leads to an overestimation of persons who are presumed to be at risk for clinically significant IgA anaphylactic reactions. This overestimation potentially includes persons among these 48 recipients who may have had an anaphylactic reaction unrelated to transfusion and a coincidental IgA antibody detected during the subsequent investigation. It is estimated that the incidence of IgA anaphylactic transfusion reactions is between 1 in 20,000 to 47,000 transfusions.⁹ In the 10-year period 1982 to 1992, 297,190 blood components were transfused at Georgetown University Medical Center, Washington, DC. During these 10 years, approximately 0.9% of all component transfusions were investigated for adverse reactions, but no cases of IgA anaphylactic reactions were identified (S. Novak, personal communication, January 1994). In Canada, the incidence of anaphylactic transfusion reactions has been estimated to be 1.3 per 1,000,000 U blood or blood products transfused.¹¹ As a consequence of this nonspecificity of PHA for IgA antibodies, an excessive number of IgA-deficient patients and healthy donors screened for IgA deficiency will be informed that they have anti-IgA in their serum *and* require IgA-deficient blood products for any future transfusions. Such a lifetime requirement may have serious implications if, for example, urgently needed blood transfusions are delayed while efforts are made to locate IgA-deficient blood products.

With regard to IgA antibodies of limited specificity, the issue of the nonspecificity of PHA is more complex. The only case report of a fatal anaphylactic transfusion reaction associated with anti-IgA that we have been able to identify in the medical literature involved a 57-year-old man who

Table 2. Anti-IgA in IgA-Deficient Blood Donors or Their IgA-Deficient Family Members

Anti-IgA Specificity	No.
No anti-IgA	156
Anti-IgA detected	102
Class-specific only	82
Anti-IgA2, only	3
Class-specific + anti-IgA2	8
Class-specific + anti-IgA2m(1)	2
Class-specific + anti-IgA2m(2)	1
Class-specific + anti-IgA2 + IgA2m(1)	4
Total	358

IgA concentration less than 0.05 mg/dL.

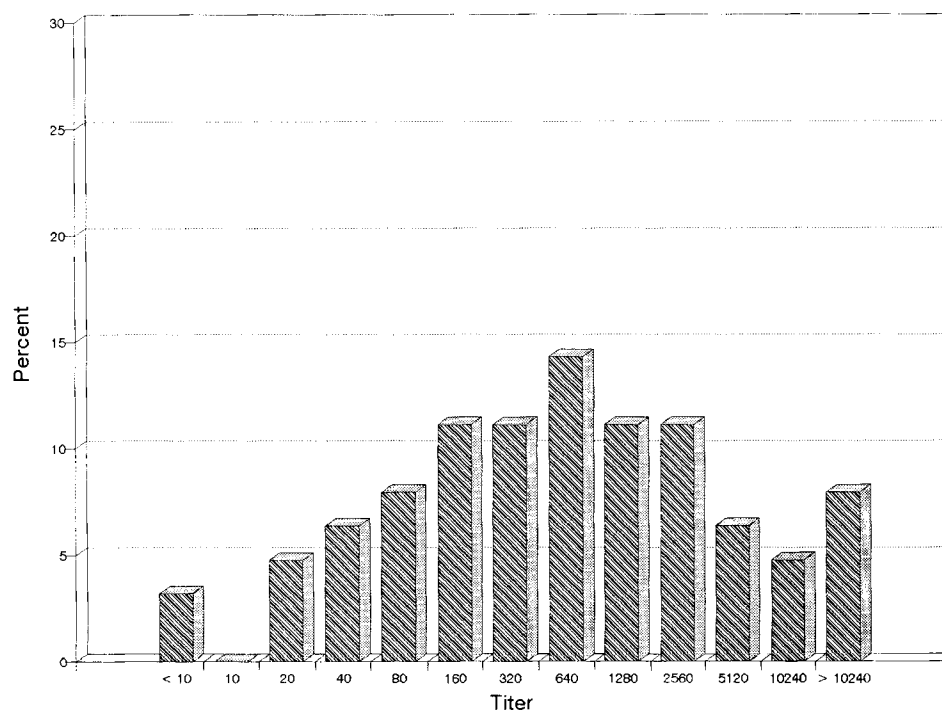


Fig 2. PHA titers of class-specific anti-IgA for 95 IgA-deficient blood donors or their IgA-deficient family members. The titer represents the reciprocal of the highest dilution of a test serum that agglutinated all 6 IgA myeloma-coated RBCs.

had a normal serum IgA concentration and an IgA antibody of limited specificity.⁹ The patient's antibody titer by PHA was 64 in the pretransfusion sample and 16 in the immediate postreaction sample. Other reports of severe transfusion reactions attributed to IgA antibodies of limited specificity have described persons who also had normal serum concentrations of total IgA.^{1,3,9,14} To a certain degree, the identification of an IgA antibody of limited specificity is a function of the number, variety, and serologic integrity of IgA myeloma proteins used to coat indicator RBCs for the PHA. In our study, we observed the frequency of IgA deficiency associated with class-specific anti-IgA to be one in 1,200 in volunteer blood donors using a PHA with 6 different myeloma-coated indicator cells: two examples each of IgA1, IgA2m(1), and IgA2m(2). Sixteen of 358 IgA-deficient blood donors or their family members had IgA antibodies of limited specificity (Table 2). Rivat et al²³ screened 1,010 healthy blood donors by PHA using 12 different serologically defined IgA myeloma proteins. They detected IgA antibodies of limited specificity in 59%, a frequency that did not differ significantly from that among patients with suspected IgA anaphylactic reactions. Our finding of a disproportionately high frequency of anti-IgA in health blood donors, compared with the rarity of confirmed IgA anaphylactic reactions in transfused patients, substantiates the impression of Mollison et al,²⁴ who suggested that the finding of IgA antibodies of limited specificity in some reports of anaphylactoid reactions "may have been, at least to some extent, coincidental." They cite a series of six patients whose plasma contained IgA antibodies of limited specificity (titer seldom >32) and who had been transfused with standard blood components without reaction.^{24,25}

Efforts to identify a more specific marker for the risk

of an IgA anaphylactic transfusion reaction have included attempts to detect IgE anti-IgA, but with equivocal results. Direct skin testing for reagin (ie, IgE anti-IgA) gave negative results in one patient with a well-documented anaphylactic reaction associated with anti-IgA.⁶ Testing for IgE anti-IgA by the Prausnitz-Kustner passive transfer technique was negative in another case.² In a previously reported study, sera from seven of 16 of our patients with anaphylactic or anaphylactoid reactions and anti-IgA by PHA had detectable IgE anti-IgA by radioimmunoassay (RIA).²⁶ RIA detected IgE anti-IgA in two patients with symptomatic hypogammaglobulinemia who had experienced well-documented recurrent anaphylactic reactions associated with anti-IgA by PHA after injections of immune serum globulin (gamma globulin).²⁶ Such approaches to a more specific laboratory diagnosis of IgA anaphylactic reactions are promising. Further research is needed to identify a marker more specific than hemagglutinating anti-IgA in patients at risk for IgA anaphylactic transfusion reactions. While the current algorithm of PHA to detect anti-IgA and PHIA to measure IgA concentration is clinically safe, it is not optimal.

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